

Aging: A Brief History

March 26, 2025

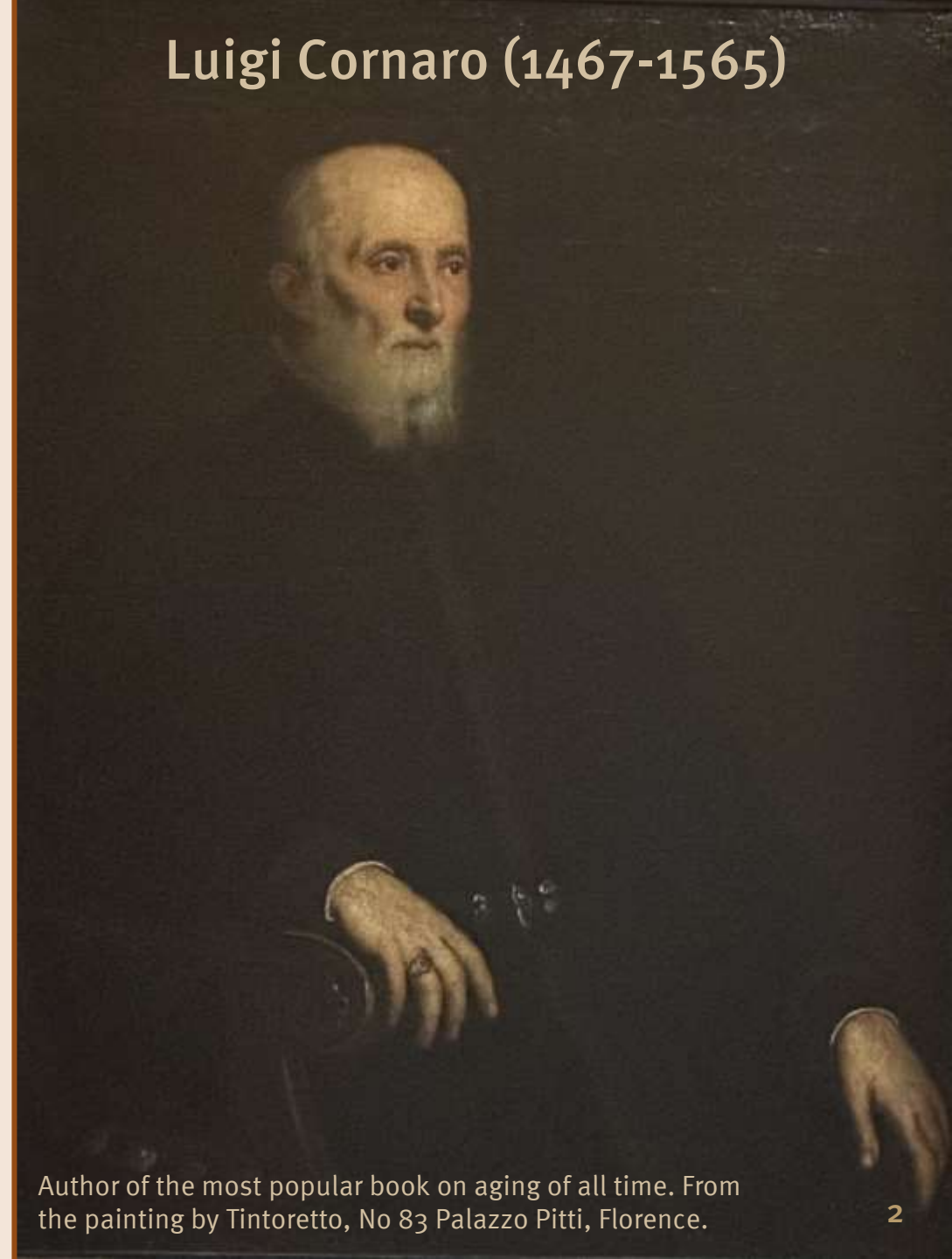
By Tim Opler (Managing Director, Stifel)



Table of Contents

Section	Page
1. Introduction	3
2. Twenty Top Historic Publications in Aging Research	20
3. Thought on Aging in Antiquity	26
4. Thought on Aging in the Medieval Period (200 to 1499 AD)	37
5. Thought on Aging in the Early Modern Period (1500 to 1800)	46
6. Thought on Aging in the 19 th Century	88
7. Thought on Aging in 1900 to 1975 Period	112
8. The Last Fifty Years of Aging Research (1976 to 2025)	160
A. Genetics and lifespan	164
B. Caloric restriction / nutrient sensing pathways	189
C. Oxidative stress, mitochondria and aging	207
D. Telomeres and senescent cells	227
E. Autophagy and aging	246
F. Parabiosis	262
G. Cellular reprogramming	272
H. Biomarkers and epidemiology of aging	290
References	305
Accessing related Stifel healthcare reports	308

Luigi Cornaro (1467-1565)



Author of the most popular book on aging of all time. From the painting by Tintoretto, No 83 Palazzo Pitti, Florence.

1. Introduction

The History of Aging Research



Leonardo da Vinci
Self-Portrait (1510)
Royal Library of Turin

Introduction: Humanity's Unending Quest for Longevity

The quest to understand aging and extend human longevity is one of the most enduring endeavors in the history of science and medicine.

For at least 5,000 years, humanity has sought to unravel the mysteries of aging, exploring its causes, consequences, and potential interventions in search of a cure. In the 11th Century, the writers of the *Regimen Sanitatis* of Salerno mused “Oh that were a medicine curing age...”

This survey traces the development of our understanding of aging, highlighting milestones, influential publications, and the intellectual progress that has shaped the field. In total, we highlight and describe a little over 200 influential and important publications on aging starting from 1700BC and ending in 2025.

This survey is the first installment of a two-part publication on aging research. We cover thought on aging from the past to the present but do not take a position on what's going to work in the science, if anything. Our upcoming second report analyzes today's translational science and where we think this field is going to take us.

The Bristlecone Pine lives for up to 5000 years



By examining the historical trajectory of aging research, we gain insight into the cumulative nature of scientific knowledge and the profound impact of past discoveries on contemporary efforts to extend human lifespan. This survey is organized chronologically, starting with ancient civilizations and progressing to the present day.

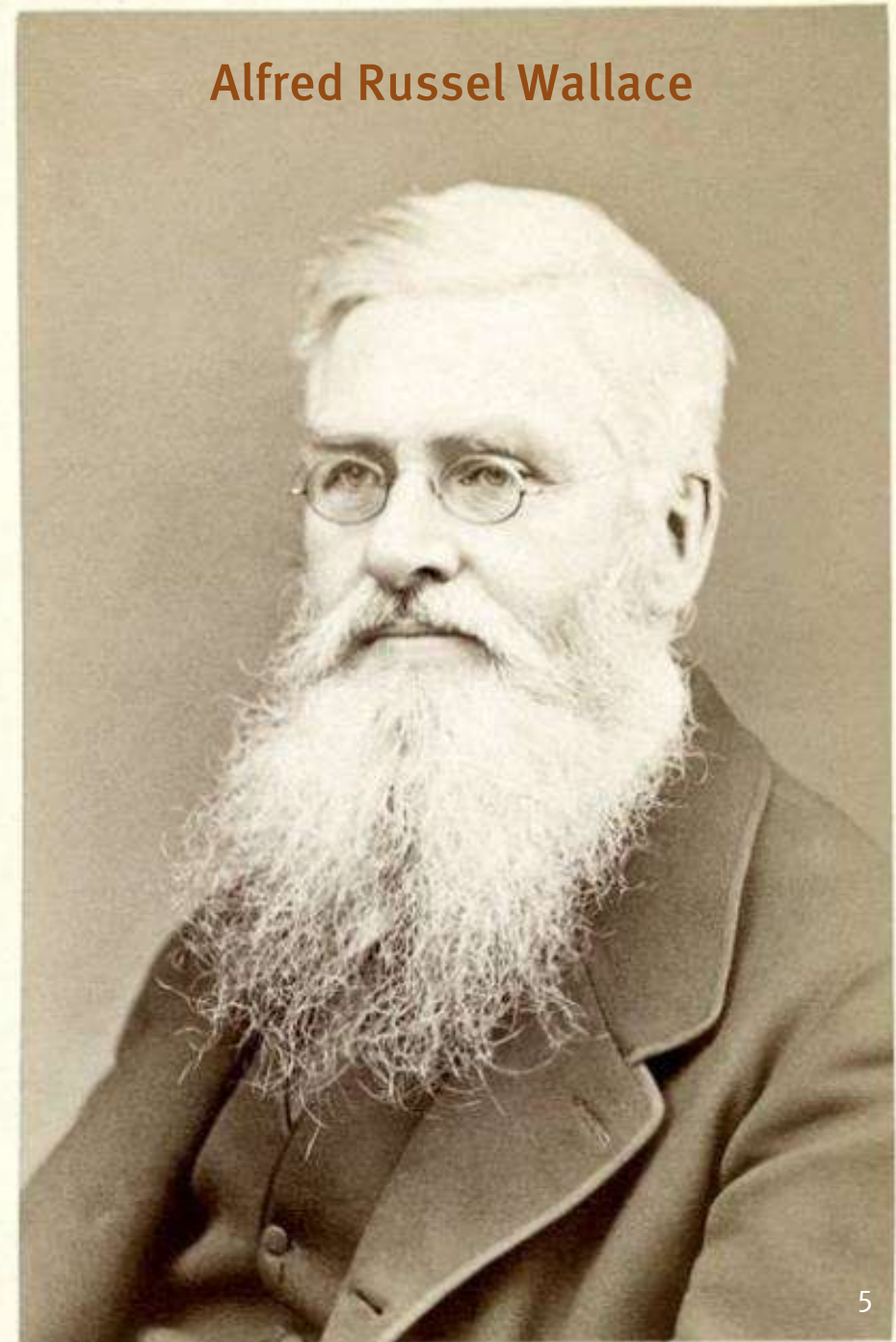
Each section focuses on pivotal works in a particular period. The intention is to provide a narration on the high points rather than a comprehensive bibliography. Together with this narrative, I have included photographs of relevant books from my personal collection of historical books on medicine, which span the last six hundred years of human history.*

These materials underscore the continuity of humanity's quest to conquer aging. It has been quite the journey, and one can come to appreciate the efforts of countless many who have contributed along the way.

It is fascinating to see how early some of the most important insights have arisen. For example, much of today's thinking about evolution and aging was explained rather well by Alfred Russel Wallace, Darwin's collaborator, in an 1865 essay. He argues that death is desirable for us as a species, once we have reproduced of course, and that we should not fear it.

* Somehow the [cat got out of the bag](#) that I have an unhealthy obsession with collecting antiquarian medical books – also known as bibliomania. In part, this survey is an effort to share a bit of what I have learned in one medical discipline by studying old texts. For those that find this a bit on the boring side, rest assured, you are not alone.

Alfred Russel Wallace



Aging Science is Exploding

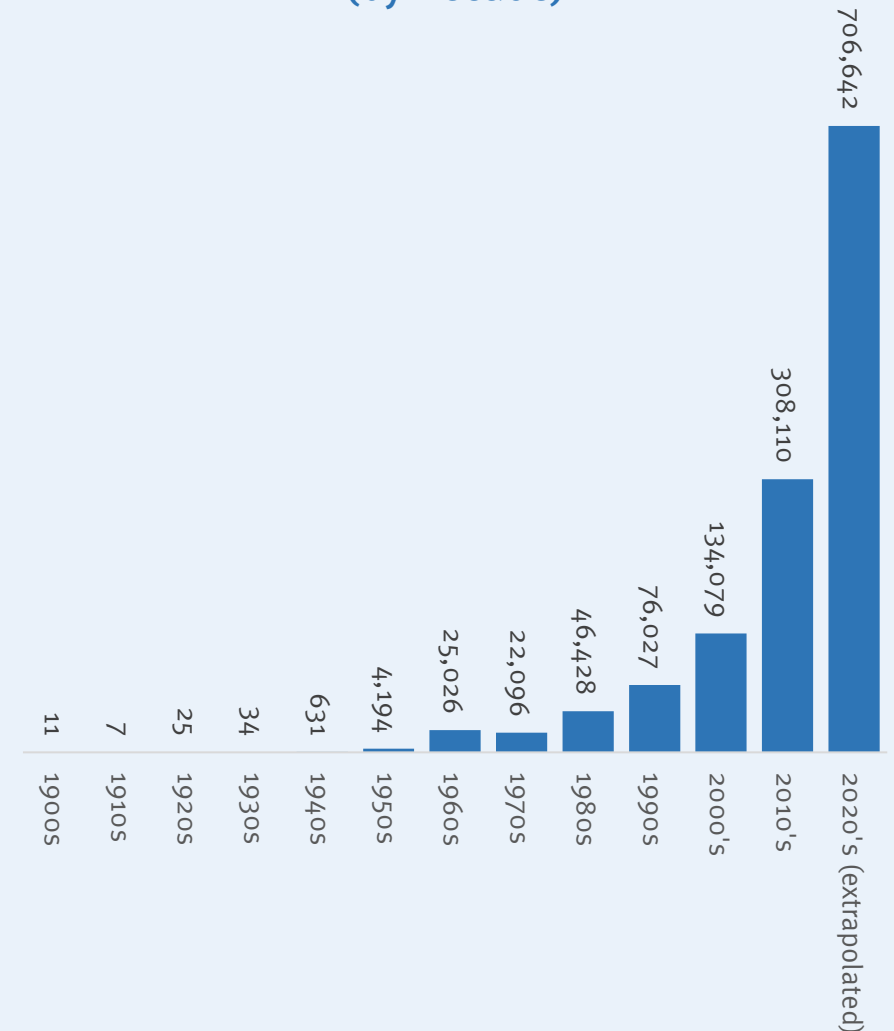
What's also notable is how the field of aging is picking up now. The sheer volume of excellent science work taking place in this decade is jaw-dropping. If one extrapolates the publication count of scientific works on aging or longevity listed in Pubmed through Mar 2025 to the end of this decade, we will see more work published on aging this decade than throughout the remainder of human history combined.

Our society has reached an inflection point in biology. As you read through this material, you will note that it is now possible to disentangle the causes of senescence at both the cellular and organismal level.

Bibliographic interest in the history of aging research has not been high. The last thorough book on the topic that we can find was published by Joseph Freeman in 1979 and is dated in light of recent contributions.

Thus, unlike other fields of medicine, it has not been difficult to purchase the many interesting historical books pertaining to human aging– starting with texts from the 15th and 16th centuries. Copies of key works from authors such as Giorgio Baglivi, George Cheyne, Pierre Flourens, Benjamin Gompertz, Barbara McClintock and Pliny are available and in my history of aging book collection.

Count of Publications on Aging or Longevity in Pubmed, 1900 to 2025 (by Decade)



Source: Pubmed. Accessed Mar 19, 2025

History Highly Relevant Today

As it happens, much of the advice on how to live a long life has been unchanged for thousands of years: eat in moderation, exercise often, avoid heavy drinking and get plenty of fresh air. A friend and colleague refers to this as “the advice your mother gave you.”

Thousands of books have been written repeating this advice - dating from the time of Christ to the present day (i.e., from Galen to Attia). To avoid monotony, I have been selective in which books to include from this genre.

The course of history is also highly relevant in estimating where ongoing efforts will lead us as today’s efforts to identify modifiable causes of mortality are very much a function of what has been understood before.

We, for example, start by reviewing the thoughts on aging of around 5000 years ago and conclude with what’s going on today at companies like [Calico](#) in the biology of calorie restriction and at [Altos Labs](#) and [Retro Biosciences](#) the field of cellular reprogramming.

ALTOS LABS



CALICO LABS



The Story of Acceleration of Biological Innovation is Stunning

While it can be ponderous to flip through a 300 page plus deck reviewing thousands of years of biology, we think there is something valuable that may emerge: a tangible and highly textured appreciation for the innovation itself. The acceleration of discovery, understanding and thought surrounding the causes of aging is stunning.

We start with scrolls, then manuscripts, then early books. We end up with preprints and a world where books are all but gone as dissemination of science research has moved online.

The quality of today's thinking and current scientific rigor is immensely better than what was going on in aging research fifty years ago and is incomparable to what we saw centuries ago.

To illustrate, Calico's Oct 2024 [publication](#) in *Nature* showed how the effect of calorie restriction on aging in mice depended on the [genetics](#) of the mice. The translational lessons learned is what those genetics were and why they mattered.

The logo for Nature Calico is displayed within a dashed green rectangular border. The word "nature" is in a black, lowercase, serif font and is underlined with a solid black line. Below it, the word "Calico" is in a large, bold, green, sans-serif font.

Research collaboration sheds new light on the relationship between genetics, diet, health and longevity.

It's Easy to Miss What Really Matters in an Era of “Inflection Points”

It's important to pause and imagine the quality of innovation that our children will work with – and more importantly yet, their children. The implications for our society of bioscience innovation could not be more profound.

It is easy in the Spring of 2025 to get discouraged by the news. Elected officials in the U.S. appear to be putting the brakes on government support for basic scientific research – at the same moment that other countries like China are stepping on the gas.* And we should fight for government funding for basic research and reimbursement for drugs developed by the private sector.

The time for this is now.

We live in a moment in history where the typical super busy public biotech investor is focused on “inflection points” that might unfold in the next two or three months. And we are all bathed in unending to-the-minute news events flashed at us from our phones. It is easy to lose sight of the fact this barrage of data and news flow might induce a myopia of sorts – one that masks the fact that research underway in the current decade has the promise to change the future of our species.

Many biotech investors have a focus on predicting outcomes of clinical trials and regulatory decisions in the short-term.



* We highlight in this survey two very good Chinese university labs working on reprogramming for cell rejuvenation with a novel small molecule approach. These groups are at the forefront of aging science, they are state-funded and may be positioned to give groups like Altos Labs a run for their money. To state the obvious, the consequences for the United States were Chinese-government funded entities to achieve a superior or blocking IP position in effective anti-aging drugs could be profound in any number of ways.

The Study of Aging is the Study of Ourselves

Specifically, science is making major progress in beating back the inevitability of death.

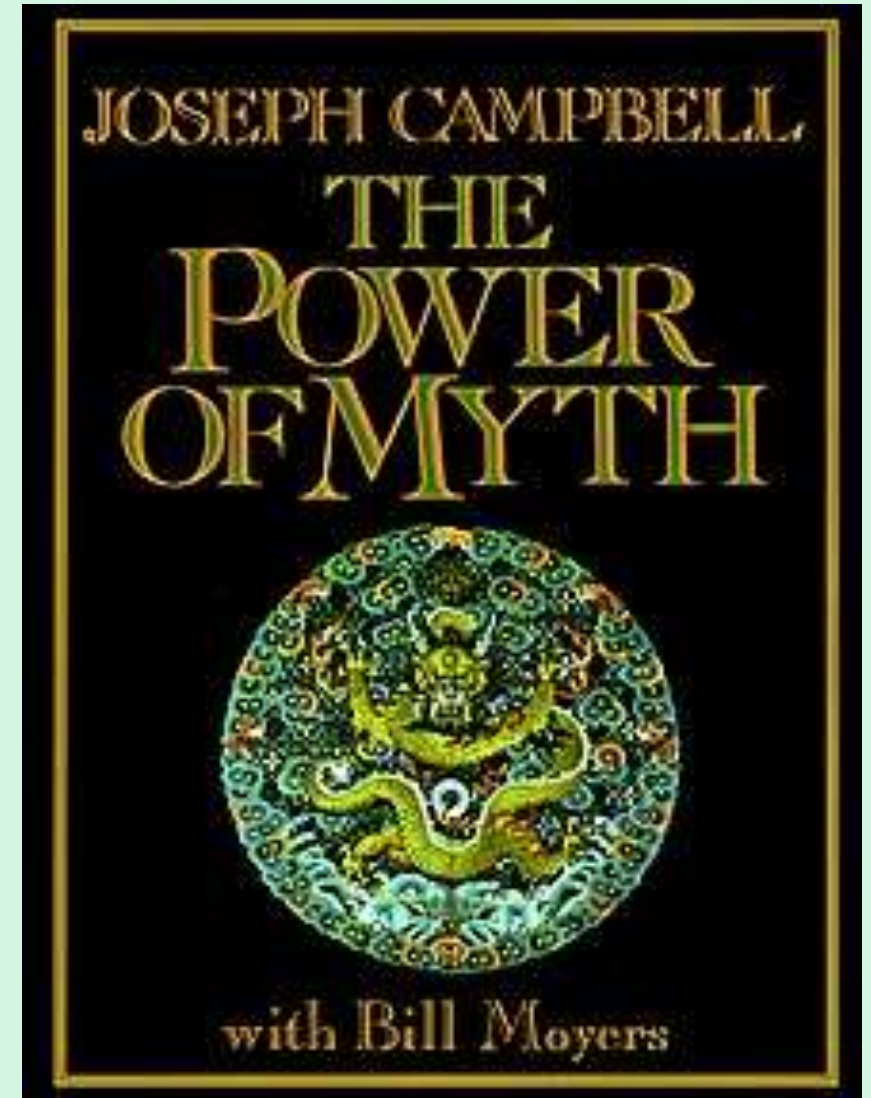
But the timescale is different. We're not talking about instant news flow here. Progress on aging is a matter of years and decades – rather than days and weeks.

Part of what makes aging such an interesting topic is that the study of aging is the study of ourselves.

The desire for more and better life is the essence of being human.

As emphasized by Joseph Campbell in his book *The Power of Myth*, ancient themes and symbols continue to be reshared today – particularly when it comes to death.

Today, more than a few Hollywood movies tell stories about death that resonate with ancient myths. Aging mythology universally teaches us that he who shall try to conquer death and achieve immortality shall regret it. A corollary to Alfred Russell Wallace's theorems on how life works is that we should all learn to accept death. "Be careful what you wish for" counsels myth.



Culture, Myth and Religion Teach Us to Accept Mortality

Specifically, culture, myth and religion teach us to accept our mortal fate. This is reflected throughout literature, film and art. For example, the virtues of mortality are reflected in films like *Frankenstein* (1931), *The Picture of Dorian Gray* (1945), *Death Becomes Her* (1992) and *Interview with a Vampire* (1994). Most recently, in *The Age of Adaline* (2015), Adaline Bowman, who never ages, is miserable and unable to build normal relationships with contemporaries, particularly love interests. A similar theme arises in *The Curious Case of Benjamin Button* (2008) when the protagonist experiences a profound loneliness as he ages in reverse.

Likewise, in an ancient Mesopotamian epic, Gilgamesh embarks on a quest for eternal life after the death of his friend Enkidu. He ultimately learns that immortality is reserved for the gods and that accepting mortality is key to living a meaningful life. A similar fate awaits Tithonus who in the 8th Century BC story “Hymn to Aphrodite” wishes to be immortal and is granted his wish but lives as a ravaged old man. Joseph Campbell argues that all religions teach us not to fear death, saying “The conquest of the fear of death is the recovery of life’s joy. One can experience an unconditional affirmation of life only when one has accepted death, not as contrary to life, but as an aspect of life.”

Some refer to this philosophy of accepting mortality as mortalism.

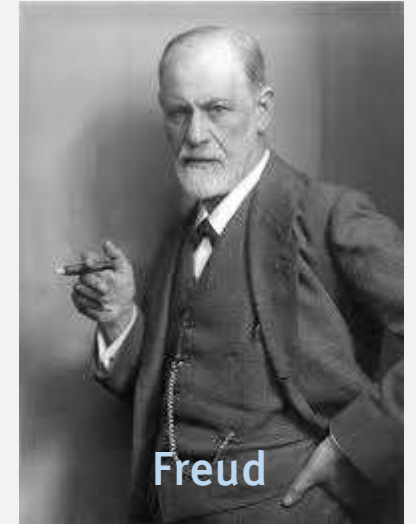


Aging Research May Lead Us to Profound Social Adaptation

In an upcoming publication, I will argue that there is a reasonable chance that we will obtain pharmaceuticals that can meaningfully extend human lifespan in the next ten to forty years. This is but a blink of an eye on the historic scale. Given this, we must start to think about what this all means for mortalist religion, culture and our myths – built up and sustained over millennia. I, for one, believe that culture, religion and myths will adapt to the new circumstance of longer, possibly indefinite life. Movies and books might start to tell us it's ok not to die, for example.

While perhaps controversial, it has long been argued by anthropologists such as [Bronisław Malinowski](#) that rituals, myths and religion are adaptive and allow man to have a sense of control in the face of uncertainty and death. Psychologists have expressed similar views. Sigmund Freud in his book *The Future of an Illusion* (1927) and Ernest Becker in *The Denial of Death* (1973) argue that culture, including religion and mythology, is driven by the need to manage the terror of mortality.

Sociologists like [Émile Durkheim](#) argue that religion is a collective expression of societal values and norms and a way to reinforce social fabric. Similarly, in *The Protestant Ethic and the Spirit of Capitalism* (1905), [Max Weber](#) argues that religious beliefs help individuals to accept their fate and find purpose in their struggles. Philosophers have weighed in on this topic as well. Friedrich Nietzsche argued in his book *The Genealogy of Morals* (1887) that religion might prevent active engagement with life while [Albert Camus](#) in *The Myth of Sisyphus* (1942), acknowledges the value of myth in an indifferent and seemingly absurd universe.



Core Aspects of our Society such as Private Property Law Are Built Around Humanity's Finite Life

I don't have a particular position here, other than to note that our myths may need to adapt as humanity starts to break the bounds imposed by death – and I see no reason why we can't do this. Admittedly, Camus and Nietzsche don't fit terribly well with an optimist's disposition.

I have previously argued that biotech, essentially capitalist efforts to develop and commercialize disease interventions based on scientific insights, can be [civilization changing](#). There is no greater opportunity for this than in the field of aging, where daily advances in somewhat esoteric fields like DNA damage repair mechanisms, autophagy and cellular biology have implications far beyond the usual scientific concerns of experimental design, assay validation and appropriate clinical trial methods.

It is fair to say that almost all major aspects of human society, including organization of life at work, at home, with friends, lovers, and religious organizations, is built around the reality of finite life. Our legal systems also reflect our mortality. Laws surrounding private property and inheritance taxation are designed around the reality that no one seems to make it past 110 years of age. Capitalism itself, of course, only functions well with private property.



Historical Figures Foreshadowed Today's Aging Theories

Homo sapiens is the only species known to be both aware of its mortality and to be undertaking organized efforts to delay mortality through research into its underlying causes.

Perhaps not surprisingly, many of the ideas underlying today's aging research were anticipated long ago. Key researchers who helped to see what was to come include [Charles S. Minot](#) late in the 19th century, [Robert Boyle](#) in the 17th century and [Leonard Hayflick](#) in the 20th. For example, current progress in cell senescence was foreshadowed more than 200 years ago by Xavier Bichat and the amazing work today on cell reprogramming was foreshadowed 60 years ago by John Gurdon.

Of course, writing of respiration and aging in the 1670s, Boyle was neither aware of the existence of cells nor of mitochondria. He had no access to modern tools with which to conduct his investigations.

But as discussed in this survey, his experiments, intuition and insights made at the dawn of the Enlightenment are surprisingly relevant today.



Writing in the 1670's, Robert Boyle got strikingly close to today's theory of oxidative stress as a driver of aging.

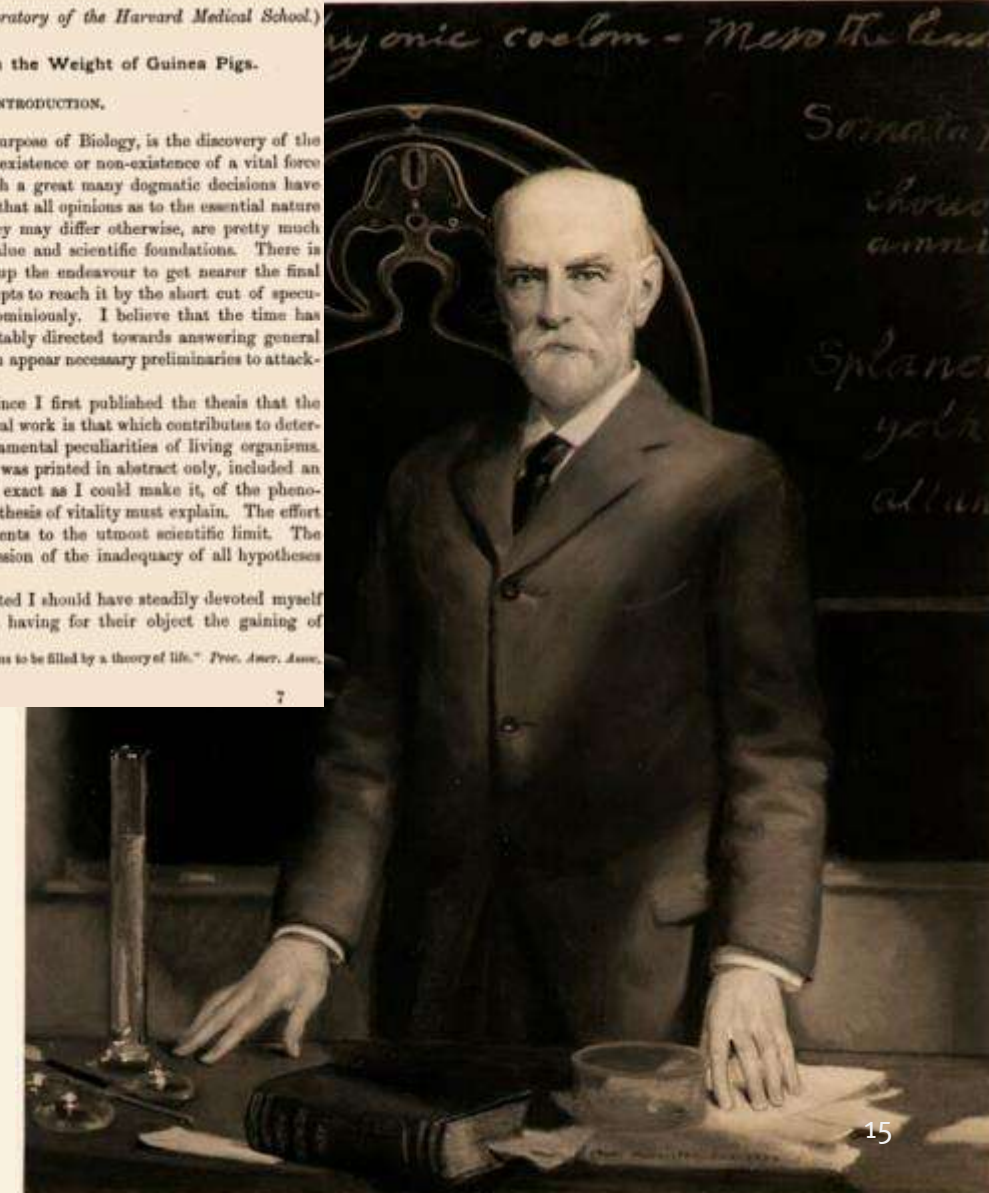
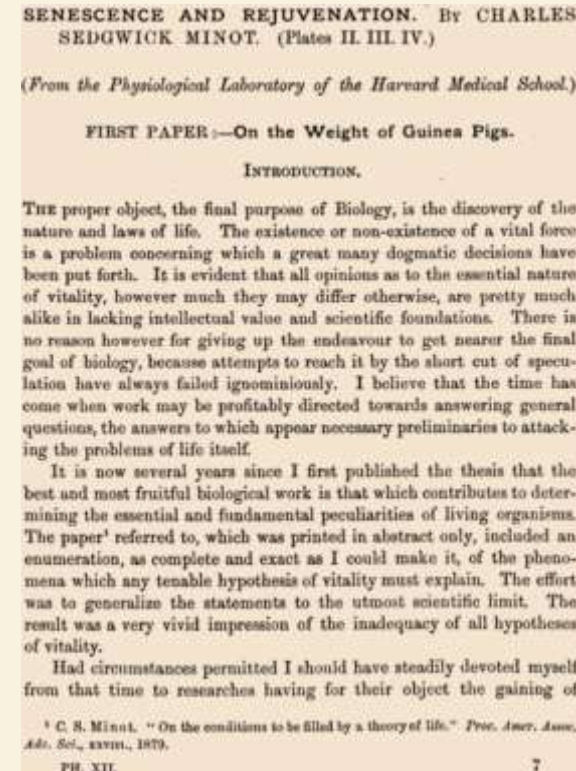
Scientific Process Crucial

The processes of the modern scientific enterprise are themselves crucial to progress. Less than optimal research methods have historically made it difficult to build and confirm hypotheses as to the causes of aging.

I am reminded, for example, of the failure of an early effort to understanding the cause of aging in guinea pigs carried out in the 1890's by Charles Sedgewick Minot of Harvard Medical School. Minot was perhaps the leading U.S. academic biologist of his day. At some expense, Dr. Minot had acquired a hundred or so guinea pigs for this research but had failed to follow what today would be called “Good Laboratory Practices” and AAALAC rules that pertain to vivarium management. He writes:

“For greater safety my animals were kept in a locked room where the floor of was divided into large and commodious pens. ...unfortunately, the janitor of the building had a bull terrier which... he wished to isolate for two or three days from other dogs and therefore chained her up in the “animal room”. During the first night the terrier broke loose and the next morning we found 94 guinea pigs lying dead, leaving only four alive.”

Charles Minot Ran into a Serious Problem with His Guinea Pig Experiments in the 1890s.



* See Charles Sedgewick Minot, “Senescence and Rejuvenation,” *Journal of Physiology*, Vol XII:2, 1891.

New research tools in areas like mass spectrometry, binding assays, single cell analysis, flow cytometry, AI, Cryo-EM, transcriptomics, sequencing and the like have made it possible to carry out far more detailed and directed investigations than ever before.

Powered by new research tools, the field of aging research has gone from a niche area populated by iconoclasts and curious generalists to one that is in the scientific mainstream.

Importantly, real progress is being made. We hope that our survey of the history's top texts on aging will give you a sense of the advancements underway in addressing humanity's limited life span. This progress, of course, has not been easy given the underlying complexity of aging itself. To quote Venki Ramakrishnan, the 2009 Nobel Prize winner in Chemistry:

“...extending human life span – the longest we can expect to live in the best of circumstances – is a much tougher problem. Is our life span fixed, or could we slow down or even abolish aging as we learn more about our own biology?”*

We thank you for the time you are taking to peruse and consider the material herein. I hope that you can share some of the same pleasure that I have gained from contemplating our species' efforts to understand and examine its limited life span. We are poised for transformative breakthroughs in aging research.

The convergence of advances in genomics, artificial intelligence, and regenerative medicine holds the promise of unprecedented progress in extending human lifespan.

By understanding and modulating the biological processes that underlie aging, we may be able to delay or even prevent age-related diseases, improving the quality of life for billions of people.

The history of aging research chronicled here is a testament to humanity's enduring curiosity and ingenuity.

Tim Opler

New York, March 2025

* Venki Ramakrishnan, *Why We Die*, William Morrow: New York, 2024, p. 5.

Acknowledgements

In preparing this survey I have benefitted from the advice and comments of many and am grateful for excellent suggestions along the way. I would particularly like to thank Joe-Betts LaCroix, CEO of Retro Biosciences, for literature suggestions; David Blaustein, physician and hedge fund analyst for thoughts on the cell reprogramming literature and encouragement to look into aging in the first place; Robert Chevalier, retired biologist/medical historian from the University of Virginia, for comments and for helping me to locate a set of Minot's offprints; Andrew J. Dannenberg Jr., of Emerald Bioventures, for linking the current proteomics literature to theories of aging; Vandon Duong of Stanford for pointing to the extraordinary work ongoing there in Marius Wernig's lab; Bill Greene, Chief Investment Officer of the Hevolution Foundation for suggestions on the literature on geroscience and health span; Ann Kurth, President of the New York Academy of Medicine, for suggestions on the health span literature and conversations about how to go forward with aging research in the "real world"; Mario Molina, member of the Board of Governors at The Huntington Library, for suggestions on several modern contributors to aging research; Jeremy Norman, science historian and book dealer for locating a set of Hayflick's personal offprints; Gloria Olivier, of the Mayo Clinic, and tireless advocate for the extraordinary work there on cell senescence; David Perlmutter, Dean of the School of Medicine at Washington University, St. Louis for generously guiding me through the recent aging literature; Dan Perry, Founder of the Alliance for Aging Research, for a number of suggestions of seminal books in the history of gerontology; Nils Regge, General Partner at Apollo Health Ventures for suggestions of landmark papers in the field of caloric restriction and aging genetics; Vittorio Sebastiano, Associate Professor, Stanford University for thoughts and guidance on the cell reprogramming literature; Arlene Shaner, Historical Collections Reference Librarian, of the New York Academy of Medicine for pointing me to missing material in earlier drafts, current thinkers on the history of aging and relevant reference sources and Tal Zaks of OrbiMed for pointing to some contemporary items.

Editing assistance from Brandon Chang, Chris Cook, Masaki Doi, Nick Haddad, Jenna Hill, Stephanie Leouzon and Nate Oliver is very much appreciated.

This survey has further benefitted from much work on the history of aging prepared by those that have come before me, and I have included a section of bibliographic references at the end.

Joseph Freeman's 1979 book *Aging: Its History and Literature*, especially his list of 100 distinguished works on aging of which roughly half are represented here, was particularly helpful. I went through his list in detail and selected the subset that had the greatest scientific importance.

I have gained greatly from a list of important books related to the history of aging research from an exhibit put on by the [New York Academy of Medicine](#) (NYAM) in 1928. In total, they listed 159 books and many on their list appear here.

Gerald Gruman's 1966 monograph was very useful in building the narrative on pre-modern literature and myths regarding longevity. Nathan Shock's comprehensive 1950 bibliography; William Bailey's 1987 bibliography and William Osler's 1929 *Osleriana* were also valuable resources.

Several contemporary sources have been most helpful, and I would specifically point to the excellent 2020 review of the literature on [aging history](#) prepared by Ilia Stambler of Bar Ilan University in Israel. David Boyd Haycock's 2009 book *Mortal Coil* was extraordinary and provided substantial detail missed by others on the aging literature in the 17th to 20th Centuries.

I would be particularly remiss if I did not acknowledge the extraordinary 2024 survey of aging science entitled *Why We Die* by [Venki Ramakrishnan](#). I would have struggled greatly to assemble the intellectual history of aging research of the last 75 years without access to his book.

A Few Quotes

“It is astonishing to realize that the human species survived hundreds of thousands of years, more than 99 percent of its time on this planet, with a life expectancy of only eighteen years.”

Leonard Hayflick

“If you know you are on the right track, if you have this inner knowledge, then nobody can turn you off... no matter what they say.”

Barbara McClintock, 1983 Nobel Prize in Physiology or Medicine

“Remembering that you are going to die is the best way I know to avoid the trap of thinking you have something to lose. You are already naked. There is no reason not to follow your heart.”

Steve Jobs

“In nature’s infinite book of secrecy, a little I can read.”

William Shakespeare

“Life loses its dynamism from the moment we lose the passion with which to live it. No matter what our age, we cannot afford to let the flame within our heart grow dim. . . . We do not become unhappy because we grow old. We become unhappy only when we grow ever more unwilling to change as we age.”

Daisaku Ikeda

“No-one gets out of here alive.”

Jim Morrison

It is impossible to imagine the Height to which may be carried in a 1000 Years the Power of Man over Matter. We may perhaps learn to deprive large Masses of their Gravity & give them absolute Levity, for the sake of easy Transport. Agriculture may diminish its Labour & double its Produce. All Diseases may by sure means be prevented or cured, not excepting even that of Old Age, and our Lives lengthened at pleasure even beyond the antediluvian Standard.

Benjamin Franklin Letter to Joseph Priestley (1780)

“Mythology is the womb of mankind’s initiation to life and death.”

Joseph Campbell

"Well, I figured that if I'm wrong and go down in flames, I'll have important company".

Leonard Hayflick

“This is the true joy in life, being used for a purpose recognized by yourself as a mighty one. Being a force of nature instead of a feverish, selfish little clod of ailments and grievances, complaining that the world will not devote itself to making you happy. I am of the opinion that my life belongs to the whole community and as long as I live, it is my privilege to do for it what I can. I want to be thoroughly used up when I die, for the harder I work, the more I live. I rejoice in life for its own sake. Life is no brief candle to me. It is a sort of splendid torch which I have got hold of for the moment and I want to make it burn as brightly as possible before handing it on to future generations.”

George Bernard Shaw

Some Poetry

“Do not go gentle into that good night,
Old age should burn and rave at close of day;
Rage, rage against the dying of the light.

Though wise men at their end know dark is right,
Because their words had forked no lightning they
Do not go gentle into that good night.

Good men, the last wave by, crying how bright
Their frail deeds might have danced in a green bay,
Rage, rage against the dying of the light.”

Do not go gentle into that good night
Dylan Thomas

“Because I could not stop for Death –
He kindly stopped for me –
The Carriage held but just Ourselves –
And Immortality.
We slowly drove – He knew no haste”

Because I could not stop for death
Emily Dickinson

“Darkling I listen; and, for many a time
I have been half in love with easeful Death,
Call'd him soft names in many a mused rhyme,
To take into the air my quiet breath;
Now more than ever seems it rich to die,
To cease upon the midnight with no pain,
While thou art pouring forth thy soul abroad
In such an ecstasy!”

Ode to a Nightingale
John Keats

Section 2:

Landmarks in Aging Research

20 Landmark Publications that Have Shaped Aging Research Over the Last 2500 Years



Twenty Landmark Publications in Aging Research

#1



Aristotle

350 BC

Aristotle linked aging to respiration and heat. Argued for lifestyle moderation.

Shaped subsequent early thought including Galen. *Editio princeps* of his works *de Animalibus* published in 1476.

#2



Luigi Cornaro

1558

Popularized the notion that caloric restriction leads to long life.

Published *da Vita Sobria* in 1558 which became the best-selling aging book of all time. Still discussed today.

#3



Francis Bacon

1623

More than any one individual developed the empirical scientific method.

Argued in his 1623 book *de Vita et Mortis* that the conquest of aging should be the central purpose of science.

#4



Robert Boyle

1672

Presents experimental evidence that life depends on respiration and hypothesizes that lifespan is linked to respiration.

His 1672 book *Tracts* was highly influential in chemistry and physics.

Twenty Landmark Publications in Aging (#5 to #8)

#5



Lazzaro Spallanzani

1768

Presented experimental evidence that the regenerative ability of animals declines with age.

Anticipated the cellular senescence literature and his findings have been replicated many times since.

#6



Alfred Russell Wallace

1865

Linked evolution to human aging in an 1865 essay. Noted that long lived persons could compete for resources that slow down reproduction. The essay was published in 1882 by August Weissman who expanded his ideas.

#7



Max Rubner

1908

He suggested that organisms have a fixed metabolic potential over their lifetime.

He argued that a higher metabolic rate leads to a shorter lifespan. This crucial observation was refined by Raymond Pearl in 1920.

#8



Clive McCay

1935

First researcher to test caloric restriction theory in mammals.

He found that prolonged periods of growth, coupled with lower caloric intake, could delay aging in rats.

This work was highly influential.

Twenty Landmark Publications in Aging (#9 to #12)



= Nobel Prize

#9



Barbara McClintock

1937-41



McClintock demonstrated that DNA sequences at the ends of chromosomes (called telomeres) play a crucial role in preventing genetic damage during cell division.

This was laid out in three consecutive papers.

#10



Denham Harman

1955

Argued that the central cause of aging was oxidative stress to cells. In 1972 extended this to indicate that mitochondria were the main victims of this stress. Father of oxidative stress theory.

#11



Leonard Hayflick

1961

Hayflick discovered that normal, somatic human cells have a limited number of divisions before they enter a state of senescence, a phenomenon now known as the "Hayflick limit." He is the father of "senescent cell" theory.

#12



Elizabeth Blackburn

1978



Found the DNA sequence for telomeres and described their function. Showed role in extending and maintaining the length of telomeres, shedding light on the mechanisms of cellular aging and chromosome stability.

Twenty Landmark Publications in Aging (#13 to #16)

#13



Blackburn & Greider

1985



Elizabeth Blackburn and Carol Greider reported the discovery of telomerase, an enzyme that extends the repetitive DNA sequences at the ends of chromosomes (telomeres). Provided key insights into cellular aging.

#14



Cynthia Kenyon

1993

A single mutation in the *daf-2* gene could double the lifespan of *C. elegans*. This gene was linked to metabolism via the insulin system. This electrified the field of aging research by showing the potential importance of genetics.

#15



Beth Levine

2003

Beth Levine and her team demonstrated that autophagy is essential for lifespan extension in *C. elegans*. They found that mutations in the insulin/IGF-1 signaling pathway increased autophagy, leading to enhanced longevity.

#16



Shinya Yamanaka

2006



Yamanaka and his team found that differentiated adult cells could be reprogrammed into pluripotent stem cells by introducing just four transcription factors: Oct4, Sox2, Klf4, and c-Myc (now called the Yamanaka factors).

Twenty Landmark Publications in Aging (#17 to #20)

#17



Judith Campisi

2008

Judith Campisi made a fundamental discovery in cell senescence in 2008. Specifically, she showed that senescent cells secrete cytokines that can harm nearby tissue.

Her 1995 biomarker paper was also a landmark in the field.

#18



Juan Belmonte

2008 / 2016

Belmonte showed how to induce pluripotent stem cells from somatic cells by reprogramming of keratinocytes. In 2016 he and his team extended this approach substantially by showing the value of “partial reprogramming” of somatic cells.

#19

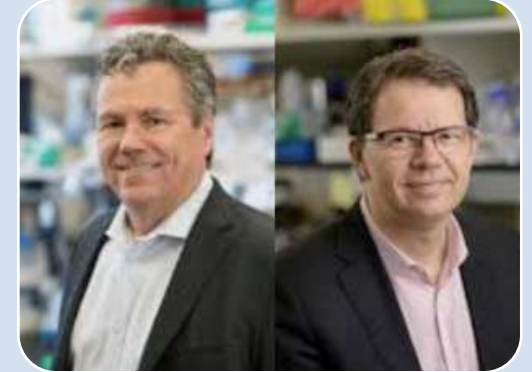


Van Deursen and Mayo Team

2011

In 2011, a team at the Mayo Clinic, including Dr. Jan van Deursen, showed that removal of senescent cells in progeroid mice can extend their lifespan by 25 to 35 percent. This electrifying finding was confirmed in 2016 using wild-type mice.

#20



Lowe and Sadelain

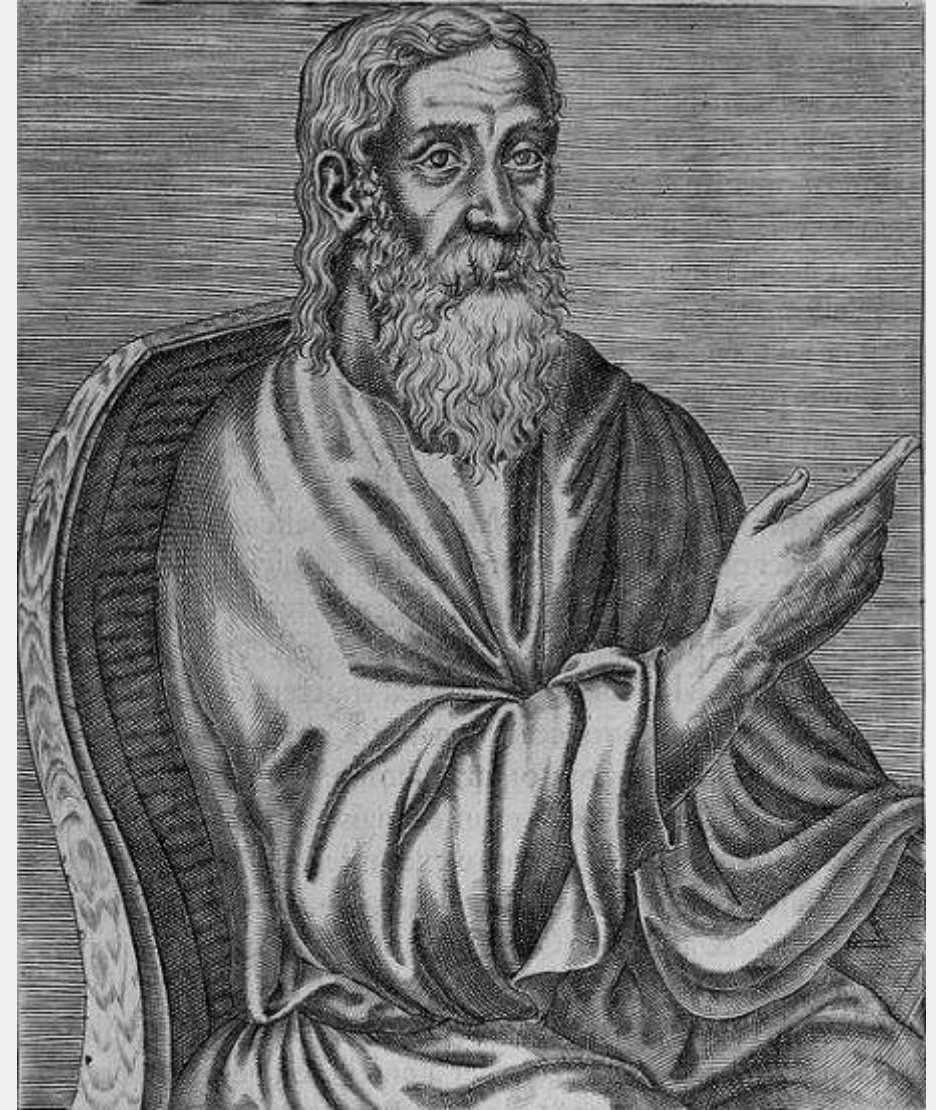
2024

Showed that could slow mammalian aging with a CAR-t cell approach that removes senescent cells. Results more impressive than those seen with first generation senolytics. This type of therapy might be tractable in humans.

Section 3:

Aging in Antiquity:

From the Cradle of Civilization



Clement of Alexandria (2nd Century AD) believed that age gave strength and confidence.

Aging in Antiquity

Joseph Freeman writes in his 1979 book *Aging: Its History and Literature* that “In every human quest, the hope and survival and the urgency to achieve an understanding of old age are important parts of science’s historical imperatives.” The period of antiquity dating from humanity’s first records to 200AD is no exception as discussions of aging and the inevitability of death are central to the many remaining writings from this period.

Nonetheless, the empiric scientific method had yet to be developed. Not surprisingly, attempts at the time to explain aging often resorted to myth or narratives that seemed to fit the circumstances. W. Andrew Achenbaum, Professor of Texas Medical Center, wrote in 2018: “Before theories of aging were developed, observers of late life created representations and myths.”*

Hammurabi, the King of Babylon, around 2250 B.C. left *The Code of Hammurabi* which makes clear that a medical class existed in the Valley of the Tigris and Euphrates. This “first historical codification of medicine” was carved on stone. At roughly the same period, Emperor Huang-ti of the Han Dynasty ordered the compilation of interviews with his sages which is “supposedly the oldest medical book extant.” As shown in the painting scroll at right, Huang-ti, the Yellow Emperor, traveled to the Kongtong Mountains to inquire about the Tao with the Taoist sage Guangchengzi.

Taoist beliefs strongly influenced Han-era views on longevity, emphasizing balance, diet, breath control, and elixirs to extend life. Alchemy and herbal medicine sought to enhance “Qi” and slow aging. The Han court employed physicians who documented aging-related concerns, such as Zhang Zhongjing’s writings on geriatric health.



Emperor Huang-ti

* Achenbaum, Ever-Emerging Theories of Aging, Oxford Research Encyclopaedia, 2018.

Aging in Antiquity (continued)

In his *Sushruta Samhita*, Sushruta (750BC) described aging as the result of bodily decline due to imbalance in the doshas (Vata, Pitta, Kapha). Sushruta argued that longevity could be estimated from bodily measurements. He advocated lifestyle changes, herbal treatments, and surgery to maintain health. Ayurveda, influenced by Sushruta, considered longevity achievable through disciplined living (Rasayana therapies).

The Assyrians in the 7th century BC viewed aging through a mix of astrology, medicine, and mythology. Their medical texts, such as those in the Library of Ashurbanipal, describe aging as an affliction treatable through prayers, incantations, and herbal remedies. Their mythology included figures like Utnapishtim in the Epic of Gilgamesh, who achieved immortality—an unattainable goal for ordinary mortals. A similar mythology held in Zoroastrian religion where the sacred *Avesta* text portrayed people who knew no aging nor death.

In ancient Greece, Aristotle saw aging as a natural, inevitable decline due to the gradual loss of innate heat, similar to a candle burning out (circa 350BC). He linked longevity to biological constitution, believing that larger animals lived longer due to greater moisture retention. While he acknowledged lifestyle influence, his views were more deterministic than interventionist.

In ancient Rome, aging and longevity were viewed through a philosophical and medical lens. Cicero, in *De Senectute*, portrayed aging as a stage of wisdom, advocating virtue as means to fulfillment. Seneca saw aging as a natural process and emphasized living with dignity and rational acceptance of death.



The Library of Ashurbanipal in the British Museum

Pliny the Elder, in *Natural History*, recorded cases of extreme longevity and attributed them to simple rural lifestyles, diet, and moderation. Galen, Rome's leading physician, took a medical approach, linking aging to the gradual decline of bodily humors and advocating for exercise, diet, and balance to slow deterioration. Roman society valued old age for its wisdom in governance and law but also saw it as a time of physical decline, necessitating both philosophical resilience and medical care.

Egyptians Well Aware of Age

“Would that my body were young again! For old age has come; feebleness has overtaken me. My eyes are heavy, my arms weak; my legs fail to follow. The heart is weary; death is near.”

The Story of Sinuhe, 1800 BC

Head of a Statue of an Older Man

Circa 2550–2460 BC (The Met Museum)



The Edwin Smith Papyrus Advocates Anti-Aging Pharmacology in the 17th Century B.C.

This papyrus tells us unmistakably that since the beginning of recorded history people have tried to minimize or avoid aging because of the diminishment of vitality and strength.

The [Edwin Smith Papyrus](#) contains the earliest known written remedy for aging, titled The Book for Transforming an Old Man into a Youth of Twenty. In this book there are recipes for a special ointment and directions for its use: “It is a remover of wrinkles from the head.

When the flesh is smeared therewith it becomes a beautifier of the skin, remover of blemishes, of all disfigurements, of all signs of age, of all weaknesses which are in the flesh.” In the margin is a note written in informal Coptic script by the scribe drawing the hieroglyphs: “Found effective myriad times.”

The ambivalence regarding growing old is clear and will echo throughout history.

Interestingly, this text may actually date back to 3000 B.C.

References: Freeman p. 74; Morley p. 1132; Stambler p. 228.



Plates vi & vii of the Edwin Smith Papyrus. From the New York Academy of Medicine.

350 BC: Aristotle Writes Extensively on Longevity



Aristotle was the first to link efficiency of respiration to longevity. Attributed senescence to waste of “innate heat” and loss of moisture.

Stagirite de Iuuetute & Senectute, Vitaque, & Obitu Liber

(Latin to English: Aristotle's Treatise on Youth, Old Age, Life and Death)

(Translated from Greek to Latin by Pietro Alcyonio), 1563

“Respiration plays a crucial role in life, as it supports vital processes and sustains the body’s functions. It is evident that air is necessary for survival, just as earth, water, and food are essential for nourishment. In perfect animals, the internal organs are structured to receive air, which enables respiration and prolongs life.

Animals with well-developed vital organs and efficient respiration tend to live longer, while those that lack these structures age quickly and succumb to natural decay. Insects, for instance, have a much simpler internal system compared to higher animals, which influences their lifespan.”

Different animals and plants exhibit varying lifespans. Some live longer due to their stronger constitutions, while others perish quickly due to their delicate nature. Longevity is often tied to an animal's internal structure, as well as its ability to resist external influences. Some species naturally renew themselves, while others degrade over time. Aristotle also observed that long-lived parents often had long-lived offspring, suggesting an early recognition of hereditary factors in lifespan.

References: Bailey 8; Freeman p. 22; Grmek p. 64; Gruman p. 15; Haycock p. 19; Hayflick p. 267; Schafer p. 13; Shock 17293; Stambler p. 230.



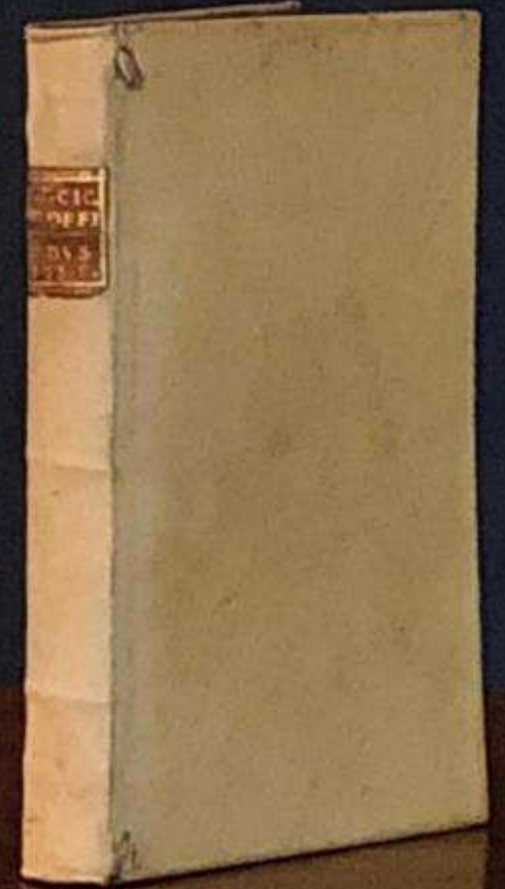
Aristoteles, *Opera post omnes quae in hunc usque diem prodierunt editiones, summo studio emaculata et ad graecum exemplar diligenter recognita*, Lyon: Antoine Vincent, 1563, p. 946 (volume 2). From the Medical Library of Tim C. Opler.

44 BC: Cicero's *Cato Maior de Senectute*

Cato Maior de Senectute (Cato the Elder on Old Age) was written by Cicero in his sixty-third year and is addressed to his friend Atticus. Cicero represents the discourse as delivered by the elder Cato (in his eighty-fourth year) on occasion of young Scipio and Laelius expressing their admiration at the wonderful ease with which he still bore the load of life. Cicero acknowledges that the sentiments put into the mouth of Cato are really his own. His purpose is to show that old age is not only tolerable, but comfortable by internal resources of happiness.

He examines the infirmities of old age under four general headings:—that it incapacitates from mingling in the affairs of the world—that it produces infirmities of body—that it stops the enjoyment of sensual gratifications—and that it brings us to the verge of death. Some of these disadvantages, he maintains, are imaginary, and for any pleasures of which the old are deprived, others more refined and may be substituted. The view of old age is optimistic.

References: Bailey 36 (1967 ed); Freeman p. 11; Grmek p. 136; Gruman p. 14; Haycock p. 21; Hayflick p. 266; NYAM p.121 (Franklin trans. 1744); Schafer p. 64; Stambler p. 230.



49 AD: Seneca - Life is Short; Make the Most of It

Lucius Annaeus Seneca the Younger (c. 4 BC – AD 65), usually known as Seneca, was a Stoic philosopher of Ancient Rome, a statesman, dramatist, and in one work, satirist, from the post-Augustan age of Latin literature.

His book *De Brevitate Vitae* (English: On the Shortness of Life) is a moral essay written around the year 49 AD, to his father-in-law Paulinus.

[Seneca](#) recognizes that life is far too short for what most people wish to accomplish.

The philosopher brings up many Stoic principles on the nature of time, namely that people waste much of it in meaningless pursuits.

According to the essay, nature gives people enough time to do what is really important and the individual must allot it properly. In general, time is best used by living in the present moment in pursuit of the intentional, purposeful life.

References: Achenbaum p. 2; Grmek p. 136; Gruman p. 75; Morley p. 1133; Shock 17328.

See: *De Brevitate Vitae* in John Basore (ed.), *Seneca Moral Essays*, Vol 2, Loeb Classical Library, 1932, From the Medical Library of Tim C. Opler



50 AD: Celsus Recognizes Chronic Diseases Rise with Aging (e.g., COPD)

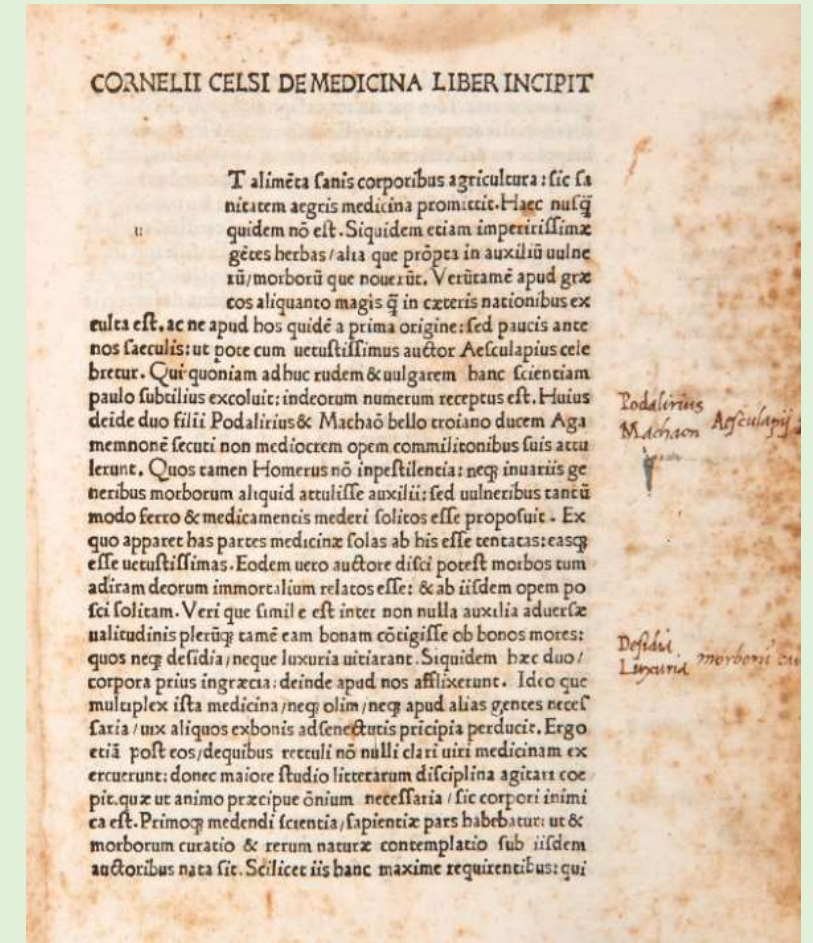
In *De Medicina*, [Aurelius Cornelius Celsus](#) discussed regimens for prolonging life, including dietary restrictions, exercise, and moderation in pleasures. He emphasized individualized medical care, recognizing that people aged differently.

Trevor Howell, “Celsus on Old Age,” *Age and Aging* 1986, pp. 378-379.

“Celsus gave a list of diseases common among the aged which may be compared with that described by Hippocrates many years before. 'In old age there occur breathing and urinary difficulties, choked nostrils, joint and renal pains, paralysis, the bad habit of body which the Greeks call cachexia, insomnias, the more chronic maladies of the ears, eyes, also of the nostrils, and especially looseness of the bowels with its sequences, dysentery, intestinal lubricity, and other ills due to bowel looseness.

Respiratory diseases must have been quite common in Roman times. In one passage we meet 'a malady which has different names according to its intensity. It consists altogether in difficulty of breathing; when moderate and without any choking, it is called dyspnoea; when more severe, so that the patient cannot breathe without making and gasping, asthma; but when in addition the patient can hardly draw in his breath, unless with neck outstretched, orthopnoea.”

References: Bailey 31; Dibner 119; Freeman p. 24; GMN 20; Grmek p. 107; Klebs 261.1; Osler 147; Schafer p. 12; Waller 44; Wellcome 1392. Also see T. Howell, “Celsus on Geriatrics,” *J Amer Ger Soc*, September 1970.



Aurelius Cornelius Celsus (25 BC-50 AD). *De medicina*. Florence: Niccolò di Lorenzo della Magna, 1478. From the Medical Library of Tim C. Opler.

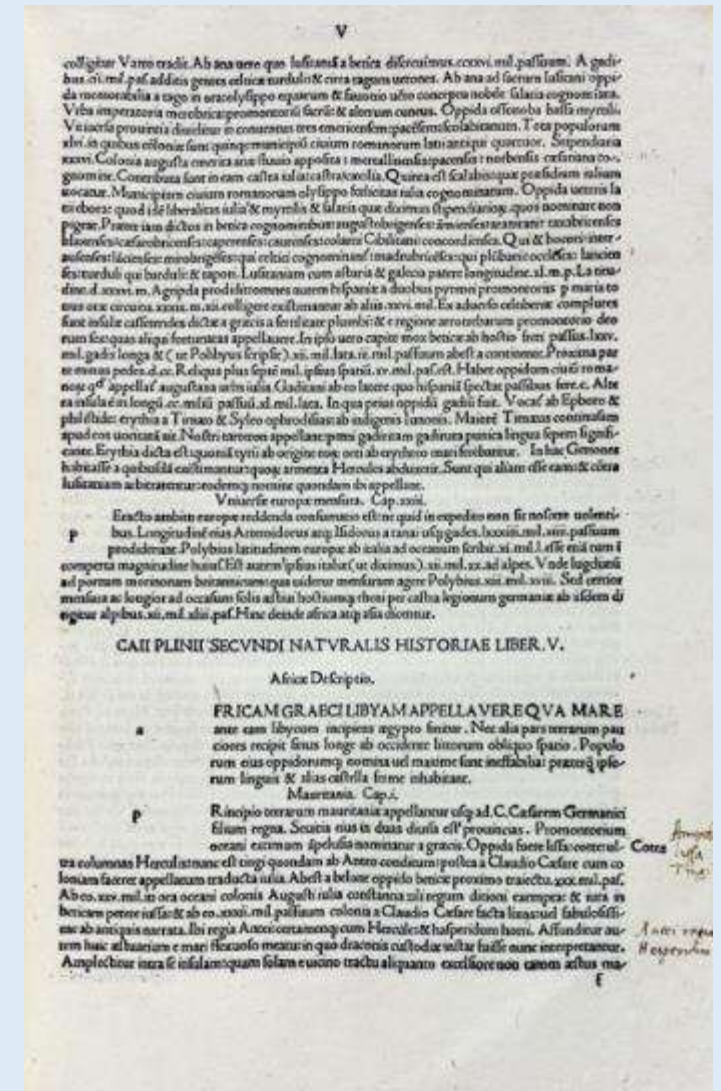
75 AD: Pliny the Elder Recognizes Humans Live Up to 110 Years of Age

Pliny's contributions to our understanding of aging are considerable. He noted that stories of long life were often wrong and that humans had an outer lifespan limit of 110 years. [Pliny the Elder](#) also noted that certain families were known for their exceptional longevity, hinting at a familial or hereditary component.

“As to the length and duration of men's life, not only geographical position but also dates and the various fortunes allotted at birth to each individual have made it uncertain. Hesiod, who first put forth some observations on this matter, placing many creatures above man in respect of longevity, fictitiously as I think, assigns nine of our lifetimes to the crow, four times a crow's life to stags, three times a stag's to ravens, and for the rest in a more fictitious style in the case of the phoenix and the nymphs. The poet Anacreon attributes 150 years to Arganthonius king of the Tartesii, 10 years more to Cinyras king of Cyprus, and 200 to Aegimius. Theopompus gives 157 to Epimenides of Knossos Cnossus.

All of these exaggerations were due to ignorance of chronology, because some people made the year coincide with the summer, the winter being a second year, others marked it by the periods of the four seasons, for example the Arcadians whose years were three months long, and some by the waning of the moon, as do the Egyptians. But to pass to admitted facts, it is almost certain that Argathonius of Gades reigned for 80 years; his reign is thought to have begun in his fortieth year. It is not questioned that Masinissa reigned 60 years and that the Sicilian Gorgias lived 108 years.”

References: Grmek p. 89; Hayflick p. 190; Waller 120 (1496 ed).



Plinii Secundi *naturae historiarum libri XXXVII*, Venice: Bernardinus Benalius, 1497. From the Medical Library of Tim C. Opler.

170 AD: Galen Argues for Healthy Lifestyle



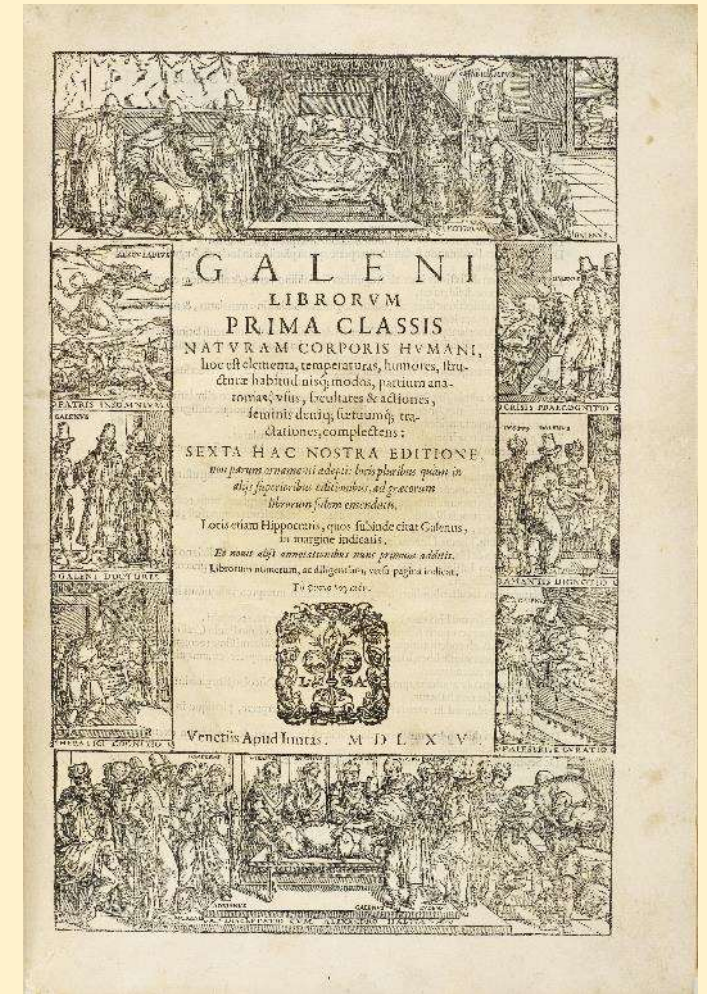
[Galen](#) (129–c. 216 CE) believed that longevity depended on maintaining a balance of the four humors—blood, phlegm, yellow bile, and black bile. He viewed aging as a gradual cooling and drying of the body, which weakened function over time. To slow this process, he emphasized a healthy lifestyle, including a moderate diet, regular exercise, and proper emotional regulation. Overeating, excessive drinking, and a sedentary lifestyle were seen as factors that could accelerate aging and lead to disease.

Physical activity played a crucial role in preserving health and preventing premature aging, but Galen warned against overexertion, which he believed could deplete vital energy. Mental well-being was equally important, as chronic stress, excessive emotions, and lack of self-discipline could weaken the body.

He also stressed the importance of environmental factors, advising people to live in temperate climates, breathe fresh air, and avoid polluted surroundings to maintain long-term health.

Galen distinguished between natural aging, which was an inevitable process, and premature aging, which could be prevented through proper lifestyle choices. His theories on longevity influenced medical thought for centuries, shaping health recommendations in medieval and Renaissance medicine.

References: Achenbaum p. 42; Freeman p. 16; Grmek p. 94; Gruman p. 17; Haycock p. 21; Morley 1133; Schafer p. 12.

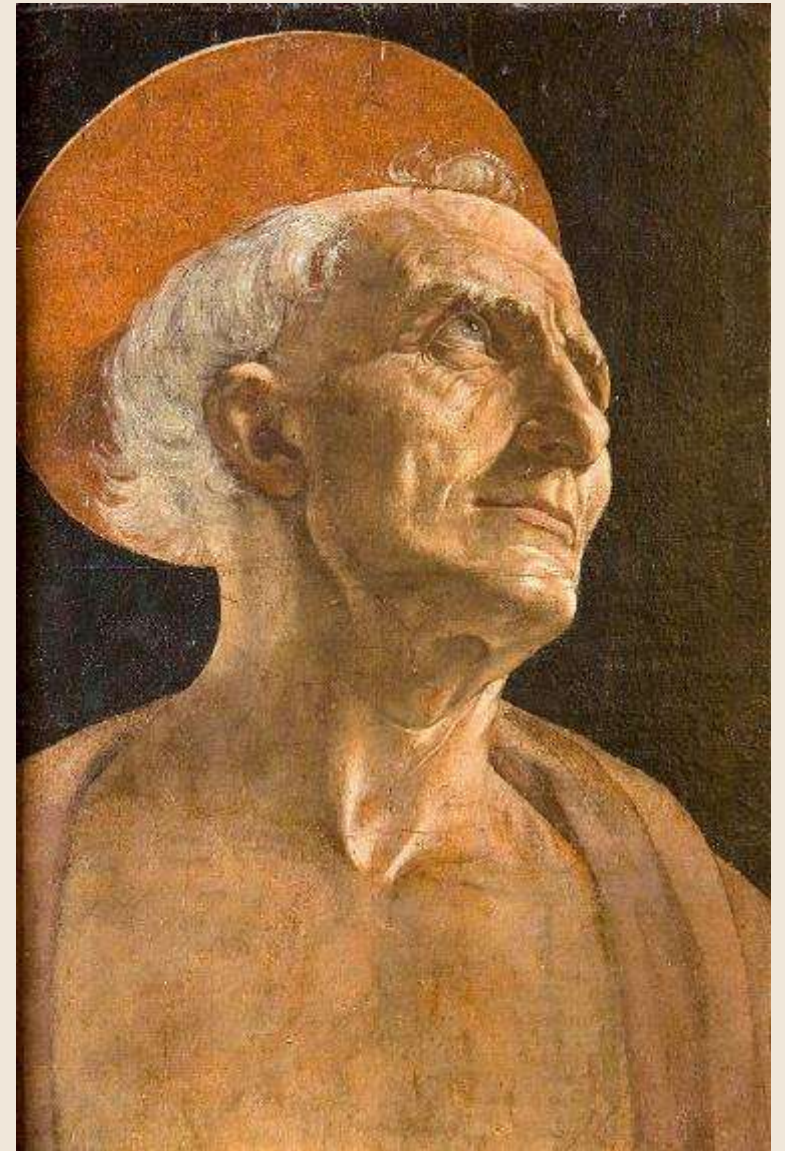


Claudius Galenus *Librorum prima classis naturam corporis humani*.... Venice: Giunta, 1565-1597. From the Medical Library of Tim C. Opler.

Section 4:

Aging in the Medieval Period

Emergence of Early Theory



St. Jerome by Verrocchio, Palazzo Pitti, Florence.

Medieval Thought on Aging

Medieval intellectual thought on aging and longevity blended classical medical theories, religious perspectives, and emerging alchemical and astrological ideas.

Many medieval medical texts were based on Galen's ideas about aging, which suggested that the gradual drying and cooling of the body led to senescence. These theories were passed down through Latin and Arabic translations of Galenic texts, particularly in the *Articella*, a compendium of medical writings widely used in medieval universities.

One of the most influential texts on aging in the medieval period was Ibn Sina's (Avicenna) [*Canon Medicinae*](#), completed in the early 11th century. Avicenna expanded on Galenic theories, explaining that aging resulted from the gradual exhaustion of innate heat and the accumulation of waste in the body. He proposed that maintaining proper digestion, avoiding excessive emotions, and following a moderate lifestyle could extend life.

The English philosopher and scientist Roger Bacon wrote *De Retardatione Accidentium Senectutis* in which he explored ways to slow down aging and extend human life. Bacon, influenced by alchemical and early experimental thought, suggested that certain elixirs, purified air, and a proper balance of humors could significantly extend one's lifespan.

The medieval Church played a significant role in shaping views on aging and longevity. Thomas Aquinas (13th century) discussed aging within a theological framework, seeing it as part of the divine order.



Page from a manuscript version of Avicenna's *Canon Medicinae*. University Library, Bologna.

Longevity in the Medieval Period

Whereas early medieval medical thought and education in Western Europe was primarily the province of the monastic orders, the growth of universities in the 11th and 12th centuries ushered in a new era. During this period medicine was increasingly influenced by academic physicians teaching in a scholastic setting.

Two European schools became important early centers of medical teaching, text translation, and advancement: the School of Salerno in Southern Italy, and the University of Montpellier in Southern France.

The learned Montpellier medical professor Arnold of Villanova (ca. 1240-1311) is known to have translated numerous texts from Arabic into Latin. But he was also one of the great medical thinkers of the Middle Ages in his own right. He is best known for his book *De Regimen Sanitatus* which taught six habits of health from the School of Salerno. Shown at right in manuscript form, this was likely the most widely distributed book on medicine in the late Middle ages.

There were, of course, many other contributors to thinking about aging in the middle ages including Bernard de Gordon, Albertus Magnus, Petrarch and Guido de Vigevano. Most of these did not make contributions that expanded thought beyond the basic homilies of Aristotle and Galen (“getting old is getting cold” and “dying is drying”).*

* These homilies were coined in Shapin, Steven, and Christopher Martyn. 2000. [How to Live Forever: Lessons of History](#). *The British Medical Journal* 321, no. 7276: 1580-1582. To explain, we examined a number of other manuscripts and their translations from the Medieval period such as Albertus Magnus' work *Juventute et Senectute* which was listed by Freeman as a top 100 publication in aging history (never printed in the modern era but viewable in c 1320 Latin [online](#) from the Bodleian Library) but found it was not intellectually additive, arguing exactly as did Aristotle 1600 years earlier, that aging was caused by loss of bodily heat and loss of moisture.



'Regimen sanitatis ad regem Aragonum' by Arnald of Villanova (Bodleian Library, 14th Century).

1010AD: Avicenna Writes that Aging Caused by Loss of Body Homeostasis

‘Ibn Sīnā’ ([Avicenna](#), 980–1037), one of the most influential physicians and philosophers of the medieval Islamic world, discussed aging and longevity extensively in *The Canon of Medicine*.

He viewed aging as a natural process resulting from the gradual depletion of innate heat and moisture, an idea derived from Galenic and Aristotelian principles. According to Avicenna, the body’s ability to maintain balance (homeostasis) weakens over time, leading to progressive deterioration of organs and bodily functions.

To slow aging and promote longevity, Avicenna emphasized lifestyle regulation, advocating for a moderate diet, proper sleep, regular exercise, and emotional well-being.

He believed that overindulgence in food and physical strain could accelerate aging, whereas a balanced regimen could preserve health for longer.

He also stressed air quality, climate, and psychological stability as key factors in longevity. Additionally, Avicenna prescribed medicinal compounds, herbal treatments, and detoxification methods to counteract the effects of aging and maintain vitality.

Avicenna’s approach combined medical science, philosophy, and preventive healthcare, laying the groundwork for later studies in gerontology and holistic medicine.

His emphasis on preventive care, moderation, and environmental influences shaped medical thought for centuries and influenced both Western and Islamic medical traditions in their understanding of aging and longevity.



Avicenna section in, *Articella nuperrime impressa cum complurimis tractatibus pristinae impressioni superadditis...* (Petrus Pomarius ed.), Lyon: Antonium du Ry, 1525. From the Medical Library of Tim C. Opler.

1270AD: Roger Bacon's "Cure of Old Age"

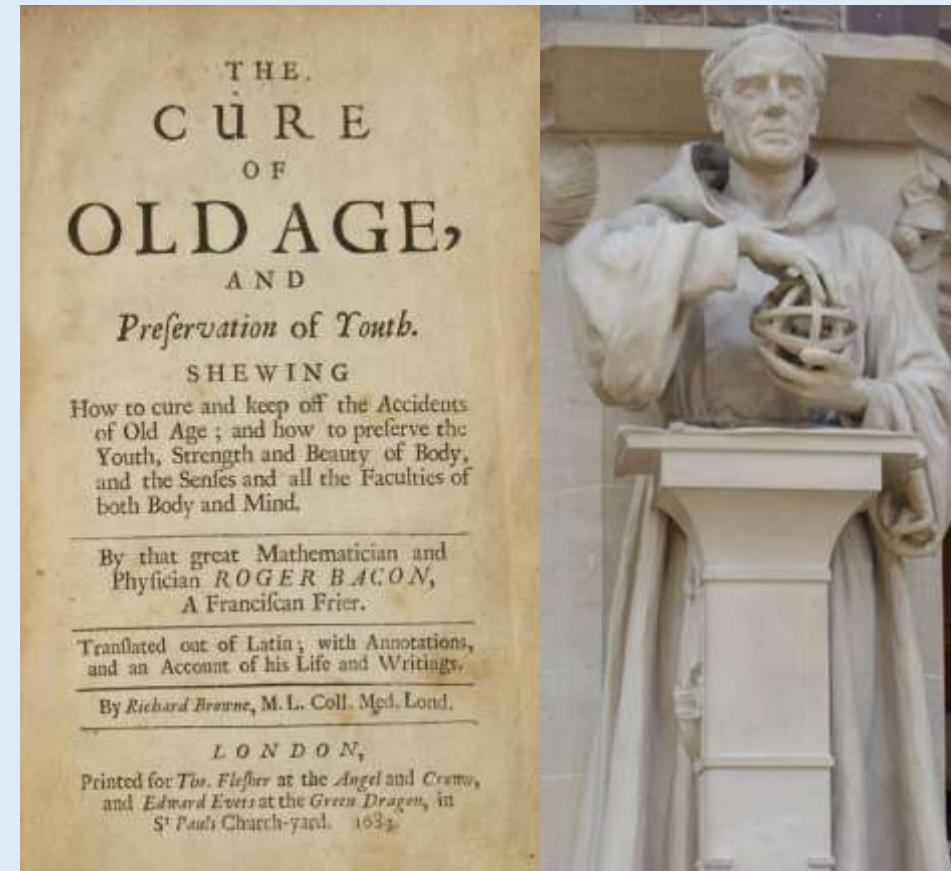
"The Accidents of Age and Old Age are, Grey Hairs, Paleness, Wrinkles of the Skin, Weakness of Faculties and of natural Strength, Diminution of Blood and Spirits, Bleareyedness, abundance of rotten Phlegm, filthy Spitting, Shortness of Breath, Anger, Want of Sleep, an unquiet Mind, Hurt of the instruments ..."

[Roger Bacon's](#) *De Retardatione Accidentium Senectutis* (The Cure of Old Age) is a 13th-century treatise that explores methods to prolong human life through scientific, medical, and philosophical principles. Drawing on classical, Islamic, and medieval medical traditions, Bacon argues that aging is not an inevitable process but one that can be slowed or delayed through proper care of the body and mind. He believed that science and lifestyle could counteract aging. Bacon provides a cryptic formula for the elixir of youth.

Bacon's work emphasizes the importance of diet, exercise, and environment in maintaining health and longevity. He advocated a balanced diet rich in natural foods, regular moderate exercise, and the avoidance of excessive mental strain. He also discussed the role of air quality, climate, and emotional well-being in delaying aging.

A distinctive aspect of Bacon's work is his scientific optimism—he believed that human longevity could be extended through advancements in alchemy, medicine, and natural science. He suggested that elixirs, medicinal compounds, and refined alchemical substances could rejuvenate the body and extend life. While some of his ideas were speculative, [De Retardatione Accidentium Senectutis](#) was one of the earliest Western texts to systematically approach aging as a medical and scientific problem, influencing later scholars in Renaissance longevity studies and the early development of gerontology.

References: Bailey 15; GMN: 10962; Grmek p. 99; Gruman p. 14; Haycock p. 23; Hayflick 271; Morley p. 1133; NYAM p. 120; Osler 501; Schafer p. 41; Shock 17296 (1658 ed); Stambler p. 229; Waller 589 (Latin ver); Wing B372.



Roger Bacon, *The Cure of Old Age and Preservation of Youth*.... London: Fleisher at the Angel and Crown, 1683. First english translation of Bacon's 13th Century manuscript From the Medical Library of Tim C. Opler. statue of Roger Bacon carrying an armillary sphere in the Oxford University Museum of Natural Science.

Alchemists and The Search for Immortality

Forgive us for a quick stop on our historical journey to discuss alchemy and aging. Roger Bacon (c. 1219–1292) was a medieval English philosopher and Franciscan friar whose work was deeply intertwined with **alchemy**, both in its practical and philosophical dimensions. While best known for his contributions to science, optics and empirical methodology, Bacon also engaged with alchemical theories, particularly those concerning transmutation, medicine and longevity.

Bacon's alchemical work reflected the common medieval belief that the Philosopher's Stone could transform base metals into gold and, more importantly, serve as an Elixir of Life to achieve immortality. It turns out that Roger Bacon was not alone. As a quick perusal of the [Wikipedia entry](#) on the history of alchemy reveals that the notion that one might be able to turn base metals into gold, or better yet, a mortal into an immortal, has been with us since ancient times.

Enormous amounts of time and resources have been spent by our predecessors in search of the Philosopher's Stone or some other magic locale or potion that will confer immortality (e.g., the “Fountain of Youth” sought by Ponce de Leon or China's first emperor Huang-Ti's search for the “pill of eternal life”, an elixir that would convey immortality).

Cynics might call this the easy way out, but alchemical works were instrumental precursors to modern chemistry. And Ponce de Leon's efforts were not completely in vain. He discovered Florida.

Just in the nick of time for an aging society.

Throughout history there has been an eager audience for books on alchemy. An example of a popular alchemical work of the last 500 years was *The Last Will and Testament of Basil Valentine* (1671) by Jonathan Thölde (apparently). Basil Valentine was a German alchemist who discussed alchemy and purification of the body for immortality. A few dragons and lots of alchemical symbols were thrown in for good measure.



Johannes Thölde (likely author), *The Last Will and Testament of Basil Valentine* 1671. Published by S.G. and B.G. or Edward Brewster, London. From the Medical Library of Tim C. Opler.

1305AD: Arnaldus De Villa Nova

Between 1305 and 1308, Arnald of Villanova wrote, in Latin, a “regimen of health” for his king, James II of Aragon entitled *Regimen sanitatis ad inclitum dominum regem Aragonum*. Broadly speaking, *regimina sanitatis* are practical hygiene handbooks aimed at ensuring the maintenance of health and ensuring longevity by offering a general regimen for living.

[Arnaldus de Villa Nova](#) (also called Arnau de Vilanova in Catalan, his language c. 1240–1311) was a physician and a religious reformer. His main advice was prevention of disease and achievement of longevity through healthy living.

His book *De conservanda bona valetudine, opusculum* proved to be one of the most reliable sources of healthy living for centuries. This [book](#) offered practical advice for living a long and healthy life such as avoiding large meals before bed and eating healthy food such as nuts. This work was Arnaldus’ commentary on the medical advice given from the School of Salerno.

The first certain historical information regarding the Salerno medical school dates to the beginning of the 9th century. The study of medicine in Salerno was eminently practiced: the art of health was practiced by monks who handed down the teaching orally. The school was undoubtedly the most ancient institution in Western Europe for teaching medicine and other disciplines, and for many centuries it was the most famous.

First published in Louvain in c.1480, after an extensive medieval circulation in manuscript form, Villa Nova’s book was printed hundreds of times and translated into several languages.

References: Bailey 11 (1912 ed); Freeman p. 27; Grmek p. 81; Gruman p. 62; Haycock p. 51; Iowa 43; NYAM p. 121 (1585 ed); Schafer p. 20; Stambler p. 230; Wellcome I:3968 (1510 ed).



Arnaldus de Villa Nova, *De conservanda bona valetudine. Opusculum Scholae Salernitanae...*, Paris: Cavellat, 1558. From the Medical Library of Tim C. Opler. Also shown is the earliest drawing of Arnaldus de Villa Nova, Bertran Boisset 'La siensa de destrair', manuscript, 1401

1489: Ficino's *Da Vita*

Attributed human aging to Galenic ideas on humors, vital spirits and cosmic forces. Argued for exercise, diet and contemplation.

Marsilio Ficino (1433-99) was an Italian Renaissance philosopher, theologian, priest, and physician, best known for his translations and exegeses of the works of Plato.

Marsilio Ficino's *De Vita Libri Tres* (Three Books on Life), published in 1489, is one of the earliest Renaissance works dedicated to health, longevity, and the connection between the body and the soul. Ficino, a Neoplatonist philosopher and physician, wrote this treatise primarily for scholars and intellectuals, who, he believed, suffered from the physical and mental toll of excessive study.

The work combines elements of ancient Greek medicine, astrology, and Platonic philosophy, emphasizing the balance of bodily humors, celestial influences, and spiritual well-being as key to maintaining health and prolonging life.

Ficino argued that proper diet, moderate exercise, herbal remedies, and even music and contemplation could enhance vitality. He also explored the influence of planetary alignments on human temperament and aging, blending medical advice with astrological speculation.

The three books address different aspects of life and longevity. The first book, *De Vita Sana*, focuses on maintaining health through diet, rest, and lifestyle. The second book, *De Vita Longa*, is dedicated to longevity, outlining ways to counteract aging through specific regimens, herbal medicines, and even alchemical preparations. The third book covered astral influences.

References: Freeman p. 36; Grmek p. 151; NYAM p. 121; Schafer p. 43.



Illuminated page from codex of Marsilio Ficino, *Da Vita Libri Tres*, 1489. From the Medicea Laurenziana Library, Florence. Originally commissioned by the Medici family. The illuminations are by Attavante Attavanti.

1489: Zerbi's Gerontocomia

Gabriele Zerbi's *Gerontocomia*: On the Care of the Aged is a seminal Renaissance work on geriatrics. Zerbi argued for specific measures to care for the elderly.

Zerbi offers an in-depth examination of aging, addressing its causes, symptoms, and the responsibilities of caregivers. He emphasizes the importance of a balanced diet, appropriate living conditions, and the use of beneficial medicines to promote the well-being of the elderly.

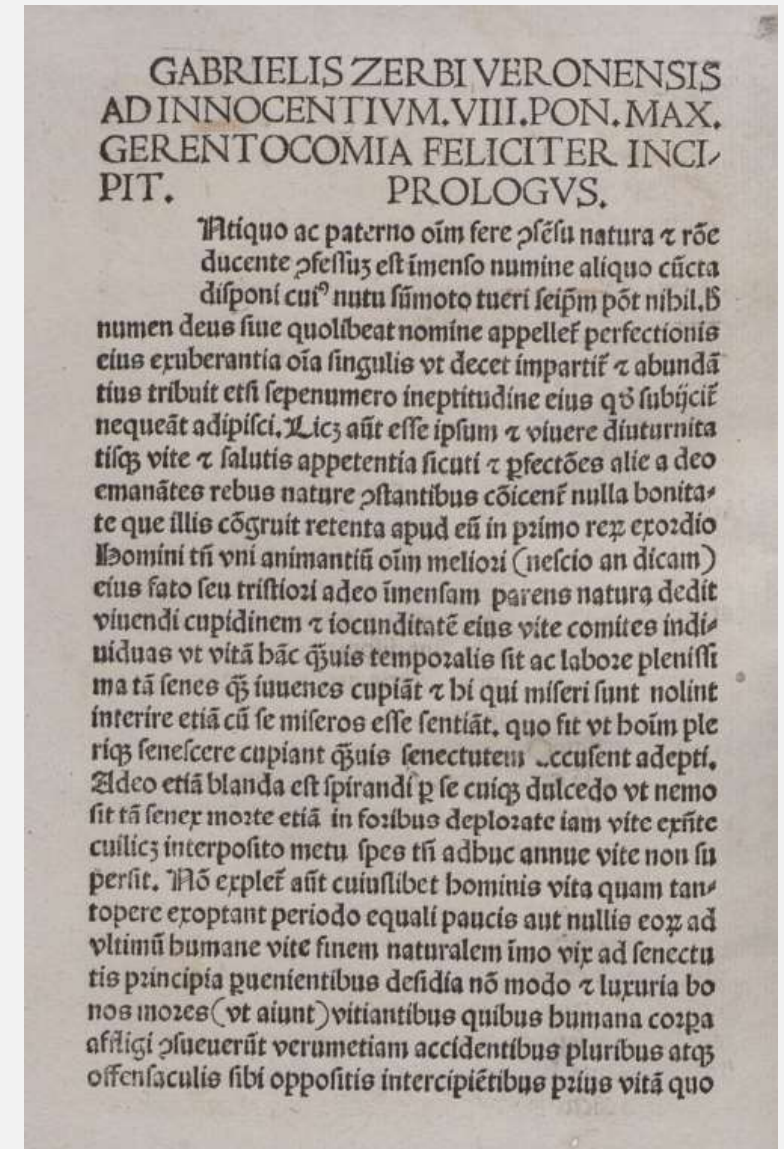
His holistic approach integrates medical knowledge with practical advice, aiming to enhance the quality of life for older individuals.

In *Gerontocomia*, Zerbi delves into dietary recommendations tailored to the aging body, environmental factors conducive to health, and specific medicinal practices to alleviate common ailments associated with old age. He adheres closely to Galenic theories of the four humours and the notion that aging involves loss of moisture.

Zerbi believed that a person's life span depended upon their horoscope and bodily constitution. He emphasized that life cannot be extended beyond its natural limits so one should aim only at retarding the aging process. Favored treatments included meat of vipers, distillate of human blood, and medicines containing gold and precious stones.

Tragically, [Gabriele Zerbi](#) never got to live to an old age. He was summoned to treat Skander, the chief minister of the Ottoman Turks, who was suffering from severe dysentery. After the Minister's death, Zerbi was executed by sawing him in half.

References: Freeman p. 32; Grmek p. 82; Iova 137; Schafer p. 53; Stambler p. 231; Waller 150.



Gabrielis Zerbi, *Gerontocomia*, Eucharius Silber (Rome), 1489. From the *Bayerische Staatsbibliothek, München*.

Section 5:

Aging in the Early Modern Period (1500 to 1800)



Portrait of Pope Julius II, by Titian, 1670, Venice. Photo from Palazzo di Pitti, Florence. Courtesy of Getty Images / A. Dagli Orti.

Limited Progress in the Pre-Scientific Period

From today's perspective we appreciate aging and eventual death as the outcome of a set of complex biological processes that are increasingly well understood.

Up until around 1600 there was no use of experimental methods, hence theories of aging needed to be derived from everyday observation and intuition.

Typically, early theories were developed by *analogy* relative to nature. Writers could observe the passing of seasons or other natural phenomena and presumed that similar things might be behind human aging. Daniel Schäfer, Professor, Institute for the History of Medicine, University of Cologne, has written in the great detail about aging in this period and throughout his work reviews the various “geroconomies”, some of which are included here.* Schäfer writes in 2005:

“Ancient and early modern notions of ageing posit several reasons for its cause. Along with common intrinsic factors – steady loss of inner warmth and moisture resulting in a lack of blood and semen, as well as insufficient digestion, which in turn leads to an accumulation of putrefying waste products – extrinsic causes are also taken into account: excessive or insufficient cooling through the surrounding air, and incorrect diet. Initially, most of these concepts drew their analogies from nature: withering leaves, the cooling corpse and, above all, the metaphor of the fading lamp or flame, which has been the focus of numerous studies. These conceptions are treated with a certain rigidity in early modern Galenism and proved astonishingly stable.”**

Essentially, Aristotelian and Galenic concepts of longevity went unchallenged for millennia. Perhaps, new authors would throw in some astrology or elixirs in their narratives for good measure.

* [Morley](#) (2004) provides a comprehensive list of early *Gerocomias* as does Freeman (1979). After study, we excluded most of these. For example, Anselmus' 1605 book *Gerocomia* recapitulates the Aristotelean theory of aging without elaboration or critique. While few have examined the work, Andrea Zanca's 2020 [look](#) at Anselmus led to the same conclusion. Others who recapitulated Aristotle's ideas without modification included Brisiani in his 1585 *Geraeologia*; David de Pomis in his 1588 book *Enarratio brevis* and Foucherolles who plagiarized de Pomis in 1617.

** See Daniel Schäfer, “More than a Fading Flame. The Physiology of Old Age between Speculative Analogy and Experimental Method,” in [Blood, Sweat and Tears: The Changing Concepts of Physiology from Antiquity into Early Modern Europe](#), In *Intersections* Vol 25, Brill, Jan 1, 2012.

Early Modern Europe: Science and Empiricism Take Off

Things changed dramatically after 1600. The 17th and 18th centuries marked a transformative period in the understanding of aging, longevity, and health. During this time, the decline of medieval scholasticism and the rise of the Scientific Enlightenment led to new ways of thinking about the human body, disease, and aging. This period saw remarkable works by astronomers like Galileo Galilei, physicists like Isaac Newton and mathematicians like Gottfried Wilhelm Leibniz.

Most importantly, Francis Bacon emphasized the importance of observation, experimentation, and inductive reasoning in understanding the natural world. His works, such as [*Novum Organum*](#) (1620), laid the groundwork for modern scientific inquiry. He was also a major contributor to aging research. In medicine, figures such as Paracelsus and Giovanni Morgagni challenged traditional Galenic and humoral theories, emphasizing observation, experimentation, and mechanistic explanations.

While understanding of the cell was still to come, by the end of the 18th Century the idea of lifespan extension was legitimized by thinkers like Condorcet and Buffon.

There was enough empirical knowledge and physiologic insight by the end of the 18th Century that humankind was poised to begin more serious scientific experimentation and engage in deeper thought about aging in the century to come.



Stillebildes Leben in Berlin und in der ersten Hälfte des XVI. Jahrhunderts.

Early Modern Europe saw the spread of ideas regarding politics, art, and humanism and the development of empiricism and observation.

The 16th Century: Empiricism Arrives

The 16th century saw the beginnings of a shift away from medieval scholasticism toward empirical and experimental approaches.

The Alchemical and Humoral Foundations

Paracelsus (1493–1541) challenged traditional Galenic medicine by advocating a chemical and alchemical approach to health. He believed that aging resulted from the accumulation of harmful substances and that longevity could be extended through the purification of bodily fluids. His emphasis on external influences, such as toxins and poor dietary habits, introduced the concept that aging was not merely a natural decline but could be actively combated. He proposed elixirs and tinctures, including the fabled "elixir of life," as means of prolonging life and maintaining vitality.

The Mechanistic and Empirical Shifts

Francis Bacon (1561–1626) advocated a systematic and empirical approach to aging. In *Historia Vitae et Mortis*, he sought to classify the causes of aging and investigate methods to prolong life, hoping to add 1000 years to life. Bacon identified dietary and medical interventions that could slow aging, emphasizing the role of scientific inquiry in unlocking the secrets of longevity. His contemporaries, René Descartes and Kenelm Digby, entertained scientific strategies to achieve immortality but never succeeded.



The ideas of Paracelsus triggered a departure from Galenic thought and encouraged empirical observation into the causes of disease and aging.

Theories of Aging Develop

Santorio Santorio: Quantifying Aging and Metabolism

Santorio Santorio introduced quantitative analysis into medical study. Using a balance scale, he measured insensible perspiration, linking metabolism to aging. He argued that the regulation of bodily functions through controlled diet and lifestyle could influence longevity. His work represented a shift toward a mechanistic understanding of aging, contrasting with earlier mystical and humoral theories.

Tobias Venner and Jan Baptist van Helmont: Balancing Approaches

Tobias Venner (1577–1660), in *Via Recta ad Vitam Longam*, blended humoral theory with emerging empirical insights. He supported moderation in diet, exercise, and sleep as means to prolong life.

Jan Baptist van Helmont (1580–1644), a Flemish chemist and physician, further advanced the chemical understanding of life. He introduced the concept of the "archeus," a vital force governing bodily processes. His work bridged alchemy and modern chemistry, influencing later thinkers like Robert Boyle. He favored chemical and physiological explanation of aging. He proposed that bodily decay resulted from internal chemical imbalances, an idea that anticipated later biochemical theories. Nonetheless, he still advocated "Elixirs" that could prolong life.



Santorio Santorio (1561-1636)

Lifestyle Advice: Self-Help Books Become Popular

Luigi Cornaro (1464–1566), an Italian nobleman, offered a different perspective. In his treatise *The Art of Living Long*, Cornaro advocated for a life of moderation, emphasizing a simple diet, regular exercise, and emotional balance.

His work, based on personal experience, became a cornerstone of longevity literature and influenced later thinkers like George Cheyne. Cornaro continues to be cited in self-help books even today.

Tobias Venner (1577–1660), an English physician, focused on hygiene and lifestyle. In *Via Recta ad Vitam Longam* (The Straight Path to a Long Life), he detailed the importance of diet, sleep, and exercise for longevity, reflecting a growing interest in preventive medicine.

Leonard Lessius (1554–1623), a Jesuit theologian, combined spiritual and physical discipline in his work *Hygiasticon*. He echoed Cornaro's emphasis on moderation and added a moral dimension, arguing that a virtuous life was essential for longevity.



Luigi Cornaro counselled his readers to avoid big banquets like this one, to weigh out food before eating it and to live a life of moderation.

The 17th and 18th Centuries: The Age of Enlightenment

The 18th century saw the systematization of medical knowledge and the rise of knowledge of physiology amidst the increasing understanding of mechanism.

Giovanni Morgagni: Pioneer of Physiology

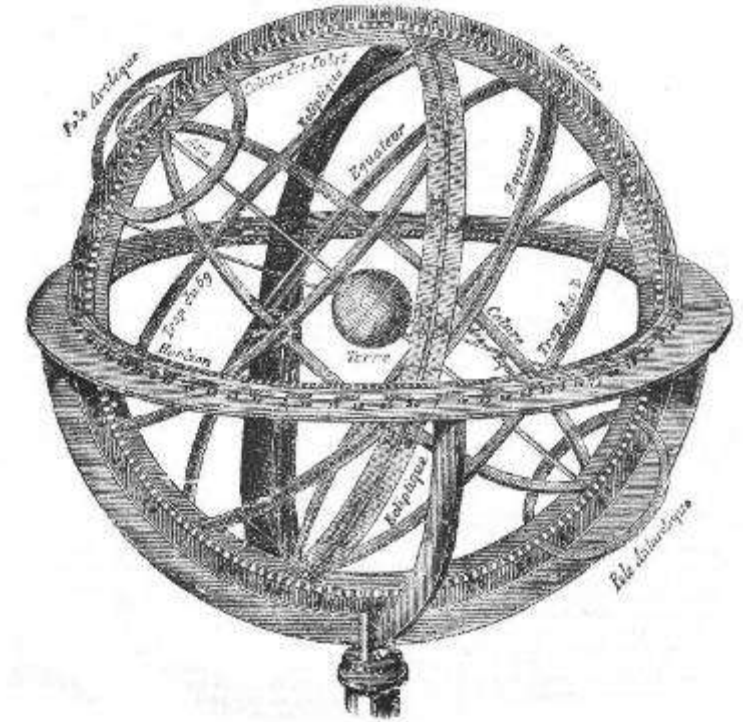
Giovanni Morgagni (1682–1771), the father of anatomical pathology, studied the structural changes associated with aging. His work *De Sedibus et Causis Morborum* (On the Seats and Causes of Diseases) linked aging to specific organ pathologies, advancing the understanding of age-related diseases.

Giorgio Baglivi: The Role of Fibers and Circulation

Giorgio Baglivi (1668–1707) advanced the theory that aging was related to changes in the body's fibers and circulatory system. He emphasized the importance of maintaining elasticity in bodily tissues and viewed physical degeneration (“wear and tear”) as a key contributor to aging. His ideas foreshadowed later developments in the study of cardiovascular aging.

John Floyer: Aging and Respiration

John Floyer (1649–1734) examined aging through the lens of respiratory function. His work linked aging to declining physiological functions, suggesting that interventions such as cold bathing could invigorate the body and slow aging.



The 1700's saw widespread experimentation and growing optimism driven by the Scientific Enlightenment.

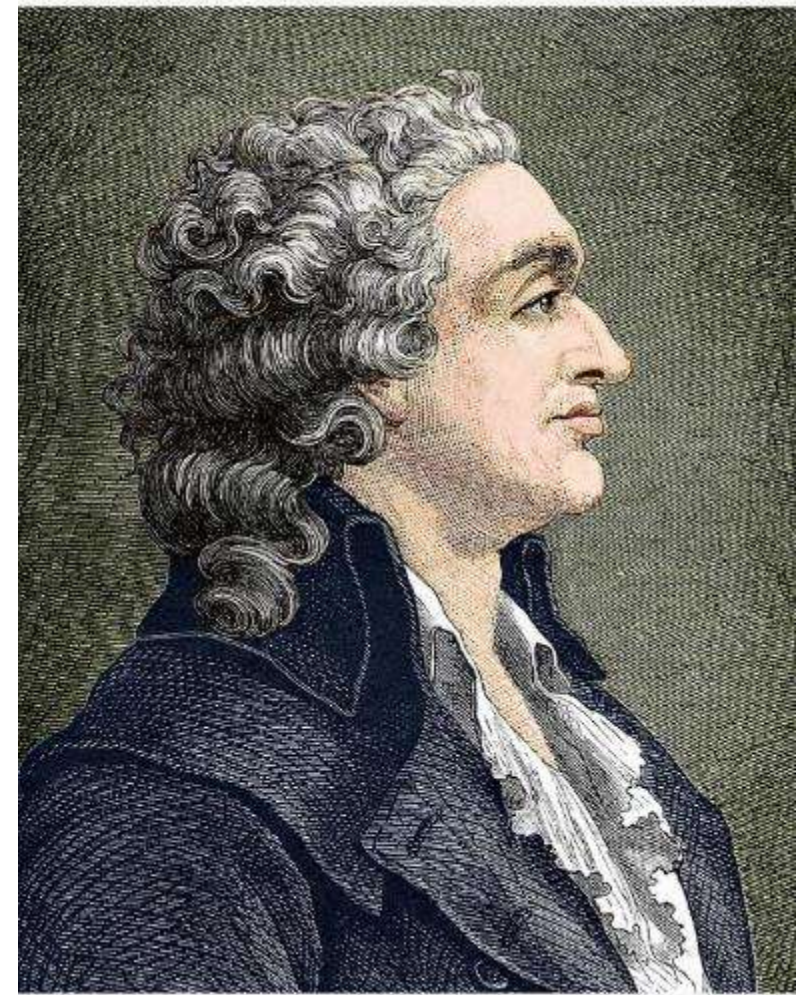
The 18th Century: Visionaries and Optimism

Condorcet: An Extraordinary and Early Visionary

Nicolas de Condorcet (1743–1794), a French philosopher, epitomized Enlightenment optimism. In *Sketch for a Historical Picture of the Progress of the Human Mind*, he envisioned a future where scientific progress would extend human lifespan greatly. He theorized that societal advancements in medicine, hygiene, and nutrition could extend human lifespan indefinitely. His optimistic view aligned with Enlightenment ideals, emphasizing human progress as a key to unlocking longevity.

Ultimately, A Period of Great Progress

Between the 16th and 18th centuries, theories of aging evolved from mystical and humoral explanations to mechanistic, anatomical, and statistical models. Figures like Paracelsus and van Helmont introduced chemical and alchemical perspectives, while Cornaro and Lessius emphasized lifestyle and moderation. The empirical rigor of Bacon, Santorio, and Morgagni paved the way for a scientific understanding of aging, culminating in Condorcet's and Easton's demographic studies. This intellectual progression laid the foundation for modern gerontology and aging research, highlighting the enduring quest to understand and extend human life.



Nicolas de Condorcet, an early visionary on human longevity

1558: Luigi Cornaro

Cornaro's *Trattato de la Vita Sobria* became the most widely read treatise on how to live a long life ever. The English translation of this book, which counsels moderation, went through more than 50 editions. Cornaro is still cited today, for example, by Peter Attia in his excellent 2023 book *Outlive*.

[Luigi Cornaro](#) (1464–1566), a Venetian nobleman, wrote extensively on aging and longevity, advocating for a disciplined and minimalist approach to diet and lifestyle.

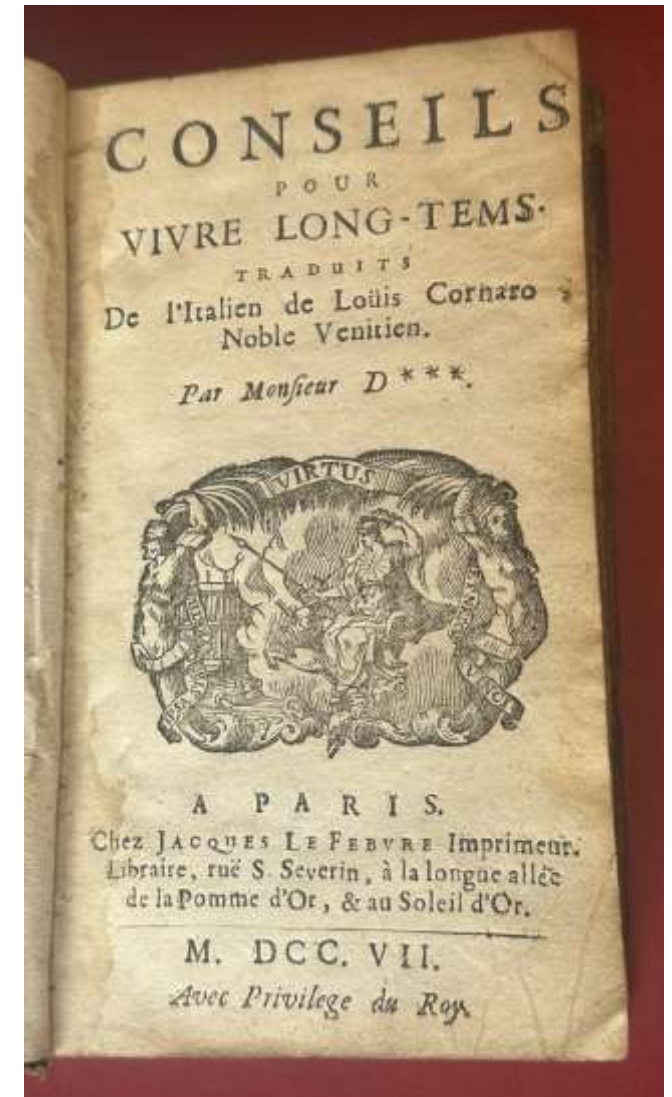
In his 1558 work, *Trattato de la Vita Sobria* (Treatise on the Sober Life), he argued that moderation in food and drink was the key to a long and healthy life.

Cornaro himself claimed to have restored his health and extended his lifespan by adopting a strict diet of around 12 ounces of solid food and 14 ounces of wine per day, avoiding excesses that he believed led to premature aging and disease.

Cornaro emphasized that overeating and indulgence weakened the body, while a simple, measured diet preserved vitality and mental clarity well into old age. He also recommended a calm, joyful and virtuous life, free from stress and excess pleasures, as a means to maintain physical and emotional well-being.

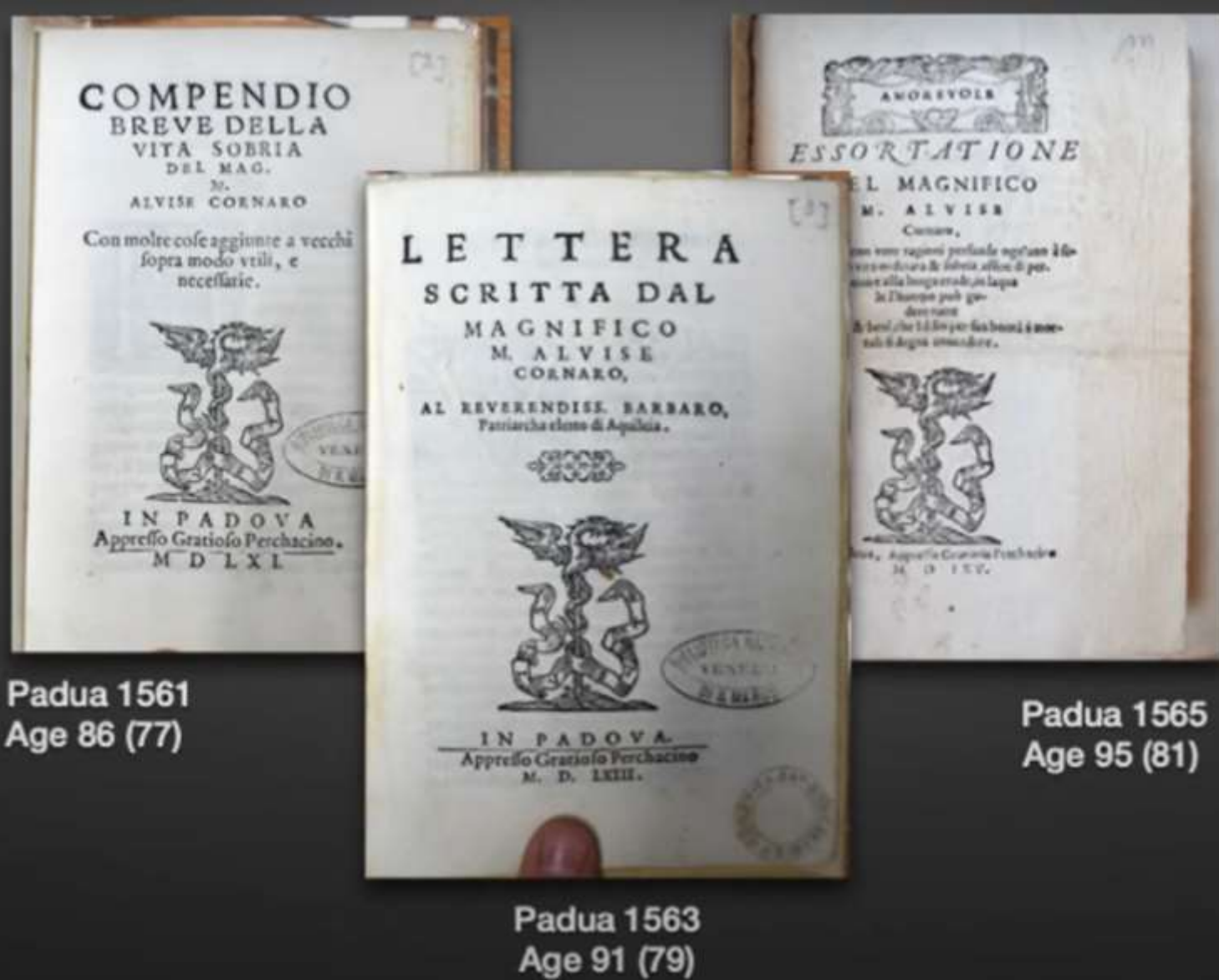
Even the cynic, Friedrich Nietzsche argued that Cornaro had a slow metabolism, and his diet resulted from this – not the other way around. This ignores, of course, his midlife switch in diet.

References: Attia p. 79; Bailey 39 (later ed); Freeman p. 36; GMN 1592; Grmek p. 119; Gruman p. 8; Hayflick p. 268; Haycock p. 24; Iowa 169.7 (eng ed); Morley p. 1138; Norman 517; NYAM p. 121 (1768 ed.); Osler 2369; Schafer p. 43; Shock 17330 (eng ed); Waller 2135 (later German ed).



Louis Cornaro, *Conseils pour vivre long-tems*, Paris, Le Febvre, 1707. From the Medical Library of Tim C. Opler.

Hannah Marcus: Cornaro Prone to Hyperbole



Cornaro appears to have been somewhat promotional and prone to hyperbole, claiming in his 1565 book that he lived to 100.

Wechsler and Marcus note he actually lived to 82.*

Interestingly, he stated age kept growing with subsequent editions of his book. This is illustrated at left in a slide from a talk on Cornaro's book by Hannah Marcus. See https://www.youtube.com/watch?v=2NY_MlYUb84

Hannah Marcus' fascinating talk covers the importance of the book and the contemporary reactions to his message of dietary asceticism. Cornaro was very much a cultural phenomenon in both early modern Italy and English-speaking countries where sales of his book took off.

* See [Wechsler and Marcus](#), 2023, "Long life: Aging and the anxieties of longevity from the premodern to the present," *Endeavor*, Sep, 47:3, 1-7

1574: Paracelsus

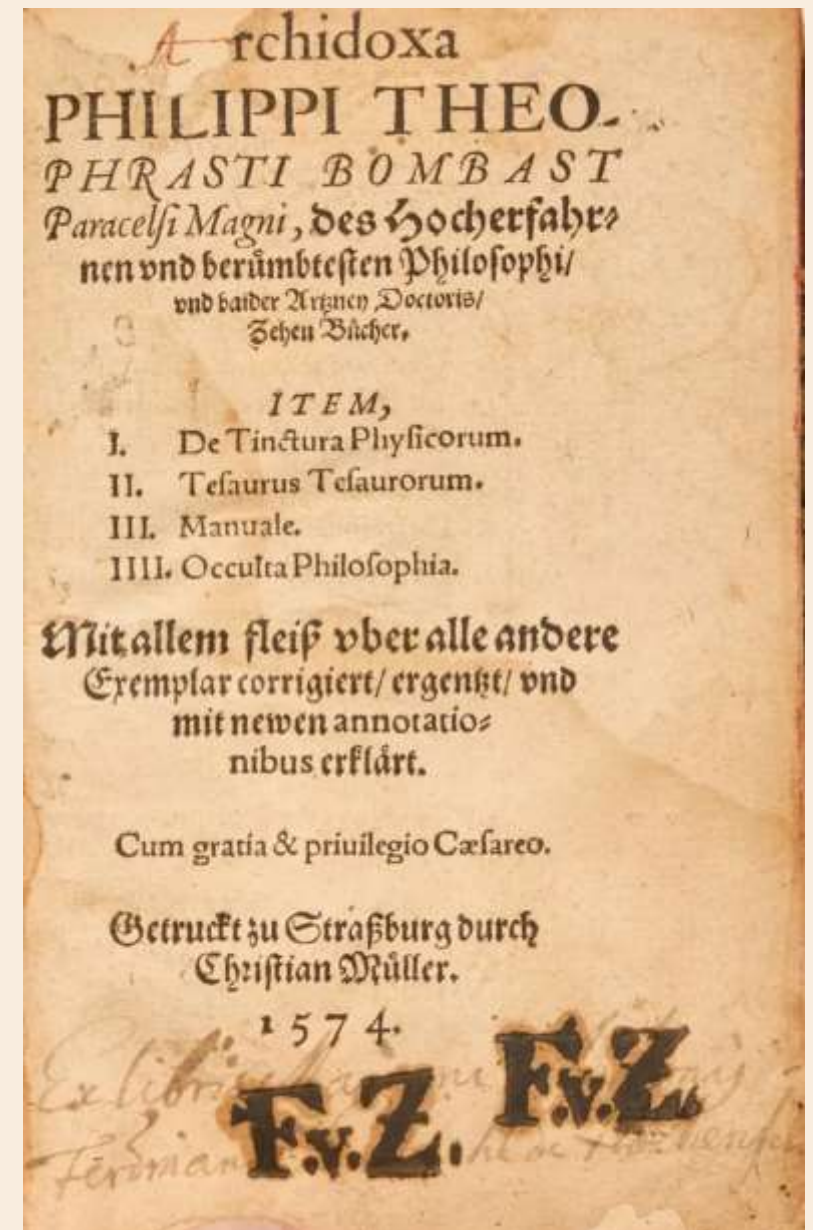
Paracelsus was one of the 16th Century's most important figures in medicine. He believed that alchemy could be used to extend life which itself was fueled by a vital force. This idea was part of his seminal book *Archidoxa*.

Paracelsus' *Archidoxa* is a work that covers a range of topics, with a focus on the practical and mystical applications of alchemy, medicine, and the natural world. He wrote on longevity and health in this book. These ideas were also expressed in his 1562 book *libri v. de vita longa*.

The key ideas are: (1) Chemical Medicine: Paracelsus is often credited with revolutionizing medicine by focusing on the use of chemicals and minerals in healing rather than herbals or bloodletting; (2) The Unity of Nature and the Microcosm/Macrocosm: Paracelsus emphasized that humans reflect the universe - he believed that by understanding the natural world (macrocosm), one could gain insight into the human body (microcosm) – leading to a holistic approach to medicine; (3) Alchemy as a Tool to Extend Life: Paracelsus applied alchemical principles to medicine, believing that transformation and purification processes could help cure illnesses and extend life and (4) The Vital Force: Paracelsus introduced the idea of the "vital force," a concept that was crucial for his understanding of health and disease. This vital force is what animates life.

[Paracelsus' life](#) is a testament to the [power of ideas](#) as he was enormously influential despite having a controversial and quite challenging personality. Henry Cuffe, a contemporary who was executed under the reign of Elizabeth I for treason, wrote that Paracelsus' ideas on longevity were "of either his false ostentation, or extreme madness." The universally kind William Osler reserves rare opprobrium for Paracelsus' writings: "One cannot speak very warmly of the practical medical writings of Paracelsus." (Silliman Lectures, *The Evolution of Modern Medicine*, 1913, p. 121).

References: Bailey 117 (later ed.); Grmek p. 157 (later ed); Gruman p. 67; Haycock p. 51; Schafer p. 43; Stambler p. 230.



Paracelsus, *Archidoxa*, Second edition, C. Muller: Strassbourg 1574. From the Medical Library of Tim C. Opler.

1586: Castore Durante

Castore Durante's *Il Tesoro della Sanità* (The Treasure of Health) is a comprehensive medical and botanical guide aimed at promoting health and longevity through diet, hygiene, and natural remedies.

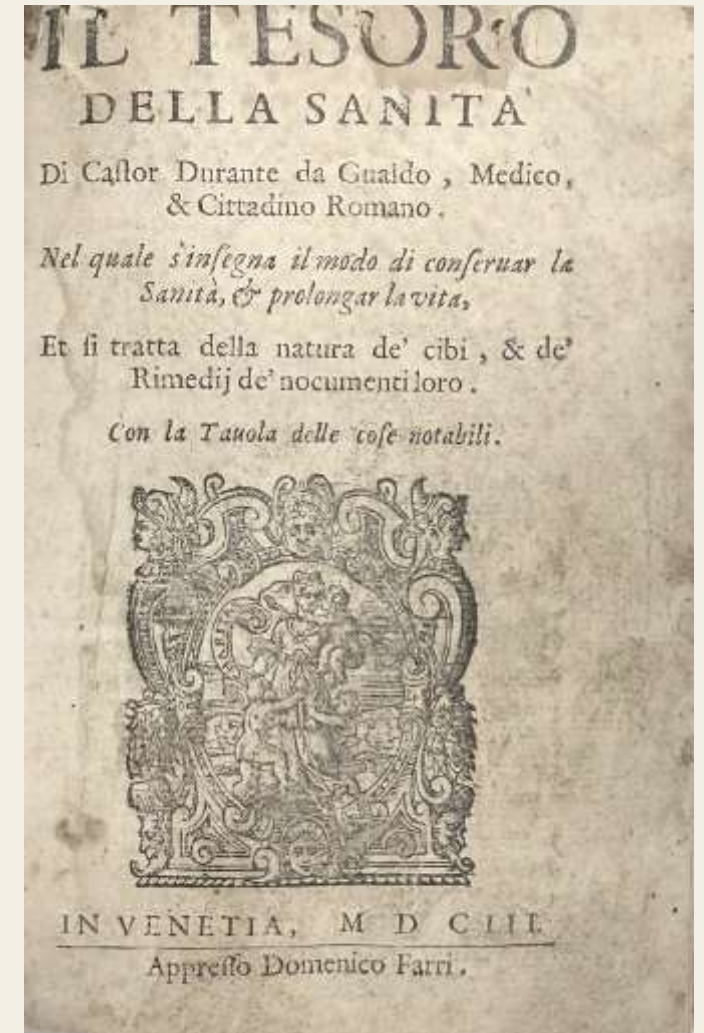
[Castore Durante](#), also called Castor Durante da Gualdo (1529 – 1590) was a physician, botanist and poet of the Italian Renaissance.

Durante organizes his work around the principles of maintaining balance in the body through proper nutrition, lifestyle and the use of medicinal plants, drawing heavily from classical sources such as Galen and Pedanius Dioscorides while incorporating contemporary medical observations.

A significant portion of the book is devoted to herbal medicine, with detailed descriptions of plants, their medicinal properties and their applications for treating various ailments. Durante, a physician and botanist, provides practical advice on preparing remedies, including herbal infusions, decoctions, and topical treatments. He also discusses the influence of environmental factors such as air, water, and seasonal changes on health, aligning with humoral theory, which dominated medical thought at the time. His meticulous attention to botanical accuracy makes *Il Tesoro della Sanità* not only a medical text but also an important work in early pharmacology and herbalism.

Durante also emphasizes the importance of a balanced diet, physical exercise, and moderation in all aspects of life to prevent illness and prolong life. He advocates for a holistic approach to well-being, combining medical knowledge with lifestyle guidance.

References: Freeman p. 77; Waller 2659 (later German ed)



Castore Durante, *Il Tesoro Della Sanità*, Appresso Domenico Farri (Venice), 1603. Second edition. From the Medical Library of Tim C. Opler.

1598: Gabriele Paleotti

Paleotti's *De Bono Senectutis* (On Good Old Age) provided a penetrating description of mental health in old age that was not matched for centuries.

[Gabriele Paleotti](#) (1522 –1597) was an Italian cardinal and Archbishop of Bologna. A candidate for the papacy in 1590, he was a prolific writer on moral and religious topics. Gabriele Paleotti's 1595 book *De Bono Senectutis* (On Good of Old Age) is a treatise that explores the virtues, wisdom, and dignity associated with aging. As a prominent Italian cardinal and scholar of the late Renaissance, Paleotti framed old age not as a period of decline but as a time of moral refinement, experience, and stoic [serenity](#). Drawing from classical philosophy, biblical teachings, and patristic writings, he argued that aging should be embraced rather than feared. And enjoying old age is an act of will (Schäfer). The book reflects the humanist interest in the value of later life, engaging with themes of longevity, intellectual maturity, and the role of the elderly in society.

In a recent talk on views of Early Modern Aging, Hannah Marcus [notes](#) that Paleotti was well aware of Luigi Cornaro's book and was aligned with his message of a good old age. *De Bono Senectutis* aligns with Renaissance discourses on aging that viewed it as a natural phase of life, best approached with virtue and acceptance. A modern review of this book by Karl Stern and Thomas Cassirer appeared in the May 1946 issue of the *American Journal of Psychiatry*. Stern and Cassirer argue that Paleotti's treatise on aging provided a fairly deep and accurate view of depression in elderly patients – unmatched in many ways well until the 19th Century: "It is astounding that the author, when speaking of depressions, makes a distinction which we find only three hundred years later again in the literature, i. e., the distinction between endogenous and exogenous mechanisms."

References: Freeman p. 96; Daniel Schäfer, "Historical Considerations on the Ethics of Aging: Examples from the Sixteenth Century," in Andrea von Hülsen-Esch (ed.), *Cultural Perspectives on Aging*, De Gruyter, Berlin, 2021.



Gabriele Paleotti, *De bono senectutis. Libri tres*, apud Minimam Societatem, Venice, 1598, From the Medical Library of Tim C. Opler.

1613: Leonard Lessius

Like his contemporary Luigi Cornaro, the Jesuit scholar Leonard Lessius counsels moderation, exercise and consistent daily habits as the best means to live a long life.

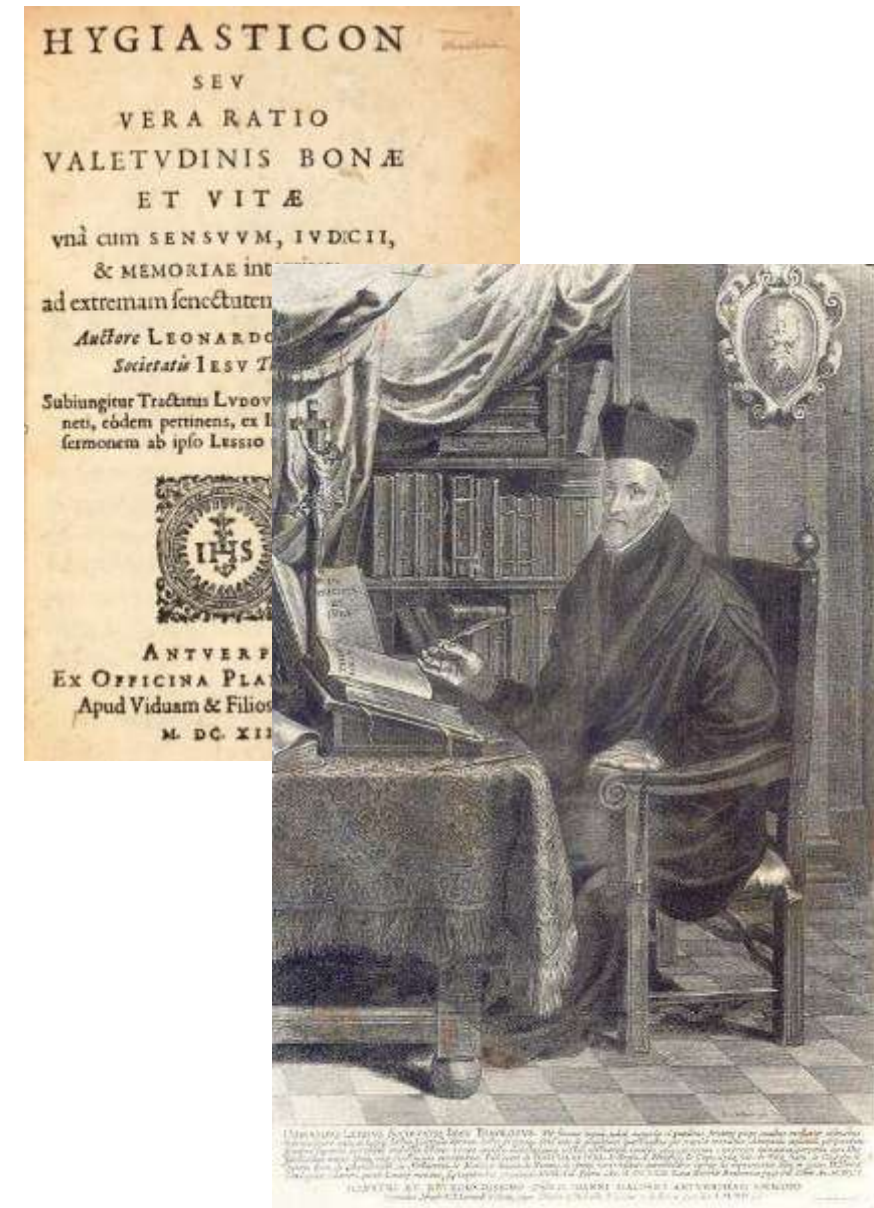
Written by the Jesuit theologian and scholar [Leonardus Lessius](#), *Hygiasticon* is a comprehensive guide to achieving a healthy and virtuous lifestyle through moderation in diet, exercise, and daily habits. Drawing on classical sources such as Hippocrates and Galen, as well as his own knowledge, Lessius emphasizes the importance of temperance in eating and drinking, advocating for a simple, frugal diet as the foundation of good health.

He also highlights the benefits of regular physical activity, adequate sleep, and mental tranquility, presenting a holistic approach to well-being that aligns with both moral and physical health.

The *Hygiasticon* was widely influential in its time, reflecting the Renaissance interest in harmonizing ancient wisdom with Christian ethics.

A key theme in *Hygiasticon* is the connection between moderation and longevity. Lessius argues that excessive indulgence in food, drink or other pleasures not only harms the body but also weakens the soul, making it harder to live a virtuous life. He provides practical advice on how to maintain a disciplined lifestyle, including specific dietary recommendations and guidelines for daily routines.

References: Bailey 100 (later ed.); Freeman p. 40; Gruman p. 72; Krivatsy 6913; Osler 3218 (english ed); Schafer p. 264; Shock 17415 (later ed.); Stambler p. 231; Waller 5745; Wellcome I, 3755.



Leonardus Lessius, *Hygiasticon*, 1625 (third edition). From the Medical Library of Tim C. Opler. Portrait of Leonard Lessius from du livre de A.Hamy: *Galerie illustrée de la Compagnie de Jésus*, 1893.

1614: Santorio Santorio

Santorio presciently linked aging to breakdown in the body's ability to exchange energy and its retention of waste products caused by the loss of "insensible perspiration" over time.

[Santorio Santorio](#), in his 1614 work *Ars de Statica Medicina* (Art of Static Medicine), presents a novel perspective that linked aging to respiration, grounded in his quantitative approach to physiology. He lived from 1561 to 1636 and was a Professor of Medicine at Padua.

He viewed the human body as a dynamic system governed by the balance of insensible perspiration—the invisible loss of bodily fluids through processes like respiration and sweating. Santorio believed that aging was closely tied to the gradual decline in the body's ability to maintain this balance.

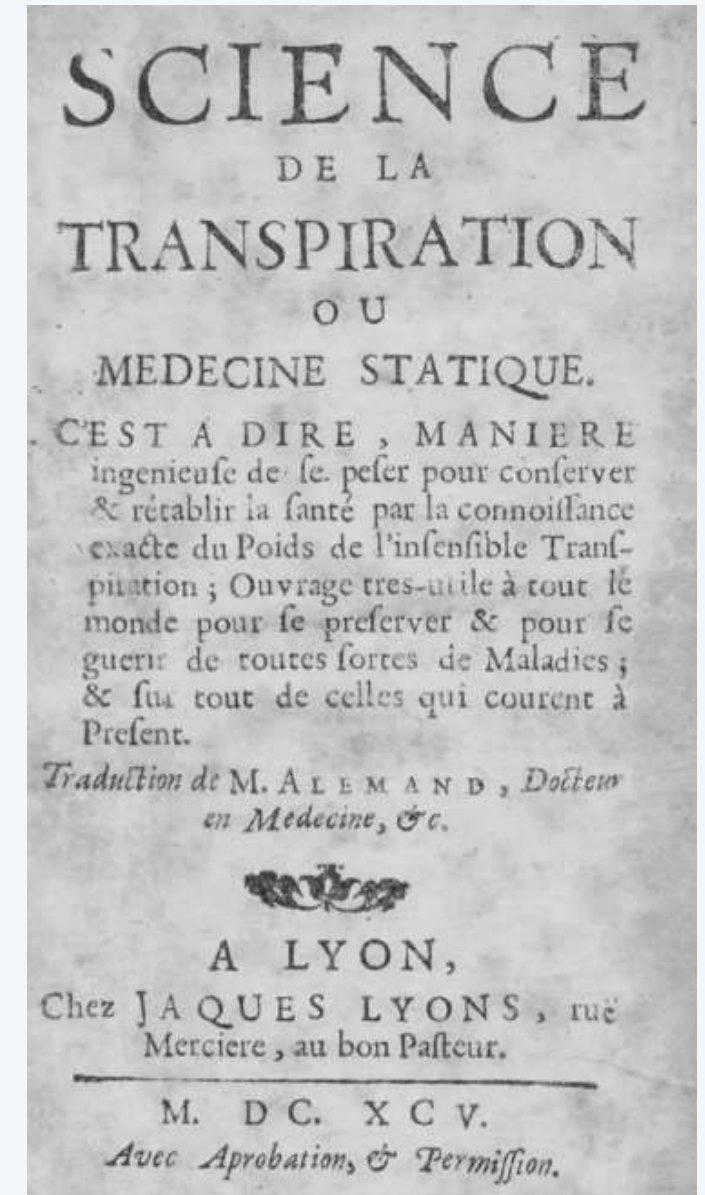
As individuals aged, he argued, their insensible perspiration decreased, leading to an accumulation of waste products and a decline in vital functions.

This view influenced his contemporary Francis Bacon and the focus on waste products anticipates later views that link cellular aging to breakdown in autophagy processes.

By measuring the [weight](#) of food intake and bodily excretions, he concluded that a significant portion of bodily waste was eliminated through respiration and insensible perspiration.

Santorio's experiments demonstrated that the body lost weight even when no visible excretions were produced, highlighting the importance of respiration in this process.

References: Freeman p. 39 (1614 ed); GMN 573; Iowa 399 (later ed); Norman 1890; Schafer p. 74.



Santorio Santorio, *Science de la Transpiration*, 1695 (first French translation of *Ars de Statica Medicina*). Lyon: Chez Jacques. From the Medical Library of Tim C. Opler. Portrait by Giacomo Piccini.

1620: Tobias Venner

Tobias Venner's 1620 *Via Recta ad Vitam Longam* (The Straight Path to a Long Life) is a well-known medical and lifestyle guide aimed at promoting longevity through diet, exercise and moderation. He favored whiskey (aqua vitae) as a remedy for old age.

Reflecting Galenic traditions, [Tobias Venner](#) emphasized the importance of the "six non-naturals"—air, food and drink, sleep and wakefulness, motion and rest, excretions and retentions and passions of the mind—as key determinants of health.

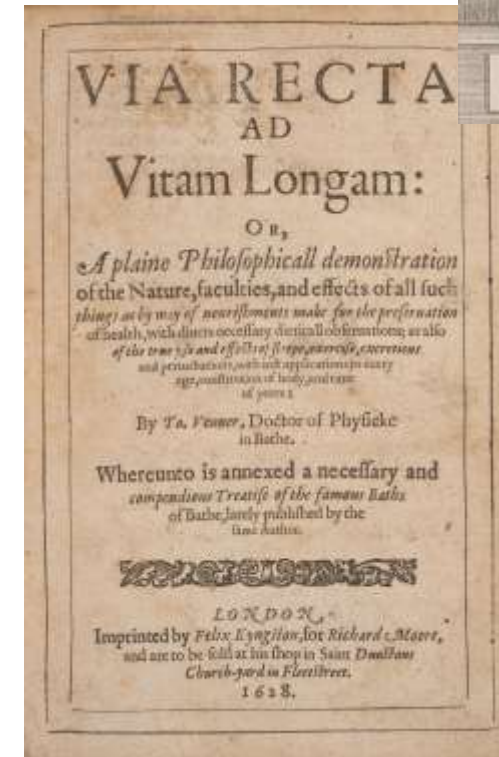
He provides detailed dietary recommendations, advocating for a balanced diet of meats, fruits and vegetables while warning against excesses such as overindulgence in rich foods and alcohol. Venner also discusses hygiene, moderate exercise and fresh air as crucial to maintaining health, particularly in an age where epidemics and chronic illnesses were common concerns.

He was strongly opposed to the use of tobacco on health grounds, perhaps being one of the first to take this position.

Words introduced to English in this book include atrophy, dietetical, excrete, flaccid, gustative, ingestion, sanguify, turgid and vomitory.

Beyond physical health, *Via Recta* (*The Straight Path to a Long Life*) delves into the psychological and social aspects of well-being. Venner acknowledges the role of emotions and balance in longevity, advising against stress, anger and melancholy. He also touches on environmental factors, arguing that clean living spaces and proper sanitation contribute to a longer life.

References: Bailey 152; Freeman p. 36; Krivatsky 12269; NYAM p. 122 (1650 ed); Osler 4163; Shock 17488 (later ed); Waller 9865; Wing V196.



Tobias Venner, *Via Recta ad Vitam Longam*, Felix Kyngston for Richard Moore: London, 1628. Third edition. From the Medical Library of Tim C. Opler.

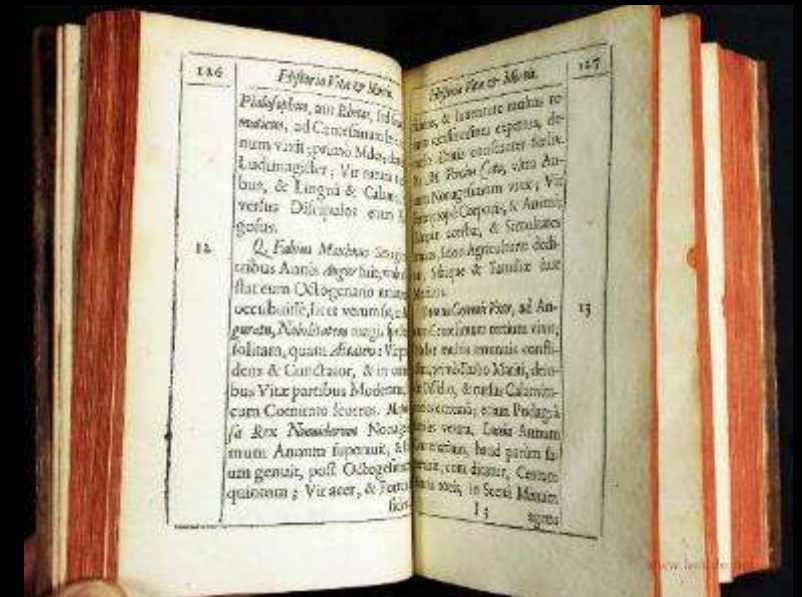
1623: Francis Bacon

Francis Bacon's *Historia Vitae et Mortis* (History of Life and Death), published in 1623, is a pioneering work in the early scientific study of aging and longevity. Part of his larger *Instauratio Magna*, which aimed to advance human knowledge, this book reflects Bacon's empirical approach to understanding nature. Bacon saw the extension of human life as an important means to improve society and advance knowledge.

Bacon analyzed observations and built theories about the factors influencing lifespan, drawing from classical sources, epidemiologic data and his own insights. Bacon replaced Galen's humoral theory with a concept of ageing based on 'spirit, or body pneumatical' through which the body functioned, and which declined in old age because of unequal repair to different parts, leading to natural death. He emphasized the role of natural processes such as the consumption of vital spirits and the accumulation of waste products (which anticipated later work on autophagy and aging).

A founding father of scientific method, Bacon investigated ways to prolong life, including diet and medical interventions. He designed "grains of youth" and "Methusaleh Water". He entertained the idea that with science life might even become eternal. Bacon's work is notable for its grand vision for why longevity extension is societally needed, its fresh ideas like the role of waste products and its synthesis of the literature (see Gemelli). Ever the experimentalist, Bacon died from pneumonia contracted when he was studying the preservation of flesh with snow.

References: Freeman p. 39; Gruman p. 35; Haycock p. 5; Schafer p. 91; Stambler p. 231. Also see Gemelli, "The history of life and death: a 'spiritual' history from invisible matter to prolongation of life," *Early Sci Med* 2012, 134-157.



Francis Bacon, *Historia vitae & mortis. Sive, titulus secundus in historia naturali & experimentalis ad condendam philosophiam : quae est instaurationis magnae pars tertia*, John Haviland: London, 1623. First edition. From the Medical Library of Tim C. Opler.

Francis Bacon On Long Life

David Boyd Hancock, Dept of Economic History, London School of Economics, “ ‘A Thing Ridiculous’? Chemical Medicines and the Prolongation of Human Life in Seventeenth-Century England,” July 2006:



Francis Bacon's entire philosophical program was designed to facilitate the growth of human knowledge such that immortality could be achieved.

“In *Historia Vita et Morbis* (1623) Sir Francis Bacon explored how human life spans might be returned to the near thousand years enjoyed by Adam and the Patriarchs.

There, he went so far as suggesting that “That which may bee repaired by Degrees, without a Totall waste of the first Stocke, is potentially eternall.”

This reflection supported his earlier (unpublished) proposal that the “true ends” of human knowledge and thus the whole purpose of his programme for the advancement of learning was, “to speak plainly and clearly ... a discovery of all operations and possibilities of operations from immortality (if it were possible) to the meanest mechanical practice.”

Indeed, Graham Rees has written that the “aim of prolonging life represented the aims of Bacon's programme as a whole,” and that he ‘marked out the prolongation of life as the first and highest objective of the new philosophy.’”

1630's: Radical Philosophers Ponder Longevity

The early and mid-1600's were remarkable years when two of the most important radical philosophers behind the Scientific Revolution, Francis Bacon and René Descartes, lived in overlapping time periods.

Francis Bacon was an English philosopher, statesman and scientist known for developing empiricism and advocating the scientific method. He believed in *inductive* reasoning in which knowledge is obtained from [systematic observation](#) and experimentation. In contrast, Descartes was a French philosopher, mathematician, and scientist who championed rationalism. Unlike Bacon's inductive approach, Descartes emphasized *deductive* reasoning, starting with fundamental truths and logically deriving conclusions. His famous statement, Cogito, ergo sum ("I think, therefore I am"), highlighted his belief in reason as the foundation of knowledge.

While Descartes and Bacon never met, they both knew the English thinker [Kenelm Digby](#). Kenelm Digby, an English natural philosopher, and occasional privateer, was born on July 11, 1603. Digby was an educated, dashing and highly catholic courtier of the royal court (which was Anglican). Because of his Catholicism, Digby spent years in voluntary exile on the continent, especially in Paris, where he spent time with the French polymath [Marin Mersenne](#), [Thomas Hobbes](#), and [René Descartes](#). Hobbes was an atomist. Eventually, Digby would publish on [atomism](#) (the idea that the universe is made up of objects composed from atoms – small indivisible parts).



Portrait of Kenelm Digby by Anthony van Dyck, 1630s

Kenelm Digby: The Original Aging Venture Capitalist?

Like Bacon, Descartes also toyed with the idea that science and knowledge might find the path to immortality. David Boyd Hancock wrote the following in 2006: “When Digby fled England during the Civil War he met René Descartes in Paris. Digby told the French philosopher that since “life was almost too short to attain to the right knowledge” of things he, Descartes, who so well understood the working of the human “machine,” ought to be searching out means of prolonging its conservation. Descartes replied that he had considered exactly this matter of “la vie eternelle,” informing Digby “that to render a man immortal, was what he would not venture to promise, but that he was very sure it was possible to lengthen out his [i.e. man’s] life to the period of the Patriarchs.”

Hancock further notes that in 1638, Descartes told Huygens of his hope that he might yet live “more than a century” longer. Hancock writes: “In fact, Descartes died in Stokholm in 1650. On hearing the news, [Samuel Hartlib](#) — who had his own ideas of how human life could be prolonged — recorded in his diary that ‘Cartes designe was to make a compleate Philosophy. In reference to this scope imagining that it was possible in nature to prolong one’s life to a thousand years.’”

He continues: “It is notable that Descarte’s early death did not chasten Digby’s quest, and Hartlib records that in 1654 Digby was considering investing £700 in a plan by Hartlib’s son-in-law, ... to establish a ‘universal laboratory’ that would “redound ... to the health and wealth

of all mankind.’ In 1660, the year the Royal Society was founded, Hartlib wrote that Digby ‘hath been up and down in Germany for the liquor alkahest the great elixir &c.’”

While Digby never succeeded in this endeavor, he did resort to carrying out his own “chymistry” experiments on aging research. “Following the lead of Paracelsus and Jacob Duchesne, Digby attempted the revivification of animals, and claimed success in re-engendering live crayfish from their calcinated ashes.

Having failed to secure physical immortality, Digby spent quite a bit of time considering the immortality of the soul in his prolific writings. He is best known for his 1645 text *Two Treatises. In the one of which, the Nature of Bodies, in the Other the Nature of Man's Soule is Looked Into*. He made a strong argument for immortality of the soul.*

In addition, this book was a landmark in medicine. Digby’s treatise contained the first fully developed expression of atomism or corpuscular theory; the first important defenses of Harvey on the circulation in English; a modern presentation of the nervous system; and groundbreaking work in embryology. It also contains the first recorded patch-test for allergy.

* Kenelm Digby, *Two Treatises. In the one of which, the Nature of Bodies, in the Other the Nature of Man's Soule is Looked Into*, John Williams: London, 1645. From the Medical Library of Tim C. Opler.

1627: François Ranchin

François Ranchin was an early advocate of elderly care and the associated lengthening of life.

[François Ranchin](#) (1564–1641) was a physician and Chancellor of the School of Medicine at the University of Montpellier.

He was Mayor of Montpellier during the plague that decimated the city in 1629-1630 and had ample opportunity to contemplate the fragility of man's life.

He was a strong advocate for proper care of the elderly. He wrote in his essay *Gerocomia*, reprinted in his 1627 book *Opuscula Medica* that:

“Not only physicians, but everybody else attending old people, being accustomed to their constant complaints, and knowing their ill-tempered and difficult manners, realize how noble and important, how serious and difficult, how useful and even indispensable is that part of practical Medicine, called Gerocomica, which deals with the conservation of old people and the healing of their diseases.”

He was a strong advocate for diet in old age, writing “that in old age, diet is not only useful but necessary as well, both for the conservation of health and for longevity.” In general, he argued for the role of medicine in preserving life in old age arguing “old age impaired by infirmities and misery is not identical with ...vigorous...old age. There are endless remedies.”

References: Grmek p. 157; Krivatsky 9376; Schafer p. 45 and Joseph T. Freeman, “François Ranchin Contributor of an Early Chapter in Geriatrics,” *Journal of the History of Medicine and Allied Sciences*, Vol. 5, No. 4 (Autumn 1950), pp. 422-431 (11 pages)



François Ranchin, *Opuscula Medica*, P. Ravau: Lyon, 1627. First edition. From the Medical Library of Tim C. Opler.

1635: John Taylor on Tom Parr

Tom Parr, an Englishman, was reputed to have lived to the age of 156. This age estimate was later discredited.

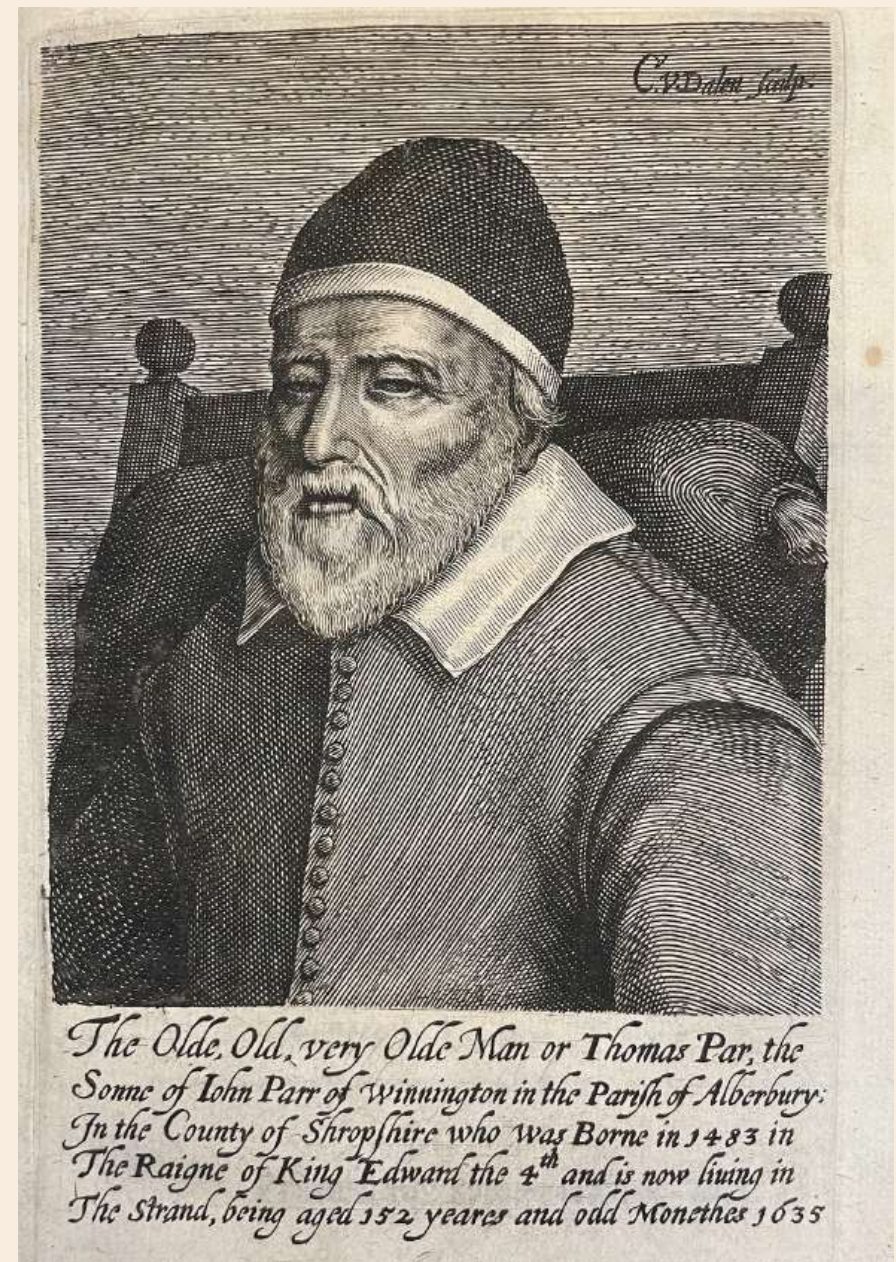
An important subgenre of the aging literature involves anecdotes of persons who lived beyond normal lifespans.

A classic of this type is John Taylor's pamphlet printed in 1635 entitled *The Old, Old, Very Old Man or the Age and Long Life of Thomas Parr*. This short pamphlet was written entirely in verse and focused on his sexual vitality even in old age.

According to the pamphlet and his headstone in Westminster Cathedral, Tom Parr was the son of John Parr of Winnington near Shrewsbury in the county of Shropshire (abbreviated to Sallop. on the stone). Thomas married his first wife Jane Taylor when he was about 80 years old and they had a son and daughter, both of whom died in infancy. At the age of 100 he did penance by standing draped in a white sheet in the parish church for being unfaithful to his wife and having an illegitimate child by Katherine Milton. Ten years after Jane's death he married Jane Lloyd but they had no children.

His recipe for long life was reputed to be: "Keep your head cool by temperance and your feet warm by exercise. Rise early, go soon to bed, and if you want to grow fat [prosperous] keep your eyes open and your mouth shut." A later 1879 analysis by William Thoms was not able to authenticate Parr's birth records from parish records.

References: Haycock p. 12.



Woodcut of Thomas Parr from John Taylor's 1635 pamphlet, *The Old, Old, Very Old Man*, London: Henry Gosson

1648: Johan Baptist van Helmont

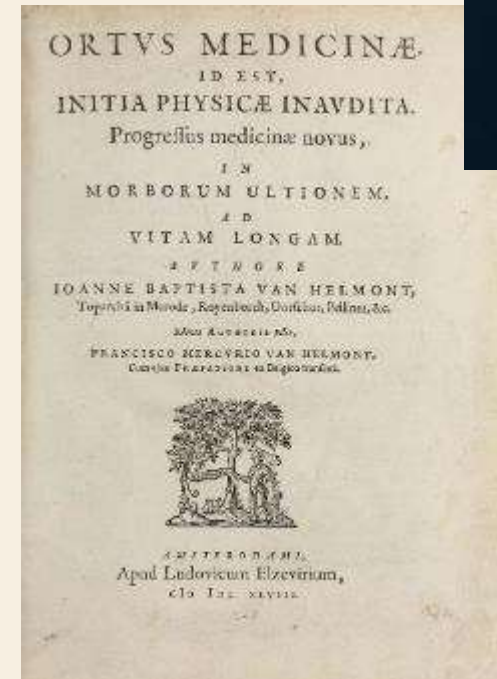
Believed in the alchemic “elixir of life” but described sources of longevity with a scientific eye. Van Helmont was one of the first experimentalists and a mystic polymath.

[Johan Baptista van Helmont](#), a Flemish physician, was born on Jan. 12, 1579 and trained at Leuven. Medicine since the time of Galen had relied almost exclusively on plant preparations as remedies, until Paracelsus in the 16th century had rebelled against what he called the Galenic tyranny and argued that disease has a chemical origin. He conducted experimental research on chemistry and physiology. He demonstrated that acid is the agent in animal digestion; and he also identified the causes of asthma. A contemporary of Bacon, he was an important influence on Boyle.

Van Helmont advocated for a holistic view of health, emphasizing the balance of bodily systems. He believed in the alchemist’s Elixir of Life but denied that it came from the Philosopher’s Stone. He argued that the Elixir could preserve life and spare the faculties from the ravages of age. He argued that the Elixir could be made from sulphurous acid and/or the essence of Lebanese cedar. He claims to have kept a 58-year-old man alive and in good health for 41 years with this Elixir.

He wrote “The life of man is a formal light.” He believed that there is the sensitive soul which is the husk or shell of the immortal mind.

References: Bailey 77 (1664 ed)hu; Freeman p. 70; GMN 665; Haycock p. 58; Krivatsy 5447; Iowa 254; Norman 1048; Osler 2929, PMM 135; Schafer p. 43; Stambler p. 230; Waller 4307; Wellcome III, 241. As for his writings on longevity see Redgrove and Redgove, *Joannes Baptista von Helmont*, William Rider: London, 1922.



Johan Baptis van Helmont, *Ortus Medicinæ [bound with] Opuscula Medica Inaudita*, Amsterdam: L. Elzevir, 1648, First ed. From the Medical Library of Tim C. Opler.

1669: Everard Maynwaring

Wrote about longevity in the context of health and history. Viewed man's estimated lifespan of 70 years as a post-flood phenomenon in a biblical sense. He advocated a modest diet to extend life.

[Everard Maynwaring](#) (c.1629–1713) was a prolific author and physician in London. He attempted throughout his life to persuade both medical and lay audiences of the superiority of experimental medicine as a mode of learned practice.

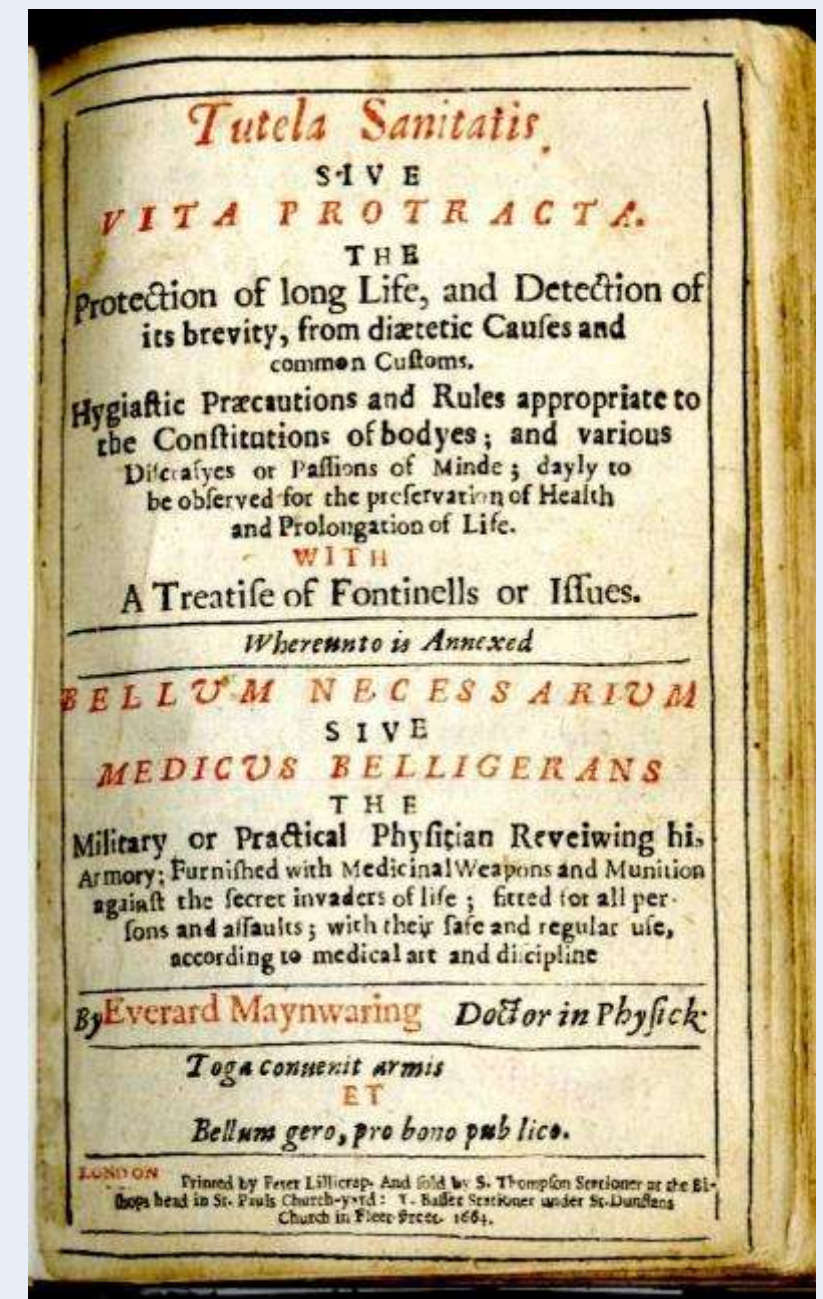
Human longevity was an important topic for him and his patients. Maynwaring explained in 1664 at the beginning of his book on *Tutela Sanitatis: Sive, Vita Protracta* (Health Care: Or, Life Extended).

“In the Primitive Age of the World, mans life was accounted to be about 1000 Years: but after the Flood, the Life of Man was abbreviated half.”

Over time it had continued to fall, so that by the time of Moses:

“the Age of Man was yet shorter, commonly not exceeding 120 Years; which also was his Age when he died ... Now the Age of Man is reduced to half that: 60 or 70 years we count upon. But although in general we find this gradual declension and abbreviation of man Life, in the several Ages of the World; yet must understand it was not equally so in all parts of the World together; but places and climates, and manner of living of a people, cause much difference in the protraction of their lives, that at the same time, some people of peculiar places, were longer lived, by a third or fourth part, then [sic] others of another Climate or Region ...”

References: Bailey 108; Haycock p. 111; NYAM p. 122, (1670 ed); Shock 17424



Everard Maynwaring, *Tutela Sanitatis: Sive, Vita Protracta*. London: Peter Lilliecrap, 1664, First ed. From the Medical Library of Tim C. Opler.

1672: Boyle's Tracts on Flame and Air

**Saw human life as similar to that of a flame. It requires air and involves a “vital force.”
Like Aristotle 2000 years before, Boyle anticipated modern theories of longevity.**

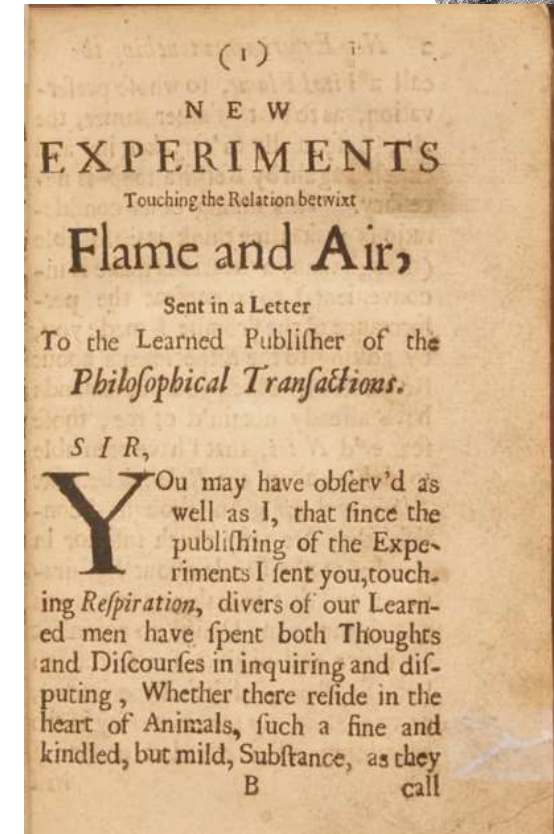
Robert Boyle's *Tractatus de Respiratione* (Treatise on Respiration) is a significant work in the study of respiration and its connection to vital processes in living organisms. In this treatise, [Robert Boyle](#), a famous chemist and “natural philosopher” best known for Boyle's law, investigates the role of air in sustaining life, drawing upon experimental observations to challenge classical theories of respiration.

He refutes Aristotelian and Galenic notions that respiration is merely for cooling the heart, instead suggesting that air contains a vital component necessary for life—a precursor to later discoveries about oxygen. Boyle considers respiration essential for maintaining bodily vigor and function. He speculates that something in the air interacts with the blood to sustain bodily heat and movement, an idea aligned with early mechanical and corpuscular theories of physiology. Boyle evidently saw aging as linked to the gradual depletion or inefficiency of vital processes. He speculates that the progressive failure of respiration and circulation diminishes the body's ability to sustain itself, leading to degeneration. Remarkably, his recognition of the declining efficacy of physiological functions aligns with later theories of aging because of cellular deterioration. Boyle's *Tractatus* is one of the most important precursors of today's theories of aging although his work is not cited in subsequent reviews of the topic.

Boyle was also involved in early blood transfusions aimed to improve health and extend life, very much in the spirit of later efforts at parabiosis.

Boyle's contemporary, [John Mayow](#), also made important contributions to the theory of respiration in his *Tractatus Quinque* (1674). He appreciated that air was needed for life.

References: Haycock p. 56.



Tracts Written By the Honourable Robert Boyle, containing New Experiments, touching the Relation betwixt Flame and Air..., First ed. London: Richard Davis, 1672. From the Medical Library of Tim C. Opler. Portrait is from the Frontispiece to *The Works of the Honourable Robert Boyle* (1744).

1693: Edmond Halley

Best known as an astronomer with a comet named after him, Halley published the first life table that allowed one to estimate conditional life expectancy.

In 1693, the English astronomer and mathematician [Edmond Halley](#) published a groundbreaking paper, "An Estimate of the Degrees of the Mortality of Mankind, drawn from Curious Tables of the Births and Funerals at the City of Breslau", in the *Philosophical Transactions of the Royal Society*.

This work marked one of the earliest applications of statistical analysis to demography and life insurance and along with [John Graunt's](#) 1662 publication *Natural and Political Observations Mentioned in a Following Index, and Made Upon the Bills of Mortality* (identifying the causes of death in London over decades) form the key first epidemiologic works that inform longevity research and associated fields of demography and actuarial science for life insurance underwriting.

Using mortality data from Breslau (now Wrocław, Poland), Halley constructed the first accurate life table, estimating the probability of survival at different ages.

A [life table](#) specifically allows one to take someone of a specific age (say 30 years) and estimate how long that person would be expected to live.

Unlike earlier attempts, which relied on unreliable population estimates, Halley's approach was data-driven, demonstrating that mortality rates increased predictably with age. His table provided a model for calculating life expectancy, which laid the foundation for modern actuarial science.

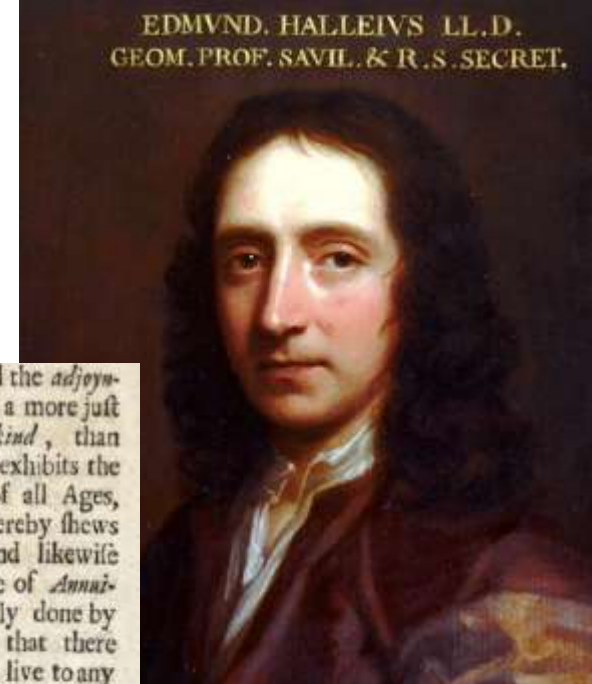
References: Haycock p. 76.

The first life table

From these Considerations I have formed the *adjoined Table*, whose Uses are manifold, and give a more just Idea of the State and Condition of Mankind, than any thing yet extant that I know of. It exhibits the Number of People in the City of Breslaw of all Ages, from the Birth to extream Old Age, and thereby shews the Chances of Mortality at all Ages, and likewise how to make a certain Estimate of the value of *Annuities for Lives*, which hitherto has been only done by an imaginary Valuation: Also the Chances that there are that a Person of any Age proposed does live to any other Age given; with many more, as I shall hereafter shew. This Table does shew the number of Persons that are living in the Age current annexed thereto, as follows:

Age Curr.	Per- sons	Age Curr.	Per- sons	Age Curr.	Per- sons	Age Curr.	Per- sons	Age Curr.	Per- sons	Age Curr.	Per- sons	Age Curr.	Per- sons
1	1000	8	580	15	528	22	485	29	539	35	481	7	5547
2	855	9	490	16	522	23	479	30	531	37	473	14	4584
3	768	10	501	17	518	24	473	31	523	38	467	21	4270
4	750	11	543	18	510	25	467	32	515	39	454	28	3954
5	732	12	545	19	504	26	460	33	507	40	445	35	3604
6	710	13	540	20	508	27	553	34	499	41	436	42	3178
7	692	14	524	21	502	28	540	35	490	42	427	49	2709
												50	2194
												63	1594
												70	1204
43	417	50	348	57	272	64	202	71	141	78	98	77	572
44	407	51	335	58	262	65	192	72	130	79	84	84	252
45	397	52	324	59	253	66	182	73	119	80	71	100	107
46	387	53	313	60	242	67	172	74	108	81	58		
47	377	54	302	61	232	68	162	75	98	82	48		
48	367	55	292	62	223	69	152	76	88	83	38		
49	357	56	282	63	212	70	142	77	78	84	28		
												Sum Total,	

Edmond Halley, "An Estimate of the Degrees of the Mortality of Mankind, drawn from Curious Tables of the Births and Funerals at the City of Breslau", in the *Philosophical Transactions of the Royal Society*, 1693. Halley's portrait from the Royal Society.



1696 and 1703: Giorgio Baglivi

Departing from Galenism, Baglivi argued that aging resulted from breakdown of the body's solid structures (loss of elasticity and resilience). He did not explain exactly how this happens.

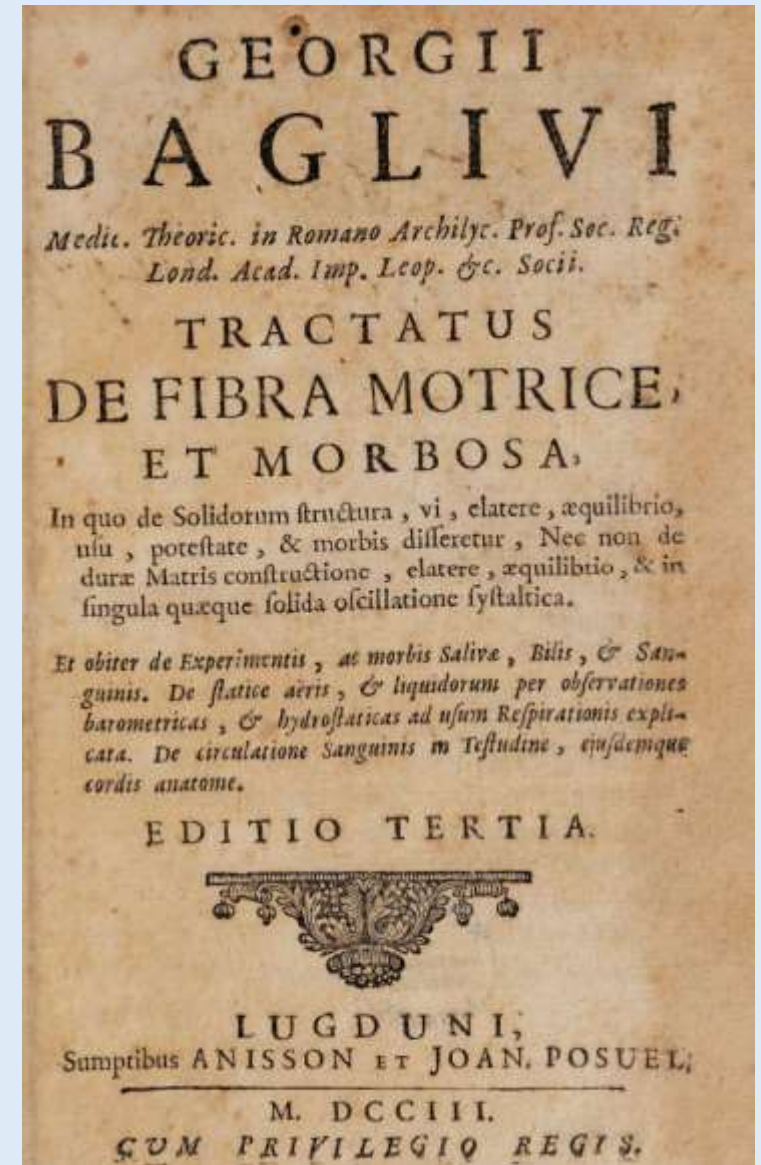
[Giorgio Baglivi](#) (1668–1707), an influential physician and anatomist, viewed human aging as primarily a mechanical and structural process rather than a purely humoral or mystical phenomenon, as many of his predecessors believed. His perspective was rooted in the iatrophysical school of thought, which sought to explain physiological processes using mechanical principles rather than relying on traditional Galenic humoral theories. This was expressed in his most well-known 1696 work entitled *De Praxi Medica*.

In his 1703 work *Tractatus de Fibra Motrice et Morbosa* (Treatise on Motor and Morbid Fibers), Baglivi expanded upon these ideas, arguing that aging resulted from the gradual loss of elasticity and resilience in the body's solid structures, particularly the fibrous and muscular tissues. He emphasized the importance of the body's "solid parts" (such as muscles, nerves, and connective tissues) over the "fluid parts" (blood and humors) in maintaining health and vitality.

In his view, aging occurred as these solid structures became progressively stiff, less responsive, and incapable of efficient function, leading to the decline of bodily processes and, ultimately, death. This mechanical degradation theory was an early precursor to later ideas about tissue degeneration and cellular aging.

Baglivi's work was another step in the transition from the humoral to the mechanistic/anatomical understanding of physiology.

References: Grmek p. 68; Iowa 737; Schafer p. 117.



Giorgio Baglivi, *Tractatus de Fibra Motrice et Morbosa*, Leiden, Anisson and Joan Posuel, 1703. From the Medical Library of Tim C. Opler.

1715: Harcouet de Longeville

Follows the Roger Bacon tradition of citing very long-lived individuals and argues that secret remedies and elixirs can be used by the reader.

Harcouet de Longeville's 1715 book, *Histoire des personnes qui ont vécu plusieurs siècles et qui ont rajeuni avec le secret du rajeunissement tiré d'Arnauld de Villeneuve* (The story of people who lived for centuries and grew younger with the secret of rejuvenation from Arnauld de Villeneuve) explores historical accounts of individuals who allegedly lived for several centuries and experienced rejuvenation.

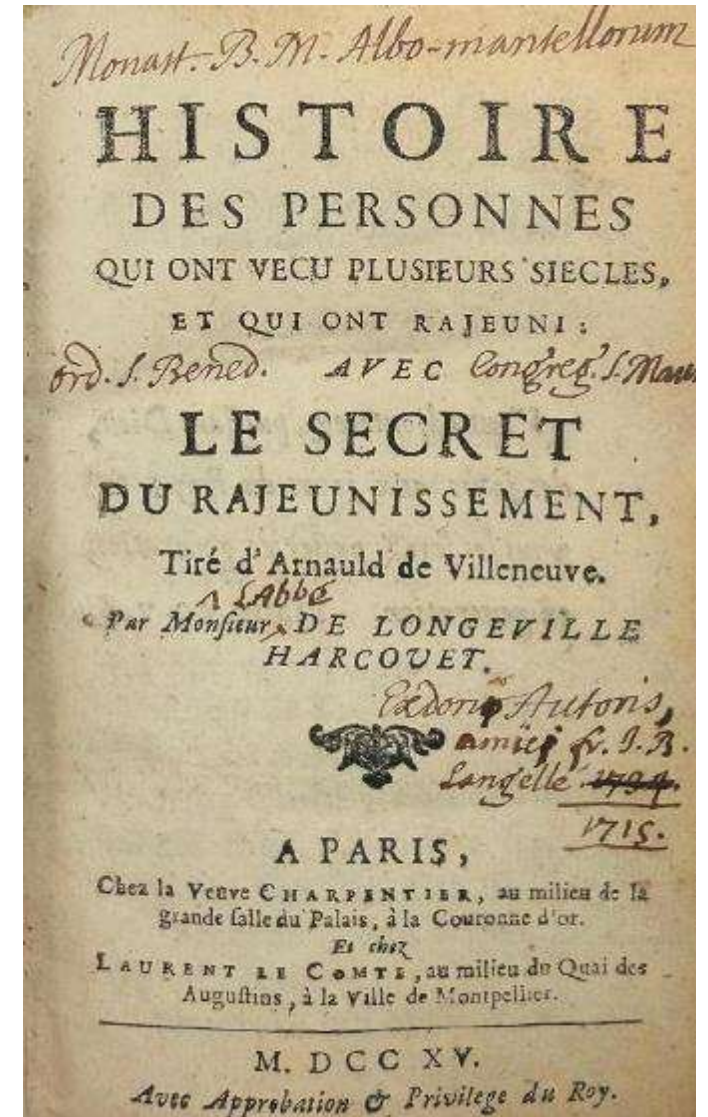
Drawing from the writings of the medieval physician and alchemist Arnau de Vilanova (Arnauld de Villeneuve), the book discusses supposed secrets of longevity and renewal, attributed to alchemical and medical knowledge.

The text blends history, legend, and speculative medicine, reflecting the early 18th-century fascination with life extension and rejuvenation. It includes narratives of long-lived figures from history and mythology, as well as purported methods for reversing aging.

The influence of alchemical traditions is strong, with an emphasis on elixirs, diets, and secret remedies. Specifically, De Longeville outlines alchemical and medical theories of rejuvenation, including elixirs of life and transformations of the body. While modern science dismisses these ideas as mythical, such works were part of a broader cultural discourse on immortality, medicine, and the limits of human lifespan.

This text is an example of early modern longevity literature, combining medical theories, mystical elements, and anecdotal reports to explore the dream of extended life and youth.

References: Bailey 104; Freeman p. 69; Schafer p. 157.



Histoire des personnes qui ont vécu plusieurs siècles et qui ont rajeuni avec le secret du rajeunissement tiré d'Arnauld de Villeneuve, First ed. Paris: Chez la Veuve Charpentier, 1715. From the Medical Library of Tim C. Opler.

1724: Herman Boerhaave

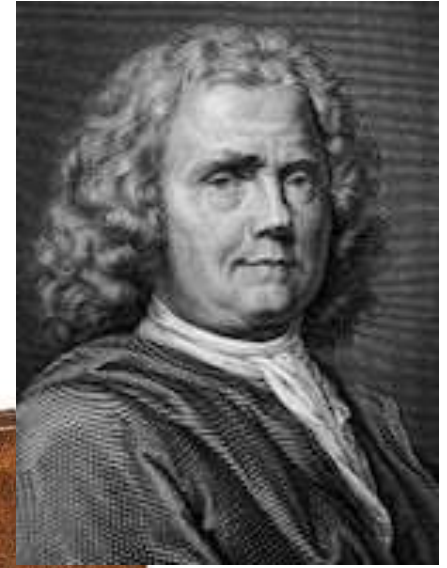
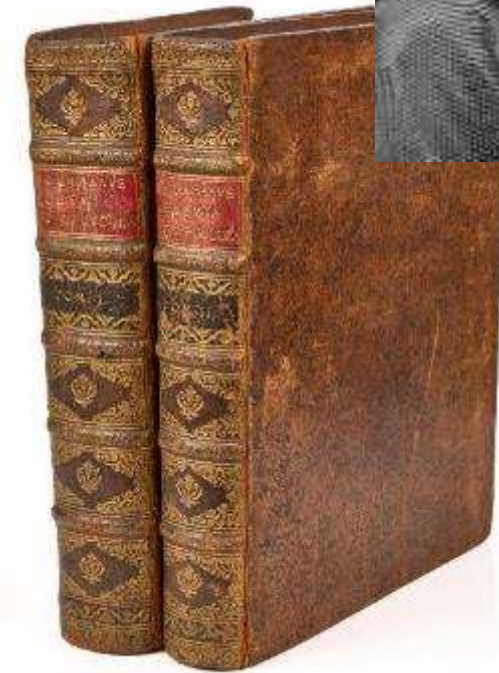
Herman Boerhaave integrated chemical medicine, empirical observation, and rejuvenation theories to understand aging. His teachings on longevity, organ regeneration, and medical elixirs shaped early modern approaches to life extension.

[Herman Boerhaave](#) was notorious for his interest in human life extension, drawing inspiration from historical and biblical precedents. One of his most remarkable interventions involved Amsterdam's burgomaster, whom he sought to rejuvenate using the biblical practice of Shunamitism. Echoing today's interest in parabiosis, this technique involved the elderly sleeping between two young women, supposedly to absorb their vitality.

In his influential 1724 work *Elementa Chemiae* (Elements of Chemistry), Herman Boerhaave detailed five distinct methods for preparing Paracelsus' *Elixir Proprietatis*. Boerhaave not only described these methods with scientific precision but also highly endorsed the elixir's efficacy, reflecting his interest in chemical medicine and its potential benefits for health and longevity.

Boerhaave's fascination with longevity deeply influenced his students, including Albrecht von Haller and Gerard van Swieten. Van Swieten, an Austrian physician (1700–1772), became one of Boerhaave's most prominent disciples and later edited his *Aphorismi*. Aging and longevity were central themes in van Swieten's extensive five-volume *Aphorismi*. In the eighth volume of *Elementa Physiologiae Corporis Humani* (1757–1766), Albrecht von Haller devoted a substantial section, titled "Decrementum," to the study of aging and longevity (published separately in 1778). This section explored the age-related decline of various organs and bodily fluids, the comparative lifespans of plants, animals, and humans, and the potential for rejuvenation, which Haller equated with organ regeneration. He also examined factors influencing longevity, such as climate and diet, and included a list of supercentenarians.

Reference: Freeman p. 63; Haycock p. 88; Schafer p. 116; Stambler p. 230.



Herman Boerhaave, 1732 (second ed), *Elementa Chemiae (quae anniversario labore docuit, in publicis, privatisque, scholis, Hermannus Boerhaave. Tome Primus. Qui continet historiam et Artis Theoriam (Tomus Secundus. Qui continet Operationes Chemicas). Cum tabulis aeneis*, Leyden: Apud Isaacum Severinum. Signed by Boerhaave. From the Medical Library of Tim C. Opler.

1724: John Floyer

Floyer discussed diet therapy in relation to the diseases of the aged, the use of hot and cold baths, and the importance of recording the pulse rate.

This is the earliest work written in English solely on geriatrics. Floyer recommends the use of hot or cold baths depending on the size and constitution of the patient. He also covers diet therapy for the elderly and stresses the importance of accurately recording the pulse rate.

[John Floyer](#) was a follower of Francis Bacon. Jean-Martin Charcot, the great physician of the 19th century, later considered this book to be the first truly modern work on geriatrics.

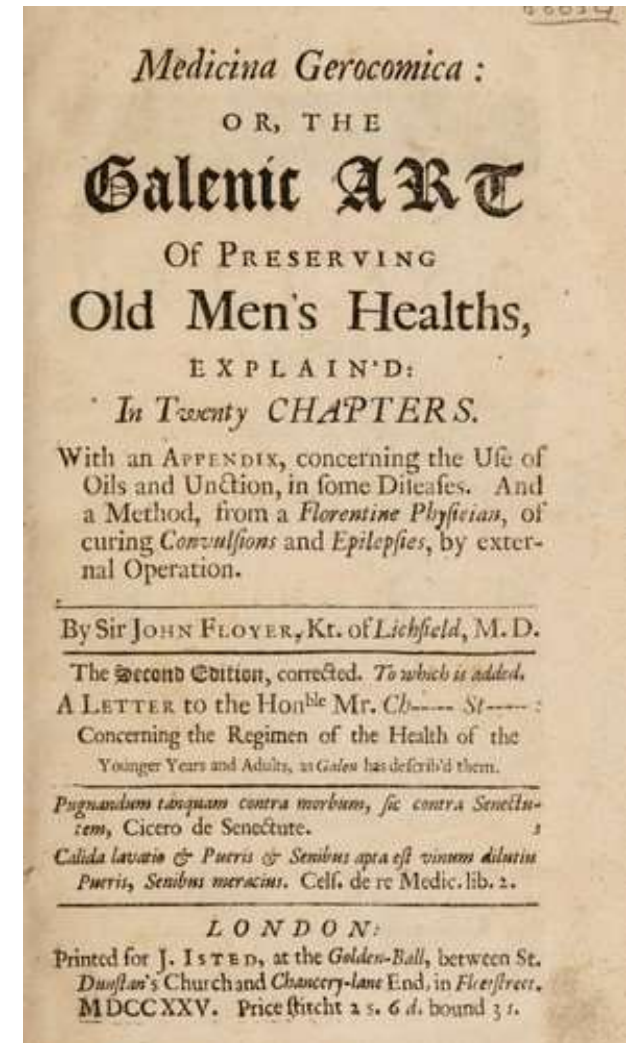
Floyer 'begins his preface by saying that "every man is a fool or becomes a physician, when he arrives at 40 or 50 years of age."

Much of the book amounts to advocacy of a commonsense approach for preservation of the aging body, with attention to fresh air, exercise, regular diet, and temperance in all things.

Throughout his life Sir John had stressed the importance of physical exercise in promoting health.

In *Medicina Gerocomica*, after giving a long list of activities that he thought too strenuous for old men, he mentions that "these are gentle exercises, sailing, pruning trees, riding, bowling, billiards, nine-pins, fishing, walking." The old men that were able to follow his advice must have enjoyed their declining years.' – D. D. Gibbs, *British Medical Journal*, 1969.

References: Bailey 56 (later ed); Freeman p. 40; GMN 1595; Grmek p. 121; Iowa 671, Morley p. 1138; Norman 808; NYAM p. 122; Osler 2616; Schafer p. 142; Shock 17368 (later ed); Waller 3091 (1725 ed); Wellcome III, p.35.



John Floyer, Medicina Gerocomica: Or, The Galenic Art of Preserving Old Men's Healths, Explain'd in Twenty Chapters With an Appendix, concerning the Use of Oils and Unction, in some Diseases. And a Method, from a Florentine Physician, of Curing Convulsions and Epilepsies, by external Operation, Second ed. London: J. Isted, 1724. To which is added, A Letter to the Honble Mr. Ch--- St--- Concerning the Regimen of the Health of the Younger Years and Adult, as Galen has describ'd them. From the Medical Library of Tim C. Opler.

1724: George Cheyne

One of the most widely published and read writers of his time. Unusually, he counselled plant-based diet and avoidance of alcohol. Published one year after Floyer's work with a self-help orientation rather than a medical one.

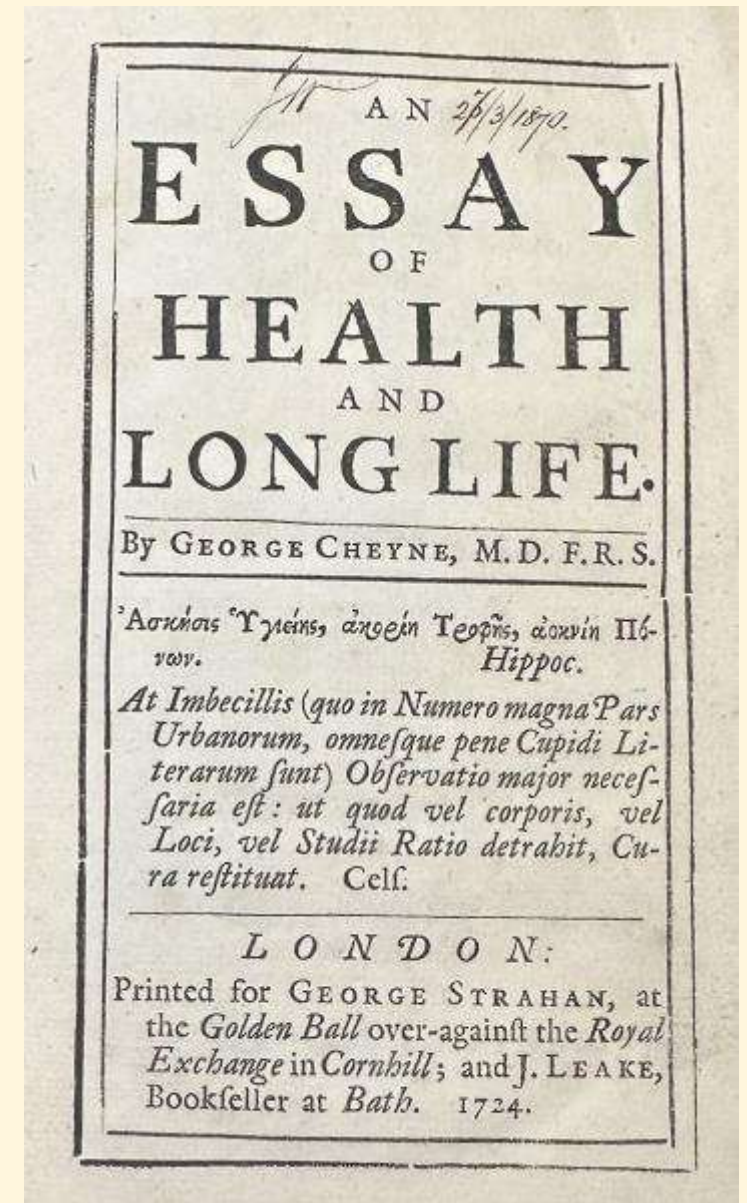
[George Cheyne's](#) *Essay of Health and Long Life* promoted a regimen of moderation and simplicity to maintain health and extend life. Cheyne, a Scottish physician and advocate of lifestyle-based medicine, integrates medical theory, personal experience, and philosophical reflections on health into this book. His central argument is that many chronic diseases, particularly those affecting the wealthy and sedentary classes, arise from overindulgence in rich foods, alcohol, and lack of physical activity. He proposes a regimen of temperance, light nourishment, and exercise as the key to longevity and well-being.

A notable aspect of the book is Cheyne's advocacy for a largely plant-based diet, favoring milk, vegetables, and simple foods over heavy meats and excessive alcohol. He bases his dietary recommendations on both humoral theory and emerging mechanistic perspectives of the body.*

Cheyne also discusses the importance of mental health and emotional stability, recognizing the connection between psychological well-being and physical health. His own struggles with obesity and health problems inform his writing, making his recommendations personal as well as medical.

References: Achenbaum p. 37; Bailey 35; Cushing C21; Freeman p. 43; Grmek p. 120; Haycock p. 92; Iowa 761; NYAM p. 122; Osler 2303 (2nd ed); Schafer p. 156; Shock 17324; Wellcome II p. 338. Also see T.H. Howell, "George Cheyne's Essay of Health and Long Life," *Gerontologist*, 9:226-228, 1969.

* Cheyne's ideas on vegetarianism were highly influential at the time impacting authors such as Percy Bysshe Shelley. An earlier advocate was Thomas Tryon, *The Way to Health, Long Life and Happiness*, 1663.



George Cheyne, *Essay of Health and Long Life*, First ed. London: George Stahan and J. Leake, 1724. From the Medical Library of Tim C. Opler.

1742: Johannes Cohausen

**Satirical book that suggested being around young people will extend life.
Anticipated today's interest in achieving longer life through parabiosis.**

[Johann Heinrich Cohausen](#) (1665-1750), born in Hildesheim, studied medicine in Frankfurt a.d. Oder, then went to Münster and was personal physician to the Bishop of Münster from 1717. He wrote three very different books on aging, one of which was *Hermippus*.

This book "Hermippus" arose from a bet with his friend Pylades, who reported to him that "an inscription on a grave had been discovered during excavations in Rome, which said that the deceased, named Hermippus, had reached the ripe old age of 114 years by breathing in young girls (*puellarum anhelitu*).

The result of the bet was the appearance of the book "Hermippus redivivus". In this work, Cohausen deals with the lifestyle of Hermippus, whom he imagines to be a teacher at a high school for girls, using the method known as 'Gerocomique' with mischievous humor. This is understood to mean the rejuvenation and preservation of the human body by bringing it into the atmosphere of youthful people. Interestingly, this book anticipates today's biotech interest in exchanging the blood of younger persons into those who are old.

In a separate 1714 book entitled *Tentaninum physico-medicorum curiosa decas* (The curiosities of the physical and medical doctors) Cohausen analyzed the different means of prolonging life, including animal, mineral, and vegetable preparations; his central tenet was that the tree of life was placed in the terrestrial paradise by God, and that in our postlapsarian state, it is possible to recover it via chemical medicine and the arcanum or life elixir.

References: Bailey 38; Grmek p. 121 Gruman p. 65; Haycock p. 98; NYAM p. 125; Osler 2337; Schafer p. 138; Shock 17329; Stambler p. 230; Waller 2038 (English ed.)



Frontispiece to Cohausen's *Hermippus*. A youthful-looking Hermippus is surrounded by children learning their lessons. *Hermippus redivivus, sive exercitatio physico-medica curiosa de methodo rara ad CXV. annos pro rogandae senectutis per anhelitum puellarum, ex veteri monumento romano deprompta, nunc artis medicae fundamentis stabilita, et rationibus atque exemplis, nec non singulari chymiae philosophicae paradoxo*, First ed. Frankfurt a.M., Andreae & Hort, 1742. From the Medical Library of Tim C. Opler.

1761: Giovanni Battista Morgagni

Unlike predecessors, Morgagni argued that aging was not itself a disease but rather a stage of life in which people are more likely to get disease.

Giovanni Battista Morgagni's book, *De Sedibus et Causis Morborum per Anatomen Indagatis* (On the Seats and Causes of Diseases Investigated by Anatomy), published in 1761, revolutionized the field of pathology and laid the foundation for modern medical research, including the study of aging.

[Morgagni](#) was sufficiently astute to point out in his study of anatomy and diseases that many diseases in old age, but especially chronic ones, can remain symptomless for many years. Two hundred years later this concept formed the basis of much clinical research on the unreported medical needs of older people.

In this monumental text, Morgagni systematically correlated clinical symptoms with postmortem anatomical findings, establishing the principle that diseases originate in specific organs or tissues. By meticulously documenting over 700 case studies, he demonstrated that structural changes in organs were the underlying causes of disease. This approach shifted medical thinking from a humoral theory of disease to one rooted in organ-based pathology.

Morgagni's work provided a framework for understanding how age-related changes in organs, such as the heart, brain, and kidneys, contribute to the development of chronic diseases and the overall decline in physiological function associated with aging. His emphasis on anatomical investigation paved the way for later research into the biological mechanisms of aging and the concept of organ-specific aging processes.

References: Freeman p. 72; GMN 2776; Schafer p. 123.



Giovanni Battista Morgagni, *De Sedibus et Causis Morborum per Anatomen Indagatis*, second ed. Naples, Typographia Simoniana, 1762. Four volumes, From the Medical Library of Tim C. Opler.

1768: Lazzaro Spallanzani

Spallanzani described regenerative capacities of remarkable complexity in animals such as the salamander and the frog, establishing the general law that an inverse ratio obtains between the regenerative capacity and age of the individual. This work anticipated the later literature on cell senescence.

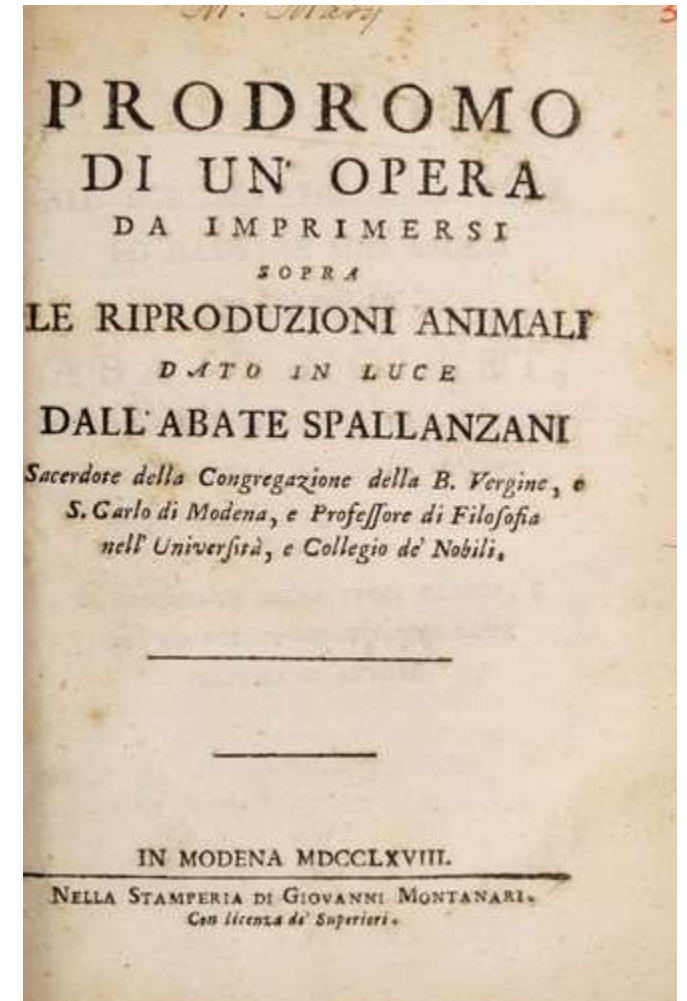
Prodromo di un'opera da imprimersi sopra le riproduzioni animali (Preliminary Work on Animal Reproduction) remains a seminal work by the Italian biologist and physiologist [Lazzaro Spallanzani](#), published in 1768. Spallanzani's meticulous observations and experiments laid the groundwork for modern embryology and reproductive biology.

Spallanzani explores the mechanisms of animal reproduction, focusing on the processes of regeneration and fertilization. He conducted extensive experiments on various organisms, including amphibians and invertebrates, to investigate how they regenerate lost body parts and reproduce. The book made the striking discovery that older animals have less ability to regenerate lost body parts, anticipating the modern interest in cell regeneration and aging.

Spallanzani's *Prodromo* is notable for its scientific rigor and innovative experimental methods. He used controlled experiments to test hypotheses about reproduction, such as the necessity of sperm for fertilization, which he demonstrated by filtering sperm from frog semen and observing the effects on fertilization.

His findings provided critical evidence against the theory of spontaneous generation and supported the idea that life arises from pre-existing life. Spallanzani's research advanced the field of biology and exemplified the application of the scientific method to biological questions.

References: GMN 101; Iowa 989.5; Waller 11962.



Lazzaro Spallanzani, *Prodromo di un'opera da imprimersi sopra le riproduzioni animali*, First ed. Modena, Nella Stamperia di Giovanni Montanari, 1768. From the Medical Library of Tim C. Opler.

1774: Comte de Buffon

Made a strong case humans have and always have had a natural lifespan of 90 to 100 years.

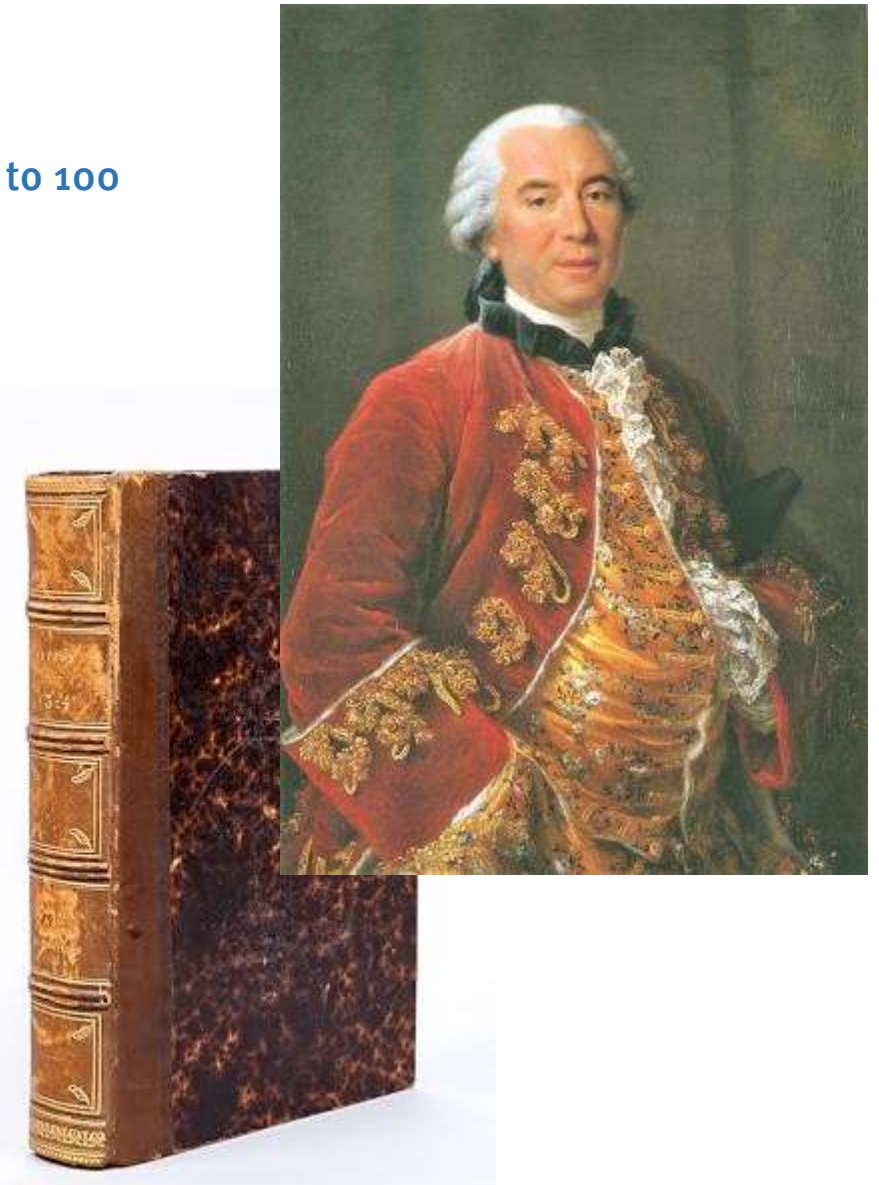
Georges-Louis Leclerc, [Comte de Buffon](#), was an influential 18th-century French naturalist, mathematician, and cosmologist, best known for his monumental work *Histoire Naturelle*. Born in 1707, Buffon was a prominent figure in the Enlightenment, blending empirical observation with philosophical inquiry to advance the study of the natural world.

Buffon's contributions laid the groundwork for later developments in evolutionary theory and comparative biology, influencing thinkers such as Jean-Baptiste Lamarck and Charles Darwin.

In the fourth volume of his *Oeuvres Complètes*, Buffon explored the concept of lifespan and aging across species, including humans. He proposed that lifespan was intrinsically linked to an organism's size, metabolism, and environmental conditions. Buffon argued that larger animals, with slower metabolic rates, tended to live longer than smaller ones, a hypothesis that foreshadowed later scientific insights into the relationship between metabolism and aging.

Buffon, is attributed with one of the first scientific formulations of the assumption that there is a specific lifespan for each species and for the human species in particular. He writes: “But if one takes the human genus in general terms, there is no difference to speak of in the duration of life; he who does not die of accidental ailments lives far and wide to ninety or one hundred years. Our ancestors lived no longer, and since the time of David, this time has varied naught.”

References: Freeman p. 64; Haycock p. 100.



Comte de Buffon, *Oeuvres Complètes, Tome Quatrième*. Entry: “Histoire naturelle de l’homme, de la vieillesse et de la mort,” Paris, E.-L.-C. Mauprivez, 1842, Third edition. From the Medical Library of Tim C. Opler. Portrait By François-Hubert Drouais - Musée Buffon à Montbard.

1786: Anthony Fothergill

Summarizes cases of long-life spans. Points to diet, drinking and lifestyle as the most important drivers of longevity.

Anthony Fothergill's *Observations on Longevity* (1786) explores the factors contributing to human longevity, drawing from case studies and historical examples.

Like many predecessors, he emphasizes the role of temperance, simple diets, and active lifestyles in prolonging life, arguing that longevity is more dependent on lifestyle choices than on hereditary factors.

[Anthony Fothergill](#) critiqued the excesses of luxury and indulgence, warning that they lead to premature aging and disease. He supports his arguments with anecdotal evidence of individuals who lived beyond the typical lifespan, often highlighting their modest living conditions, rural environments, and avoidance of intemperance.

In addition to lifestyle factors, Fothergill considers the impact of climate and occupation on longevity. He suggests that those engaged in outdoor labor, particularly in temperate climates, tend to live longer than those in sedentary professions.

His observations align with contemporary Enlightenment medical thought, which sought to rationalize health and longevity through empirical study. While some of his conclusions rely on anecdotal evidence, his work contributes to the broader discourse on preventive medicine in the late 18th century, reinforcing the idea that moderation and natural living are key to a long and healthy life.

64 ANNUAL REGISTER, 1786.

T A B L E III.
OF LONGEVITY.

Names of the Persons.	Age.	Places of Abode.	Where recorded.
Hippocrates, Physician	109	Island of Cos	Lynche on Health, Ch. 3.
Democritus, Philosopher	109	Abdera	Bacon's History, 1095.
Galen, Physician	140	Pergamus	Vall. Inst. or Lib. 3.
Albana Marc	150	Scythia	Hawkewell's ap. Lib. 1.
Dumitar Raduly	140	Haromzeck, Transylvania	Died Jan. 18, 1782, Gen. Gazetteer, April 18th.
Titus Pallonius	150	Bononia	Fulgatus, Lib. 8.
Abraham Faiba	142	Charles-town, S. Carolina	General Gazetteer.
L. Tertulia	137	Arminium	Fulgatus, Lib. 8.
Lewis Coraaro	100	Venice	Bacon's Hist. of Life, &c. p. 134.
Robert Blakeney, Esq.	114	Armagh, Ireland	General Gazetteer.
Margaret Scott	125	Dalkeith, Scotland	See Interp. on her Tomb in Dalkeith Ch. Yard.
W. Gullone	140	Ireland	Fuller's Worthies.
J. Bright	109	Ludlow	Lynche on Health.
William Poffell	120	France	Bacon's Hist. p. 134.
Jane Reeves	103	Edox	St. J. Chron. June 14, 1781.
W. Paulet, Marquis of Winchester	106	Hampshire	Baker's Chron. p. 502.
John Wilton	116	Suffolk	Gen. Gaz. Oct. 29, 1782.
Patrick Wian	115	Lebury, Northumberland	Plempius Fundamend. Sec. 4, Chap. 8.
M. Laurence	140	Oreades	Buchanan's Hist. of Scot.
Evan Williams	145	Cammarthen Work-house, still alive	Gen. Gazetteer, Oct. 12, 1782.

The antedilevians are purposely omitted, as bearing too little reference to the present race of mortals, to afford any satisfactory conclusions; and the improbable stories of some persons, who have almost rivalled them in modern times, border too much upon the marvellous, to find a place in these tables. The present examples are abundantly sufficient to prove, that longevity does not depend so much, as has been supposed, on any particular climate, situation, or occupation in life. For we see, that it often prevails in places where all these are extremely dissimilar; and it would, moreover, be very difficult, in the histories of the several persons above-mentioned, to find any circumstance common to them all, except, perhaps, that of being born of healthy parents, and of being inured to daily labour, temperance, and simplicity of diet. Among the inferior ranks of mankind, therefore,

Anthony Fothergill, *Observations on Longevity*, by Anthony Fothergill. An original article from *The Annual Register* for 1786. First Edition, London, 1786. From the Medical Library of Tim C. Opler.

1788: William Black

The most thorough and well written epidemiologic work to quantify human life span up to its time.

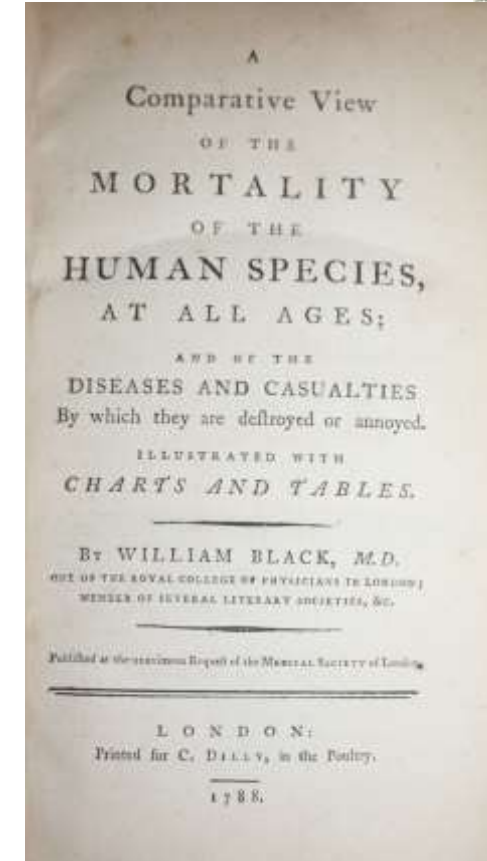
[William Black's](#) *A Comparative View of the Mortality of the Human Species* (1788) is one of the earliest systematic studies of mortality statistics, offering a detailed analysis of death rates across different ages, diseases, and external causes.

Using data from sources like the *London Bills of Mortality* and hospital records, Black categorized various causes of death and examined how they affected different demographic groups. His work stands as an early attempt to apply statistical methods to medical and demographic studies, helping to establish patterns in human mortality and disease prevalence.

A key feature of the book is its comparative approach, which examines mortality differences based on geography, social class, and occupation. Black analyzed how factors like environment and living conditions influenced life expectancy, presenting his findings through tables and charts. By classifying diseases and external causes of death, he provided insights into which conditions were most fatal at different life stages, making his study valuable for public health research.

Black's work was influential on later epidemiologists and actuaries.

His data-driven approach to understanding mortality patterns contributed to the development of public health policies and actuarial science. By emphasizing the importance of statistical analysis in studying health trends, Black's work helped to shape modern epidemiology and demographic research.



William Black, *A comparative view of the mortality of the human species at all ages*, First ed. London, P. Dilly, 1788. From the Medical Library of Tim C. Opler.

Charts from Black's Book

No one made it past 100 in London from 1702 to 1773. Less than 1% went beyond age of 70. Median age at death around 40 years. Leading causes of death were consumption (tuberculosis) and smallpox.

DISEASES AND CASUALTIES.					
	From Year, from 1701 to 1773.	From 1717 to 1732.	From 1732 to 1747.	From 1747 to 1762.	From 1762 to 1773.
Ague	80	198	82	99	109
Fever. Malignant, Spotted, Scarlet, and Purple	59,955	53,330	57,995	45,621	48,594
Small Pox	—	—	—	—	—
Measles	22,219	34,448	29,462	29,165	36,276
Quinsy, Sore Throat	1,972	2,618	2,858	3,099	3,319
Pleurisy	226	169	287	306	309
Rheumatism	384	602	811	407	321
Gout	368	447	310	175	128
	313	645	769	803	1,010
Consumption	42,541	49,680	66,009	61,749	68,949
Chin Cough, Hooping Cough, Cough	116	632	1,692	2,755	4,252
Asthma and Tiflick	5,090	7,938	9,460	5,699	6,154
Apoplexy and Suddenly	2,228	3,013	3,287	3,271	3,353
Palsy	332	550	621	1,021	1,020
Lethargy	105	126	116	105	74
Meagrimis	13	10	—	—	—
Headach	21	32	6	18	—
Lunatick	412	513	777	1,126	1,048
Spleen and Vapours	53	52	20	—	—
Rifing of the Lights	1,239	1,239	127	59	—
Stoppage of the Stomach	2,230	2,230	2,230	304	179
Vomiting and Looseness	2,230	2,230	2,230	134	130
Cholic, Gripes, and Twisting of the Guts	2,230	2,230	2,230	1,475	796
Flux	2,230	2,230	2,230	253	341
Bloody Flux	2,230	2,230	2,230	94	93
Worms	2,230	2,230	2,230	115	56
Jaundice	2,230	2,230	2,230	1,739	2,089
Gravel, Stone, and Strangury	2,230	2,230	2,230	421	429
Diabetes	2,230	2,230	2,230	10	5
	2,230	2,230	2,230	2,230	2,230

A GENERAL CHART, with different Tables, Exhibiting the Gradations of Mortality in City and Country.									
Showing the Probabilities of Life in London, from Observations on the Bills of Mortality in London for Ten Years, from 1763 to 1772. The total Number of Inhabitants, generally, about 1,000,000 in Winter. One Half near each side, Three Years of Age.					Showing the Probabilities of Life in London, from the Bills of Mortality in London for Ten Years, from 1773 to 1782. The total Number of Inhabitants, generally, about 1,000,000 in Winter. One Half near each side, Three Years of Age.				
Age	Years	Days	Hours	Minutes	Age	Years	Days	Hours	Minutes
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8	8
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37	37	37	37	37	37	37	37	37	37
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49	49	49	49	49	49	49	49	49	49
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64	64	64	64	64	64	64	64	64	64
65	65	65	65	65	65	65	65	65	65
66	66	66	66	66	66	66	66	66	66
67	67	67	67	67	67	67	67	67	67
68	68	68	68	68	68	68	68	68	68
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73	73	73	73	73	73	73	73	73	73
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99	99	99	99	99	99	99	99	99	99
100	100	100	100	100	100	100	100	100	100

1793: Benjamin Rush

America's first well-known physician opined that diet *per se* was not that important to a long life. He argued that longevity involves balancing stimuli to the body and its excitability (mental health).

[Benjamin Rush](#) (1746-1813) was an American revolutionary, a Founding Father of the United States and signatory to the U.S. Declaration of Independence and a civic leader in Philadelphia, where he was a physician, politician, social reformer, humanitarian, educator.

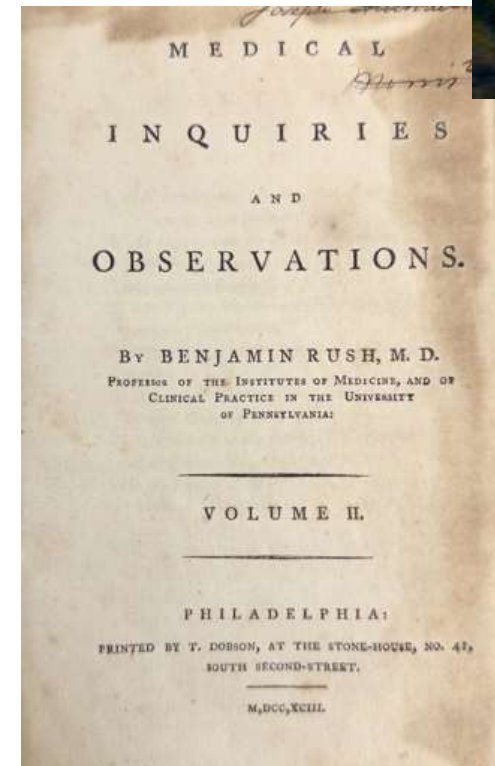
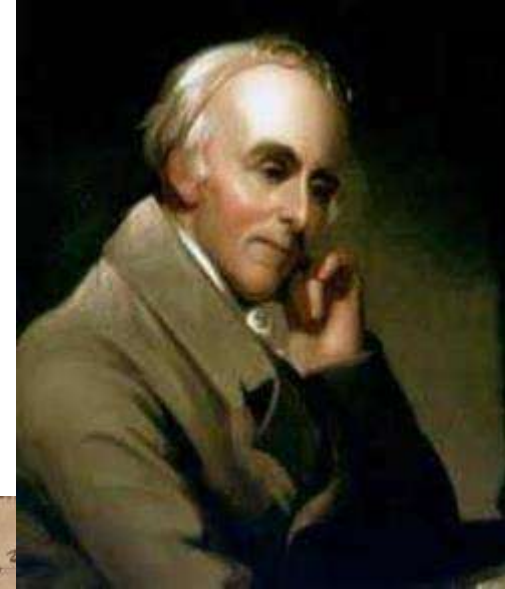
An Enlightenment intellectual, Rush was committed to organizing all medical knowledge around explanatory theories, rather than relying on empirical methods.

Rush argued that death was the result of imbalances in the body's physical system and was caused by malfunctions in the brain.

He wrote in his essay on longevity in his 1793 book *Medical Inquiries and Observations*: "I am disposed to believe, that those articles of diet do not materially affect the duration of human life... The duration of life does appear to depend so much upon the strength of the body, or upon the quantity of its excitability, as upon exact accommodation of stimuli to each of them. A watch-spring will last as long as an anchor, provided the forces, which are capable of destroying both, are in exact ratio to their strength."

Interestingly, Benjamin Rush [corresponded](#) with Thomas Jefferson and referred both to his works and that of John Sinclair, which had captured Jefferson's interest.

References: Bailey 132 (later ed.)sin; Freeman p. 43 and Schafer p. 146. Also see M. Hader and PM Schulman, "Benjamin Rush: An Early Gerontological Psychiatrist," *Gerontologist* 5:156-157, 1965.



Benjamin Rush, *Medical Inquiries and Observations*, First ed. Philadelphia: T. Dobson, P. Delly, 1793. From the Medical Library of Tim C. Opler.

1795: Nicolas de Condorcet

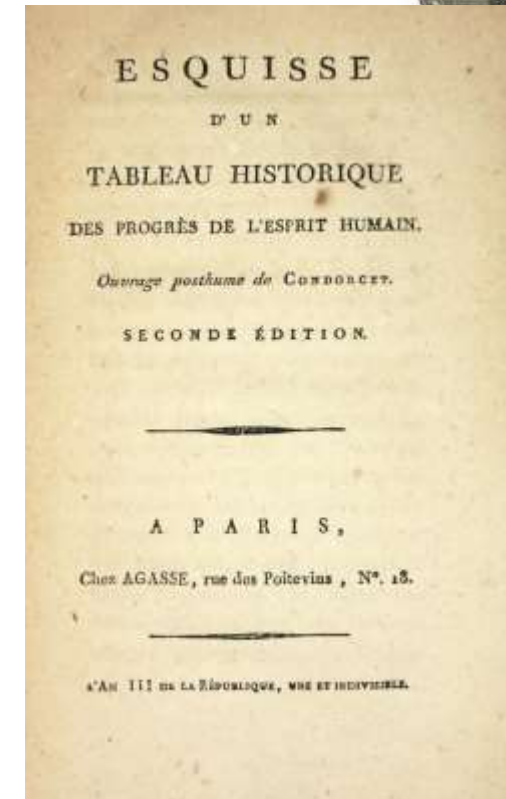
de Condorcet envisioned a future where the aging process itself could be slowed or mitigated through science and human ingenuity. The book written in the year prior to his execution, echoes Ray Kurzweil's book of 2004. Some call him the first transhumanist.

[Nicolas de Condorcet](#) was a French philosopher, political economist, politician, and mathematician. His ideas, including support for free markets, public education, constitutional government, and equal rights for women and people of all races, have been said to embody the ideals of the Age of Enlightenment.

Condorcet's 1795 book *Esquisse d'un tableau historique des progrès de l'esprit humain* (Sketch for a Historical Picture of the Progress of the Human Mind) was published posthumously. The book was written while he was in hiding following the French Revolution. The book is a theoretical treatise on perfecting the human mind, analyzing intellectual history based on social arithmetic. Condorcet articulates a vision of human progress that includes the potential for significant increases in lifespan. He believed that advances in science, medicine, and social organization would lead to the improvement of human health and the extension of life. He saw pharmaceuticals as an important way in which this might happen.

Condorcet argued that the natural lifespan of humans was not fixed and could be extended through the application of reason and scientific knowledge. He envisioned a future where diseases would be eradicated, nutrition and living conditions would improve, and the aging process itself could be slowed or mitigated. Condorcet's optimism was rooted in his faith in the power of human ingenuity and the continuous advancement of knowledge. He saw longevity as one of the many benefits that would arise from the progress of civilization, alongside greater equality, education, and well-being.

References: Achenbaum, p. 35; Gruman p 47; Hayflick p. 268; Haycock p. 80; PMM 246.



Nicolas de Condorcet, *Esquisse d'un Tableau Historique des Progrès de L'esprit Humain*, Second ed. Paris, Chez Agasse, 1795. From the Medical Library of Tim C. Opler.

1797: Christoph Hufeland

Explained aging as involving the gradual loss of vital force – a concept not unlike a car running out of gas.

[Christoph Hufeland](#), the great public educator, was court physician in Weimar and friend of Goethe, Schiller, Herder and Wieland.

This book whose title translates to *The Art of Prolonging Life*, became a classic canon of disease prophylaxis, and remains as relevant today as it was over 200 years ago. *Heirs of Hippocrates*: “The book enjoyed a phenomenal success, was quickly translated into all major languages, and went through numerous editions.”

He replaced the Galenic concept that aging involves the loss of “innate heat” the notion that aging involves loss of “vitalism”.*

This book counselled exercise and the consumption of whole foods (macrobiotics) to prolong life. The title page contains Goethe's words as the motto: “Sweet life! Beautiful, friendly habit of existence and work! - shall I part from you?” The most famous medical book of Goethe's time.

References: Bailey 86; Freeman p. 43; GMN 1602; Grmek p. 124; Gruman p. 6; Haycock p. 105; Hayflick p. 194; Iowa 1183; Morley p. 1138; Osler 3019 (1853 copy); Schafer p. 120; Shock 17391 (later ed); Waller 4973 (later ed); Wellcome III, p.310.



Christoph Hufeland, *Die Kunst das menschliche Leben zu verlängern*, First ed. Franz Haas (Vienna), 1797. From the Medical Library of Tim C. Opler.

* M.D. Grmek (1958) in his review *On Ageing and Old Age* wrote of Hufeland's contribution: “Although a very clever scientist, with a good critical sense, and an outstanding clinician and geriatrician, [Hufeland] supported a naive hypothesis, according to which senescence was due to a gradual loss of the “vital force” (or rather: *vital energy*) given in a certain measure to each organism by birth.”

1799: James Easton

Uncritically recounts many stories of exceptional long lives such as the purported 152-year life of Tom Parr.

James Easton's *Human Longevity* (1799) is an extensive record of individuals who lived to 100 years or more between AD 66 and 1799, covering a span of 1,733 years. The book documents the names, ages, places of residence, and years of death of 1,712 centenarians.

Easton's work is notable for its attempt to systematically record cases of extreme longevity, providing a historical account of human lifespan before the advent of modern medicine.

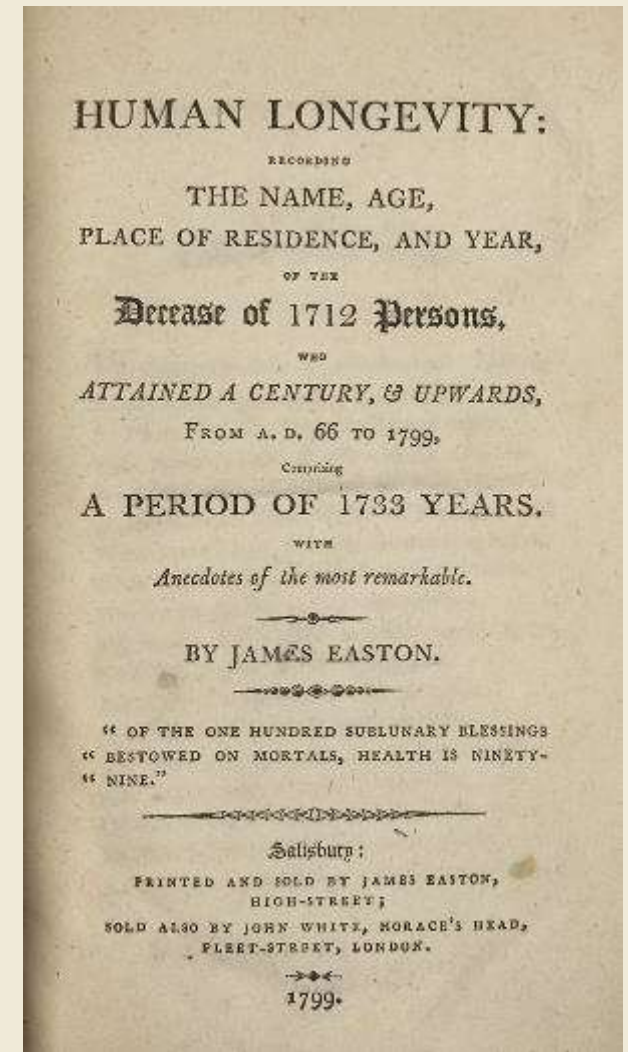
He notes one Englishman who lived 149 years and another, Tom Parr, who lived 152 years (as previously discussed), but neither case was confirmed.*

The book also includes anecdotes about particularly remarkable individuals, shedding light on their lifestyles, habits, and circumstances that may have contributed to their long lives. Some entries contain information about diet, occupation, or notable events in the lives of these centenarians. His work is but one of a long line on [longevity mythology](#).

He relates longevity to exposure to fresh air and exercise. Notes that it is not related to wealth and that it has a strong hereditary component.

References: Bailey 49; Freeman p. 53; Grmek p. 151; Haycock p. 100; NYAM p. 125; Schafer p. 157; Shock 17358.

* As noted earlier, there is substantial doubt regarding the Tom Parr case. Parr was autopsied by William Harvey. It's believed that Parr's birth record may have been confounded with that of his grandfather. Thoms (later cited) was unable to confirm the parish records of his birth. Also see https://en.wikipedia.org/wiki/Old_Tom_Parr and <https://www.demogr.mpg.de/books/odense/6/o2.htm>.



James Easton, *Human Longevity: Recording the Name, Age, Place of Residence, and Year, of the Decease of 1712 Persons, who Attained a Century, & Upwards, from AD 66-1799, Comprising a Period of 1733 Years, with Anecdotes of the Most Remarkable*, First ed. Salisbury, J. Easton, 1799. From the Medical Library of Tim C. Opler.

Section 6:

Aging Studies in the 19th Century

The Pace of Research Accelerates



Henry Peach Robinson's iconic 1876 photograph entitled "[When the Day's Work is Done](#)". Robinson wrote: One of the best models I ever employed was an old man of seventy-four. He was a crossing-sweeper. I should never have accomplished one of my best works if I had not seen him sitting at a table in my studio, waiting, till I could talk to him. I not only saw the old man there, but mentally, the old lady, and the interior of the cottage...The old man, by his attitude and expression, gave the germ of the idea; the old lady had to be found, and the cottage built, but they appeared to me then quite visibly and solidly." Source: J. Paul Getty Museum.

19th Century: Transformational for Biology

The 19th century was a transformative period for biology, marked by major advances in our understanding of heredity, cell biology, and evolution. These advances profoundly impacted the understanding of aging, shifting it from a largely philosophical or observational field to one increasingly grounded in scientific principles.

1. Heredity and the Foundations of Genetic Aging Theories

The 19th century saw the emergence of heredity as a central concept in biology, thanks to the work of scientists like Gregor Mendel and August Weismann. Mendel's experiments with pea plants (published in 1866 but largely ignored until the 20th century) laid the groundwork for understanding how traits are inherited, while Weismann's *Theory of the Continuity of the Germ Plasm* (1885) distinguished between somatic cells (which age and die) and germ cells (which are immortal and pass genetic information to the next generation). The frequency with which heredity was mentioned as a factor in aging rose throughout the 19th Century:

Weismann's Evolutionary Theory of Aging. Weismann proposed that aging results from the body's limited investment in somatic maintenance, as resources are prioritized for reproduction and the survival of the germline. This idea laid the foundation for the

“disposable soma theory” of aging, which remains a cornerstone of evolutionary biology. It suggested that aging is not an inevitable process but a consequence of evolutionary trade-offs.

Heredity and Longevity. The recognition that traits, including lifespan, could be inherited spurred interest in the genetic basis of aging. This led to early hypotheses about the role of genes in determining lifespan and susceptibility to age-related diseases.

2. Cell Biology and the Cellular Basis of Aging

The 19th century witnessed the rise of cell theory, formulated by [Theodor Schwann](#) and others in the 1830s, which posited that all living organisms are composed of cells. This was further refined by [Rudolf Virchow](#), who stated that all cells arise from pre-existing cells (*omnis cellula e cellula*, 1855). Despite these discoveries there was relatively little work acknowledging the centrality of cell biology to aging in the 19th Century.

The realization that cells are the [fundamental units of life](#) led scientists to investigate how cellular function declines with age, although changes in cells weren't explicitly linked to aging until the work of Charles S. Minot in 1907.

Advances in Biology Impact Aging Research

Tissue Degeneration: Xavier Bichat's work on tissues (early 19th century) and later studies by Carl Canstatt and Maxime Durand-Fardel described aging as a process of progressive degeneration of tissues and organs.

This tissue-based / cellular perspective provided a mechanistic framework for understanding aging as a biological process.

3. Darwin's Theory of Evolution

The publication of Charles Darwin's *On the Origin of Species* (1859) revolutionized biology by introducing the concept of natural selection. Alfred Russel Wallace arrived at similar conclusions, and together, Darwin and Wallace provided a framework for understanding how species change over time. This evolutionary perspective had profound implications for aging research.

Declining Force of Natural Selection: Wallace and later Weismann argued that natural selection weakens with age because older individuals contribute less to reproduction and survival.

In the 20th Century, this idea led to the mutation accumulation theory, which posits that harmful mutations expressed late in life are not effectively removed by natural selection, leading to aging.

Trade-offs Between Reproduction and Longevity: Evolutionary theories emphasized that organisms allocate limited resources between reproduction and somatic maintenance. This trade-off explained why aging occurs: investing in reproduction often comes at the cost of long-term somatic repair, leading to eventual decline.

Adaptive Value of Aging: Some 19th-century scientists speculated that aging might have an adaptive purpose, such as making room for younger, more fit individuals. While this idea has been largely discredited, it reflected the growing influence of evolutionary thinking on aging research.

Foundational Steps in Gerontology

Perhaps the most important advances in the 19th Century involved developing a perspective on what aging is. Notably, Anthony Carlisle, an English surgeon, contributed to the understanding of aging through his observations of degenerative changes in the human body. Carlisle noted that aging was accompanied by a progressive loss of function in organs and tissues, which he attributed to the accumulation of damage over time. His work emphasized the inevitability of biological decline but also hinted at the possibility of mitigating these effects through medical interventions.

Halting Progress Made in Aging Research in the 19th Century

Carlisle's ideas were influential in shaping early 19th-century views on aging as a process of gradual degeneration. Similarly, Carl Canstatt, a German physician, is often credited with founding the field of geriatric pathology. In his seminal work *Die Krankheiten des höheren Alters und ihre Heilung* (The Diseases of Old Age and Their Treatment, 1839), Canstatt systematically described the physiological and pathological changes associated with aging. He emphasized that aging was not a single process but a collection of changes affecting different organs and systems.

Maxime Durand-Fardel, a French physician, made significant contributions to the study of aging and the brain. In his book *Traité clinique et pratique des maladies des vieillards* (Clinical and Practical Treatise on the Diseases of the Elderly, 1854), Durand-Fardel described the structural and functional changes in the aging body and brain.

Jean-Martin Charcot, a pioneering French neurologist, expanded on Durand-Fardel's work by studying the relationship between aging and neurological disorders. Charcot's research on conditions such as Parkinson's disease and senile dementia highlighted the role of aging in the development of neurodegenerative diseases.

Despite Biological Advances, Overall Progress Was Limited

While 19th-century scientists made significant strides in understanding aging, their work was limited by the technology and methodologies of the time. Importantly, the tools to study cells and genes in detail were not yet available, limiting the ability to explore molecular mechanisms. Further, evolutionary theories of aging were largely speculative, as the genetic basis of inheritance was not fully understood until the 20th century.

Importantly, the scale of the global scientific enterprise was but a small fraction of its current scale. And standards for scientific rigor were much less developed than today. Scientific peer review was largely absent.

As a result, collaboration and communication of ideas between individual investigators was often limited and fundamental biological discoveries were often not appreciated as relevant to aging until much later. For example, [Richard Altmann](#) and [Carl Benda](#) described mitochondria in the 19th Century but the idea that their function might be a central determinant of aging did not come until well past the middle of the 20th Century.

1800: Xavier Bichat

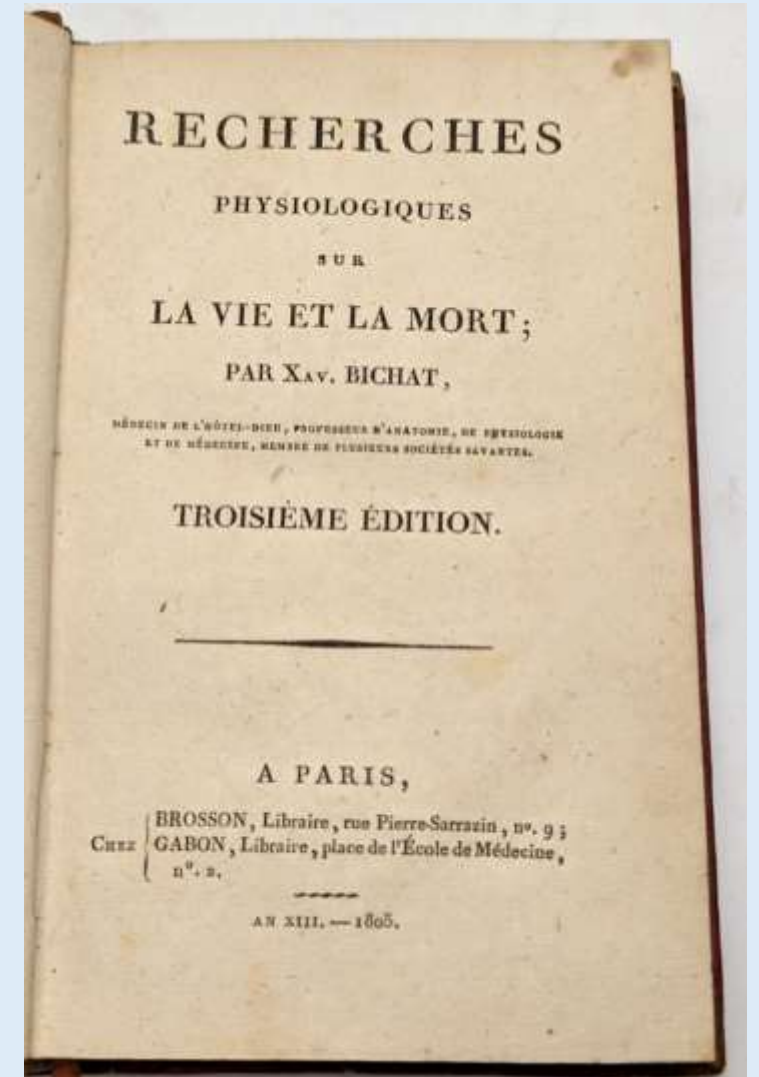
An early advocate of focusing on tissues rather than organ systems as a source of mortality, anticipating advances in cell biology to come in the decades ahead.

In *Recherches Physiologiques sur la Mort* (Physiological Research on Life and Death), [Xavier Bichat](#) introduced a fundamental distinction between organic and animal life, shaping the study of physiology. He argued that organic life, controlled by the sympathetic nervous system, includes involuntary processes such as digestion, circulation, and respiration—functions essential for sustaining life. In contrast, animal life, governed by the brain and voluntary nervous system, consists of conscious actions like movement, sensation, and perception. This showed how different organ systems operate independently yet interact to maintain life.

Bichat also redefined the concept of death, asserting that it occurs gradually, with different organ systems failing at different rates. He described three types of death: nervous system failure, circulatory failure, and respiratory failure. This hierarchical view influenced the development of emergency medicine, pathology, and resuscitation techniques. His approach changed medical perspectives on mortality by recognizing death as a systemic process rather than a single event.

A pioneering aspect of Bichat's work was his emphasis on tissues rather than organs, laying the foundation modern cellular biology. He identified 21 distinct tissue types, arguing that the body's functions depend on the properties of tissues rather than just organ structure. This tissue-based approach influenced later discoveries in pathology, cell theory, and experimental medicine, particularly inspiring figures like Claude Bernard and Rudolf Virchow.

References: Blake p. 47; Freeman p. 43; GMN 597; Schafer p. 122; Waller 1029; Wellcome II, p. 164.



Xavier Bichat, *Recherches Physiologiques sur la Mort*, Paris, Libraire Brosset et Libraire Gabon, 1805. Third edition. From the Medical Library of Tim C. Opler.

1807: John Sinclair

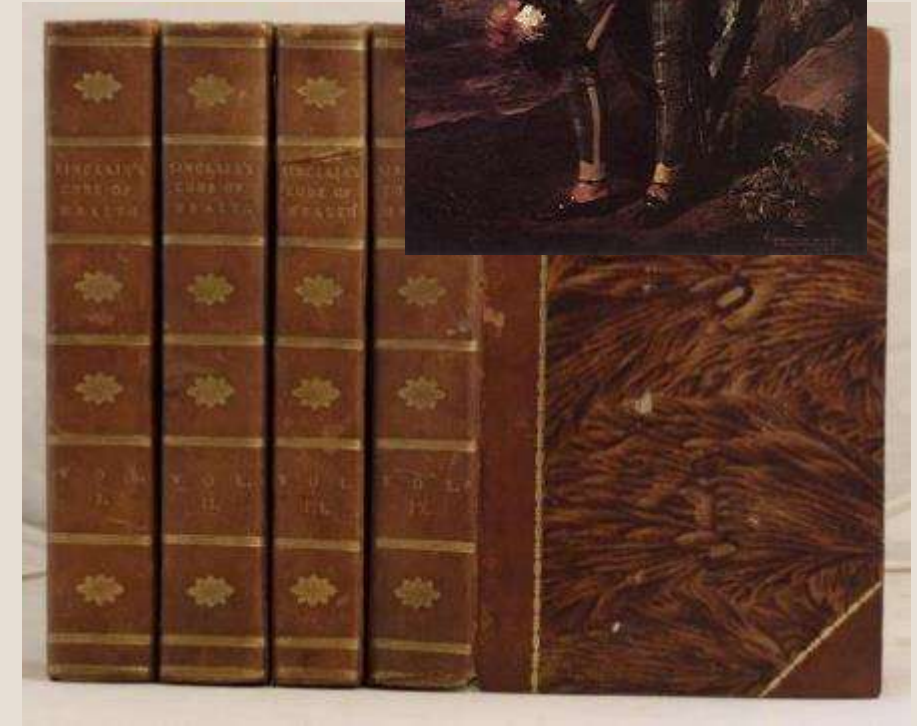
Wrote an encyclopedia on longevity, reviewing the historical literature, epidemiologic data and providing advice on moderation of lifestyle and diet.

GMN on this book: “One of the most comprehensive works on gerontology ever written, with a bibliography of 1800 references, supplemented by abstracts, translated excerpts from ancient authors, national data, etc.”

Sinclair’s book *The Code of Health and Longevity* outlines a set of guidelines and principles aimed at maintaining good health and achieving a long life, essentially a historical guide to healthy living practices. The book is structured into sections that cover a wide range of topics, including diet, exercise, hygiene, mental well-being, and environmental factors, all aimed at promoting a long and healthy life. Sinclair emphasizes the importance of moderation in eating and drinking, advocating for a balanced diet rich in natural foods, and warns against the dangers of excess. He also highlights the benefits of regular physical activity, fresh air, and cleanliness, reflecting the growing awareness of public health during his time. Additionally, the book addresses the role of mental and emotional health, stressing the need for a calm and positive mindset.

[Sir John Sinclair](#) was a Scottish politician and author known for his interest in social and agricultural reform. During sixty years he was almost constantly employed in producing more or less useful books. In 1780 he was elected to the Scottish legislature and for more than half a century Sir John Sinclair occupied a prominent position in the world of politics, as well as of science and literature. His reputation as an Agriculturist extended far and wide throughout Europe and America.

References: Bailey 135; Freeman p. 10; GMN 1602.1; Grmek p. 158; NYAM p. 125.



John Sinclair, *The code of health and longevity; or, a concise view of the principles calculated for the preservation of health, and the attainment of long life*. 4 vols. Edinburgh: Archibald Constable & Co., 1807, First edition. From the Medical Library of Tim C. Opler.

1811: Thomas Jameson

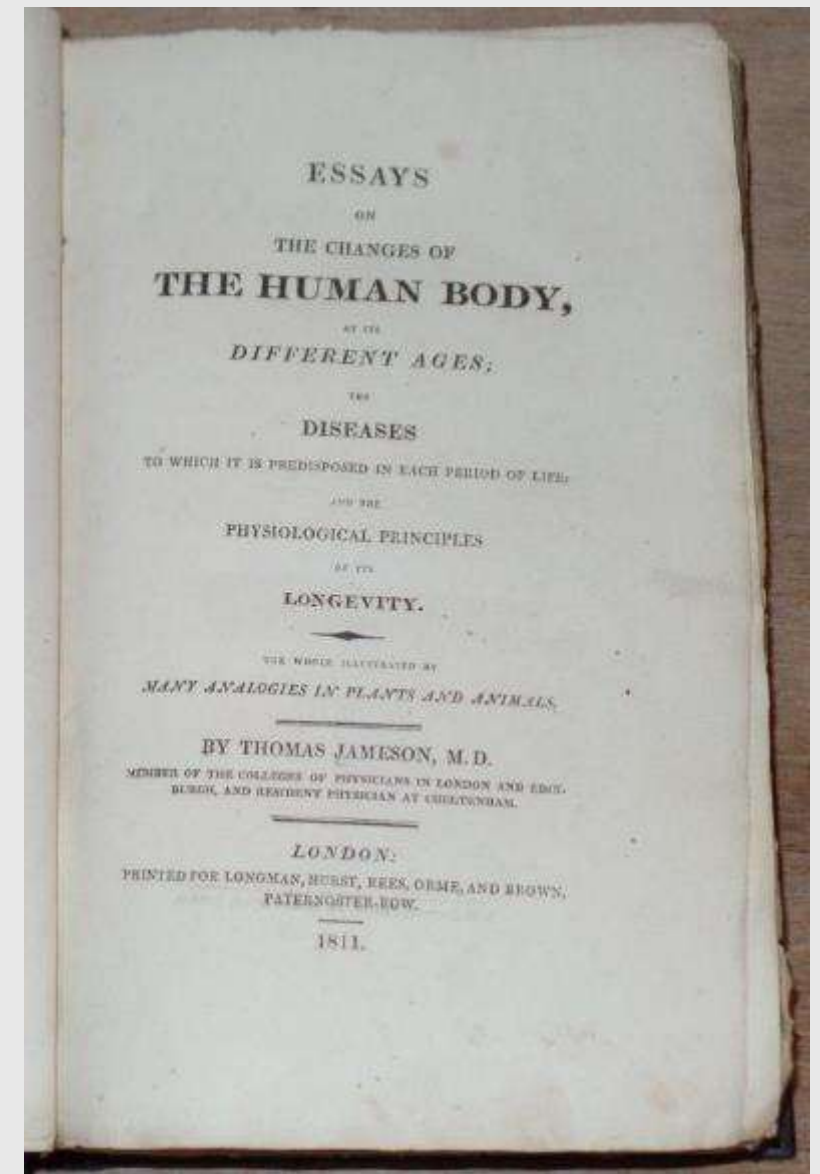
Focused on lifestyle and diet to extend life.

[Thomas Jameson's](#) *Essays on the Changes of the Human Body, at its Different Ages ... and the Physiological Principles of Longevity* is a significant early exploration of human aging and longevity from a physiological perspective. The book examines how the human body changes over time, analyzing the biological and environmental factors that contribute to aging. Jameson sought to understand not only the external signs of aging but also the internal mechanisms that govern the body's gradual decline. His work reflects an early attempt to ground the study of longevity in medical science rather than folklore or purely observational accounts.

A key theme in Jameson's essays is the concept of gradual degeneration, where aging is viewed as a progressive reduction in the body's ability to maintain its vital functions. He discusses how different life stages—from infancy to old age—are marked by distinct physiological changes, including metabolic shifts, alterations in organ function, and changes in resilience to disease. Discussed the rise of stroke, shaking palsies (later called Parkinson's) and retinopathies.

Jameson also speculates on the principles that might extend life, emphasizing the importance of temperance, proper nutrition, and overall bodily care. His approach combines elements of contemporary medical knowledge with emerging ideas about longevity, making it an early contribution to the study of aging from a scientific standpoint. His effort to systematize aging as a process with identifiable stages was forward-thinking. His emphasis on lifestyle factors influencing longevity anticipated later developments in public health and preventive medicine.

References: Grmek p. 129.



Thomas Jameson, 1811, *Essays on the Changes of the Human Body, at its Different Ages ... and the Physiological Principles of Longevity*, Longman: London, First edition. From the Medical Library of Tim C. Opler.

1817: Anthony Carlisle

An early book describing diseases of the elderly in detail.

[Anthony Carlisle](#) (1768-1840) was a prominent English surgeon and anatomist. GMN: “Carlisle, a distinguished surgeon and anatomist, advised young people to adopt a sound regimen early in life to secure longevity. Addressing himself directly to old people he described diseases common to the elderly and paid particular attention to the problems of performing surgical operations on the aged.”

Carlisle identifies the physiological changes and disorders commonly associated with old age, such as diminished organ function, weakened muscles, and cognitive decline. He blends medical knowledge and practical observation, emphasizing the importance of understanding the natural processes of aging to improve the quality of life in later years.

Carlisle also critiques the medical practices of his time, advocating for more compassionate and informed care for the elderly. His work reflects an early recognition of aging as a distinct area of medical study and underscores the need for tailored interventions to address the unique health challenges faced by older individuals. His comments on surgical problems of the old were incorporated in one of the first specialty books on the subject. he pointed out that 'dangerous operations are rarely advisable in advanced age'. because of the reduction in vitality and the presence of constitutional disorders". (Freeman Aging: Its History and Literature p45). In addition to diagnosing the disorders of old age, Carlisle offers practical advice on how to prolong life and maintain health in later years, emphasizing diet, exercise, and mental engagement, in promoting longevity.

References: Achenbaum p. 37; Bailey 31; Freeman p. 45; GMN 1604.1; Grmek p. 129; NYAM p. 124 (1818 ed); Schafer p. 147; Shock 17318; Wellcome p301.



Anthony Carlisle, 1817, *An Essay on the Disorders of Old Age and Means for Prolonging Human Life*, Longman: London, First edition. From the Medical Library of Tim C. Opler. Previously, from the Library of the English Dermatologist Arthur Rook (1918-1991) with bookplate on the inside front board.

1818: Joseph Taylor

Counselled exercise and good diet for longevity

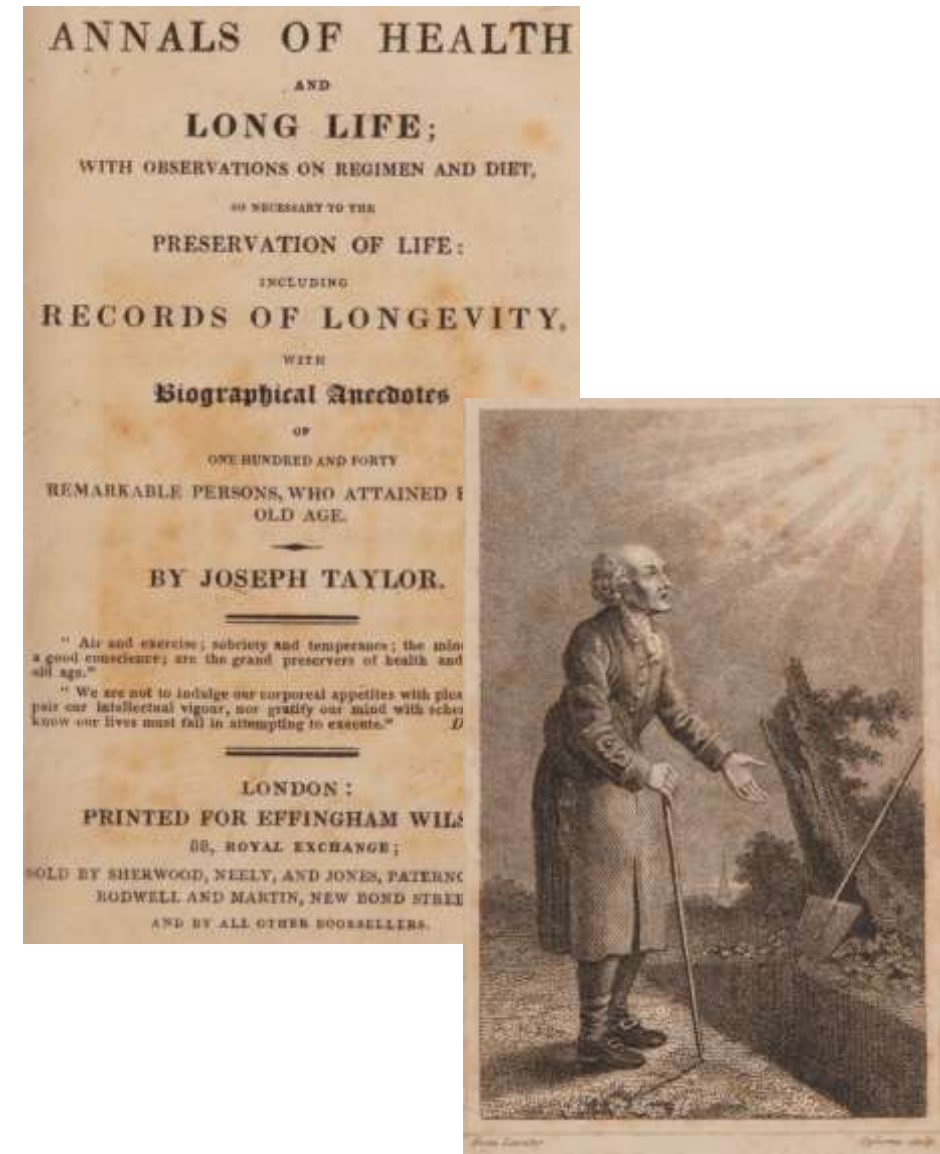
Joseph Taylor's 1818 book *Annals of Health and Long Life; With Observations of Regimen and Diet, So Necessary to the Preservation of Life; Including Records of Longevity* explores the determinants of health and longevity.

Taylor discusses the importance of diet, lifestyle, and environmental influences in maintaining vitality and extending life. He provides detailed observations on the role of moderation in eating and drinking, the benefits of physical activity, and the impact of mental well-being on overall health.

The book includes case studies and records of individuals who achieved remarkable longevity, offering insights into the habits and conditions that may have contributed to their long lives. Taylor's work reflects the growing interest in preventive medicine and the belief that a disciplined and balanced lifestyle could significantly enhance health and lifespan.

In addition to its practical advice, *Annals of Health and Long Life* serves as a historical document, capturing the medical and cultural attitudes toward aging and health in the early 19th century. Taylor emphasizes the importance of understanding the body's needs and adapting one's habits to promote longevity. His work is notable for its holistic approach, combining scientific observations with anecdotal evidence and philosophical reflections on the nature of life and aging.

References: Bailey 141; Osler 4083.



Joseph Taylor, 1818, *Annals of Health and Long Life; With Observations of Regimen and Diet, So Necessary to the Preservation of Life; Including Records of Longevity*, Effingham Wilson & Co.: London, First edition. From the Medical Library of Tim C. Opler.

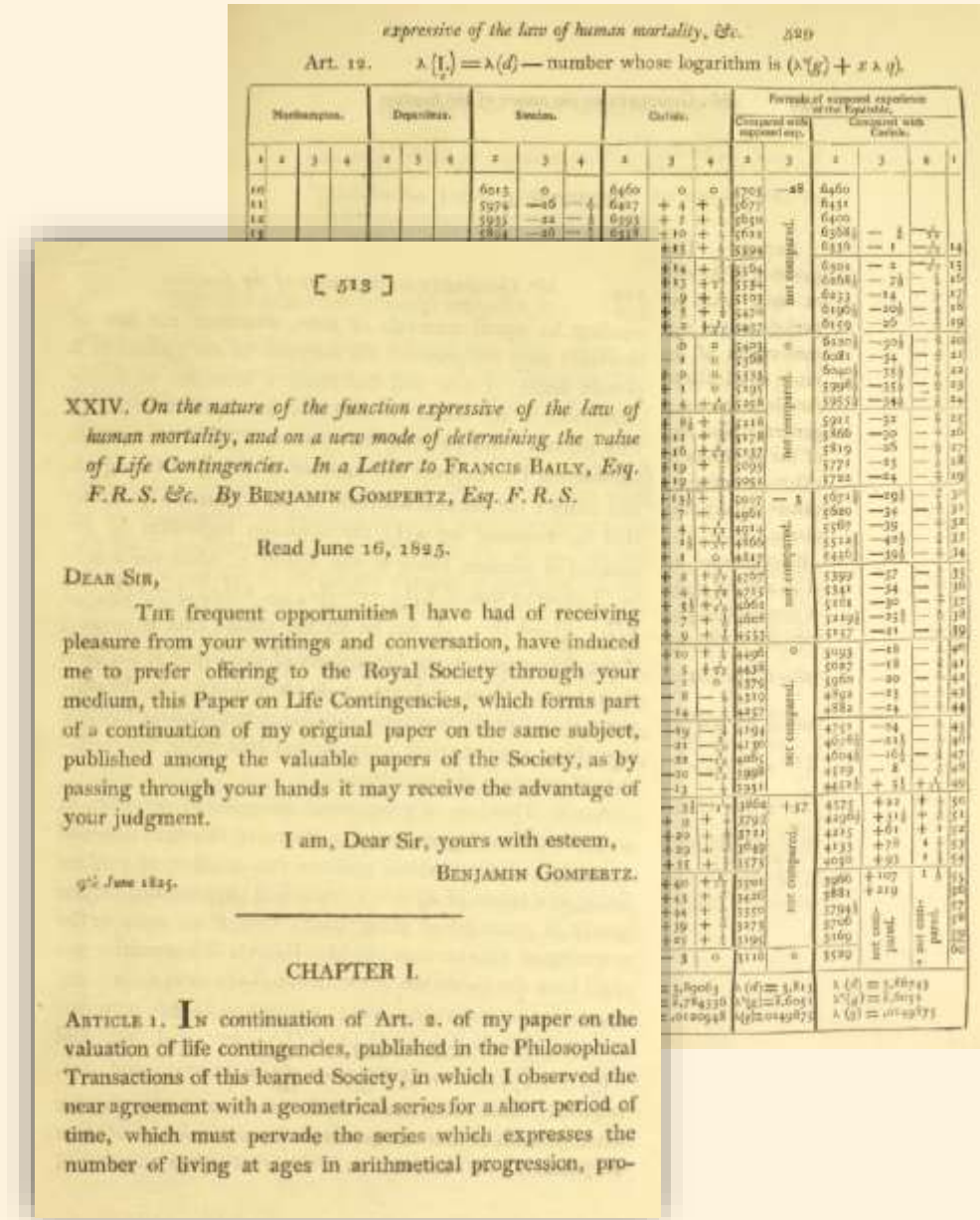
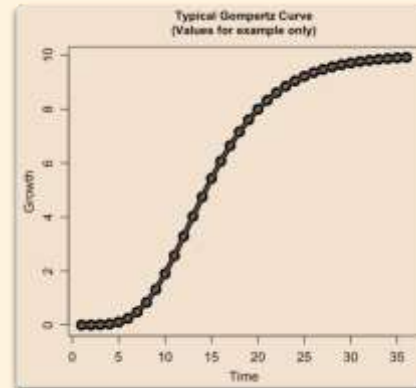
1825: Benjamin Gompertz

The Gompertz Curve proved to be a foundational model for epidemiologic work in demography, aging and the life insurance industry.

Gompertz' Law states that "the average exhaustion of man's power to avoid death to be such that at the end of equal infinitely small intervals of time he lost equal portions of his remaining power to oppose destruction which he had at the commencement of these intervals".

Thomas Kirkwood, University of Newcastle (2015):
 "Gompertz showed that over much of the adult human lifespan, age-specific mortality rates increased in an exponential manner. Gompertz's work played an important role in shaping the emerging statistical science that underpins the pricing of life insurance and annuities. The [Gompertz](#) mathematical model of mortality provided a powerful stimulus to examine the patterns of death across the life course not only in humans but also in a wide range of other organisms. Gompertz's way of representing the function expressive of the pattern of much of adult mortality retains considerable relevance for studying the factors that influence the intrinsic biology of ageing."
<http://rstb.royalsocietypublishing.org/content/370/1666/20140379>

References: Bailey 64; Freeman p. 69; GMN 8375; Haycock p. 136; Hayflick p. 64; Ramakrishnan 34.



Benjamin Gompertz, "On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies," *Phil. Trans.*, 115, 513-583, 1825. From the Medical Library of Tim C. Opler.

1827: William Kitchiner

Like Cornaro 220 years earlier, this was a self-help book that advised eating healthy foods in small portions. Ironically, he invented the potato chip.

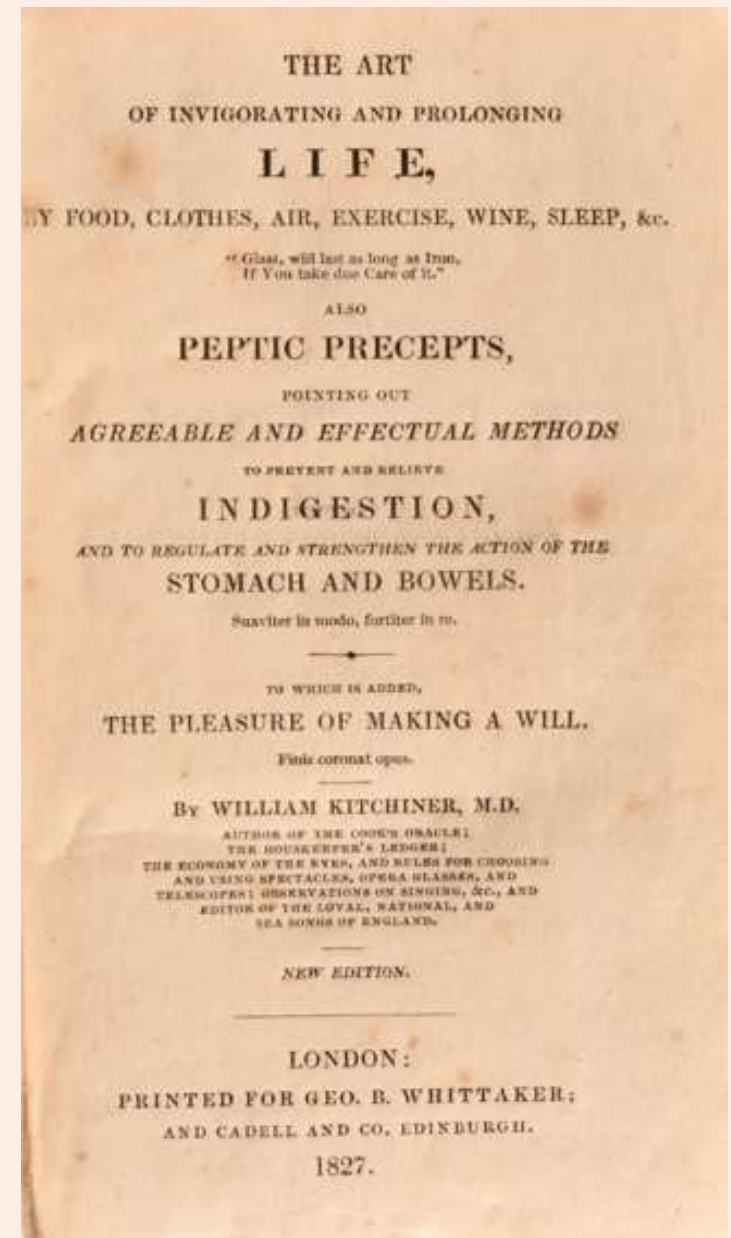
William Kitchiner's *The Art of Invigorating and Prolonging Life* (1827) is a practical and accessible guide to maintaining health and longevity, blending medical knowledge with common-sense advice. [Kitchiner](#), known for his work on cookery and domestic science, applies a similarly methodical approach to health, advocating for moderation, routine, and a balanced lifestyle. His book covers a wide range of topics, including diet, exercise, sleep, mental well-being, and even the benefits of laughter and social interaction. Unlike many medical texts of his time, Kitchiner writes in a conversational and engaging style, making health principles easy for the general public to understand and apply.

A major theme of the book is the importance of dietary habits in sustaining vitality and preventing disease. Kitchiner promotes the idea that digestion is central to health, advising readers to eat simple, well-prepared meals in moderate portions.

He discourages excessive alcohol consumption, overeating, and irregular mealtimes, warning of their long-term effects on the body. He also emphasizes the role of exercise, particularly walking, as a daily necessity for maintaining circulation and preventing lethargy.

In addition, Kitchiner advises on the benefits of fresh air, cleanliness, and proper sleep patterns, presenting a holistic approach to well-being that aligns with the emerging health-conscious culture of the 19th century.

References: Osler 2369



William Kitchiner, *The Art of Invigorating and Prolonging Life*, London: Geo. B. Whittaker, 1827. From the Medical Library of Tim C. Opler.

1839: Carl Friedrich Canstatt

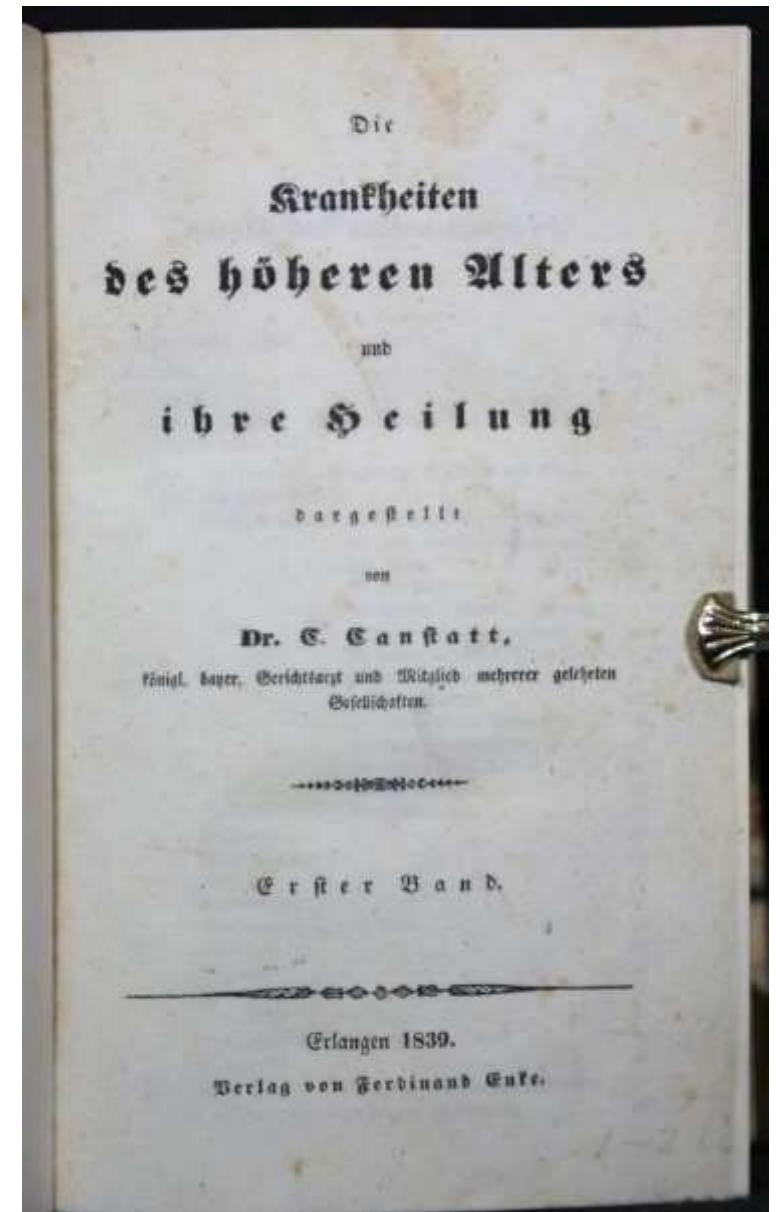
An early and pathbreaking work in the field of geriatric medicine. He theorized that death of individual cells was linked to eventual organismal death.

"Canstatt's book is one of the most important in the history of geriatrics, summarizing all previous work on the subject" (GMN 1605.1).

[Carl Friedrich Canstatt's](#) *Die Krankheiten des höheren Alters und ihre Heilung* (The Diseases of Advanced Age and Their Treatment), published in 1839, is a seminal work in the early study of geriatrics and the medical understanding of aging. Canstatt, a German physician, was one of the first to systematically categorize and describe the diseases and physiological changes associated with old age. His work emphasized that aging is not a uniform process but involves distinct pathological conditions that require specialized medical attention. Canstatt detailed a wide range of age-related ailments, including cardiovascular diseases, respiratory disorders, and cognitive decline, and he explored their underlying causes and potential treatments. His approach was both clinical and observational, combining medical theory with practical insights into the care of elderly patients.

Canstatt's work was groundbreaking for its time, as it challenged the prevailing view that aging was an inevitable and untreatable decline. He advocated for a more compassionate and scientific approach to the care of older adults, emphasizing the importance of understanding the unique needs of this population. Canstatt also highlighted the role of lifestyle, environment, and preventive measures in promoting health in later life. His contributions laid the groundwork for the development of geriatrics as a medical discipline and influenced later pioneers in the field, such as Ignatz Nascher.

References: Freeman p. 45; GMN 1605; Grmek p. 81; Schafer p. 244; Waller 1751.



Carl Friedrich Canstatt, *Die Krankheiten des höheren Alters und ihre Heilung* (2 volumes), Erlangen: Verlag von Ferdinand Enke, 1839. From the Medical Library of Tim C. Opler.

1847: Samuel Sheldon Fitch

Fitch Advocates the “Fresh Air” Theory

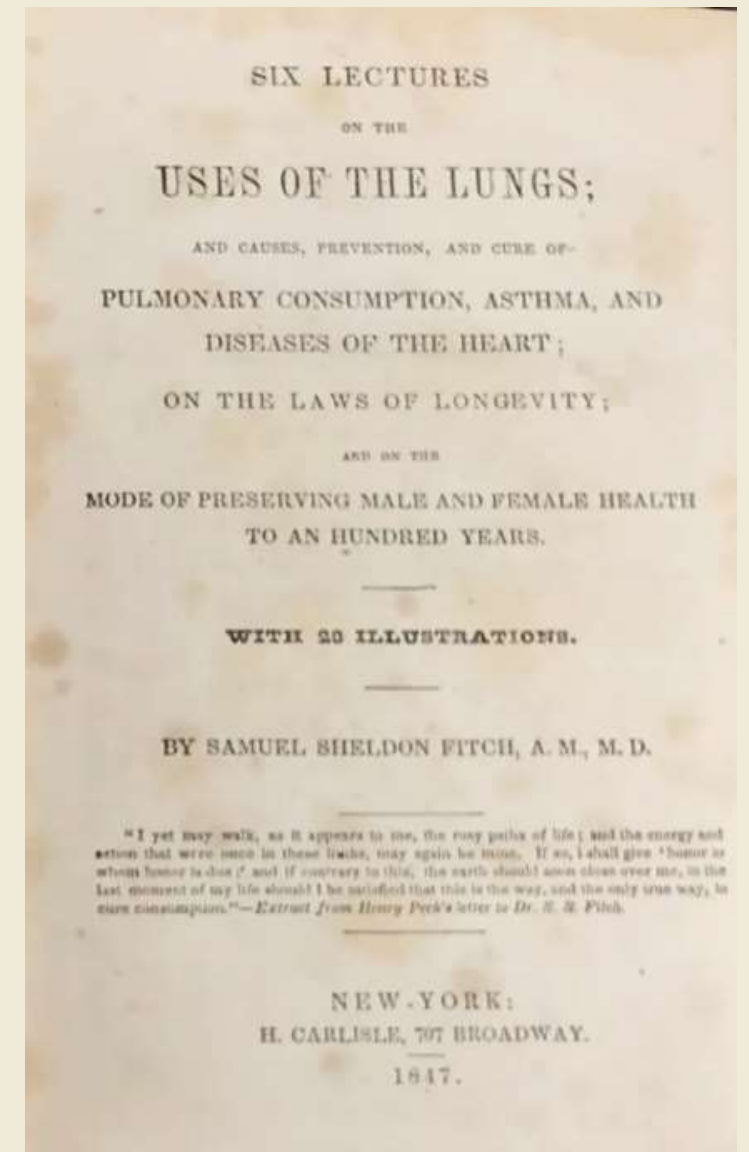
In his 1847 book [Samuel Sheldon Fitch](#) presented a holistic approach to health and longevity, arguing that a long life—potentially reaching a hundred years—is achievable through proper care of the lungs, heart, and overall body.

He emphasizes that maintaining good respiratory function is central to longevity, as oxygenation is vital for sustaining energy, preventing disease, and ensuring the proper function of all bodily systems. Fitch highlights the importance of correct posture, deep breathing, and avoiding restrictive clothing (such as tight corsets) to allow full lung expansion and optimal oxygen intake.

Fitch also advocates for a balanced lifestyle that includes proper nutrition, moderate exercise, and mental well-being. He stresses that a diet rich in wholesome foods, regular physical activity, and sufficient rest can help prevent premature aging and disease. Additionally, he warns against harmful habits such as smoking, excessive alcohol consumption, and exposure to polluted air, all of which he believes contribute to respiratory and cardiovascular decline.

A key aspect of Fitch’s longevity philosophy is the prevention of chronic diseases, particularly pulmonary conditions like tuberculosis and asthma, which were prevalent at the time. He advises early intervention, the use of fresh air, and strengthening exercises to build lung capacity and resistance to illness. He also touches on mental and emotional health, suggesting that maintaining a positive mindset and avoiding excessive stress contribute significantly to a long life.

References: Bailey 34.



Samuel Sheldon Fitch, *Six Lectures on the Uses of the Lungs; and causes, prevention, and cure of Pulmonary Consumption, Asthma, and Diseases of the Heart; On the Laws of Longevity; and on the Mode of Preserving Male and Female Health to an Hundred Years*, New York: H. Carlisle, 1847. From the Medical Library of Tim C. Opler.

1848: John Bell

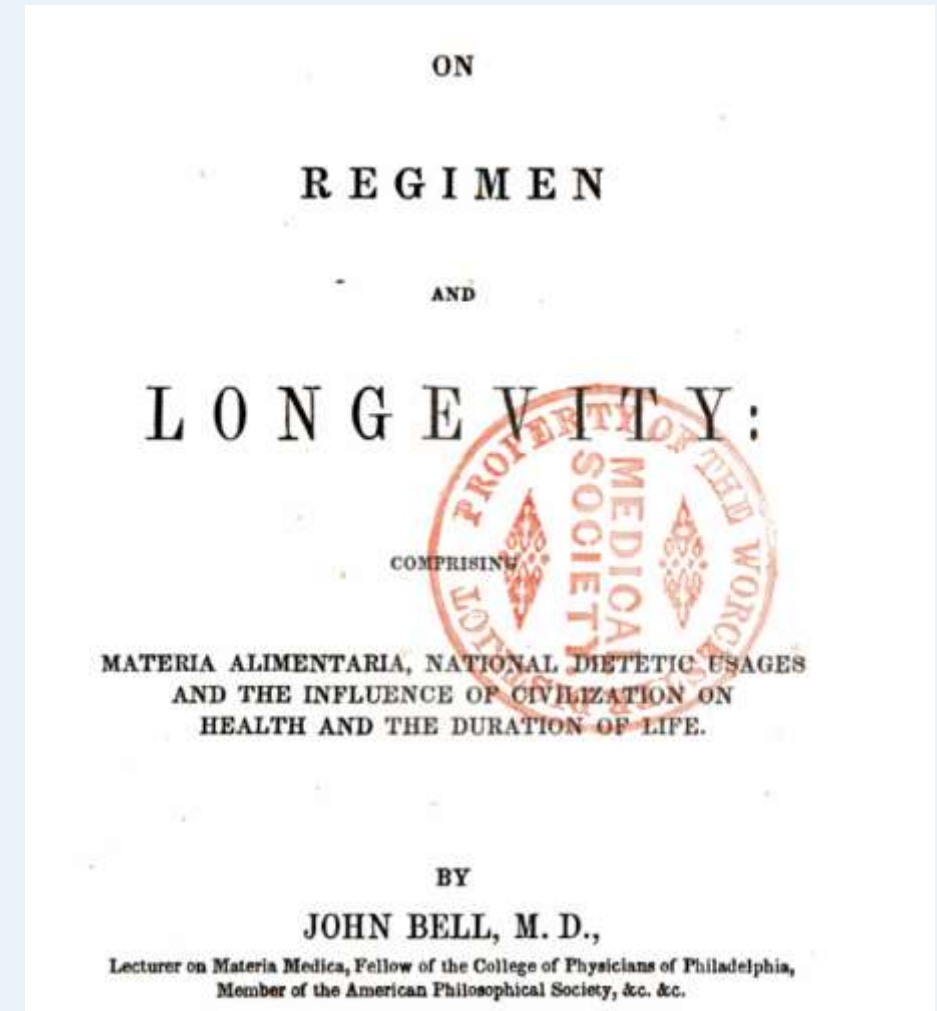
Documents the steady increase of longevity as civilization has progressed. Discusses the linkage with diet and social order. First to note that, on average, women live longer than men.

[John Bell](#) was a physician at the College of Physicians in Philadelphia and an early American writer on the determinants of longevity. His insights were particularly pertinent. Bell has been confused in the literature with John Bell (1763 to 1820), the famous Scottish surgeon, anatomist and prolific author.

John Bell of Philadelphia was the first to demonstrate that, on average, women live longer than men. In this book *On Regimen*, he wrote of the importance in social order and stability in promoting longevity:

“In presenting these historical examples of the connection between good government and social order on the one hand and health and longevity on the other my object has been to fix the attention on the principle involved Its confirmation is now placed beyond all doubt by the results of numerical calculations of the relative age of persons in the same countries and in the same city at different and remote periods. We learn from those calculations that with the diffusion of intelligence based on Christian ethics and a better though still very imperfect practice of the Christian virtues in states and communities and with a greater security of property and person more certain returns to industry in agriculture commerce and the arts better protection against the vicissitudes of weather and more abundant and better food than in former times the average duration of life is greater now in nearly all civilized countries than in any antecedent period in the history of the world.”

References: Achenbaum p. 35; Bailey 21; NYAM p. 124.



Bell, John. *On Regimen and Longevity : Comprising Materia Alimentaria, National Dietetic Usages and the Influence of Civilization on Health and the Duration of Life*. Philadelphia: Haswell & Johnson, 1842. From the Medical Library of Tim C. Opler.

1849: George Edward Day

One of the earlier books on gerontology and elderly care in English.

Day's book is a comprehensive guide focused on the care and health of elderly individuals. [George Edward Day](#), a respected physician, combined medical knowledge with practical advice to address the unique challenges of aging.

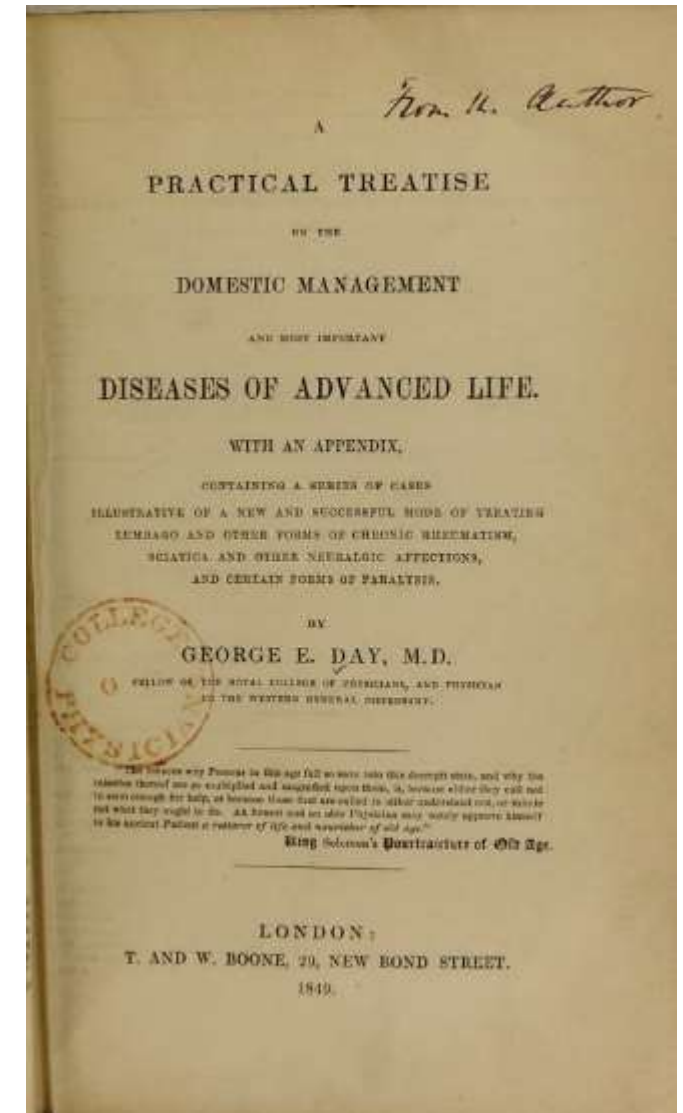
The book covers a wide range of topics, including the physiological changes associated with old age, common diseases such as arthritis, heart conditions and cognitive decline and strategies for managing these conditions at home.

Day emphasizes the importance of a supportive and understanding environment for the elderly, advocating for proper nutrition, gentle exercise and mental stimulation. His work reflects a compassionate approach to geriatric care, highlighting the need for both medical intervention and thoughtful domestic management to improve the quality of life for older adults.

In addition to its medical insights, the treatise provides detailed guidance for caregivers and family members on how to create a nurturing and healthy environment for the elderly. Day discusses the role of hygiene, comfort and emotional well-being in promoting longevity and reducing the burden of age-related illnesses.

He also addresses the social and psychological aspects of aging, recognizing the importance of companionship and respect in maintaining the dignity of older individuals.

References: Bailey 47; Freeman p. 46; GMN 13167; Grmek p. 158; Morley p. 1133; NYAM p. 124. Also see CJ Scott, "George Edwin Day and Diseases of Advanced Life," *Practitioner* 214:832-836, 1975.



George Edward Day. *A practical treatise on the domestic management and most important diseases of advanced life. With an appendix, containing a series of cases illustrative of a new and successful mode of treating lumbago and other forms of chronic rheumatism, sciatica and other neuralgic affections, and certain forms of paralysis.* London: T. and W. Boone, 1849,

1854: Maxime Durand-Fardel

A pathbreaking book that detailed the diseases of age in great detail.

An early effort to describe diseases of old age in detail. Durand-Fardel's book *Traité des Maladies des Vieillards* (Treatise on Diseases of the Elders) is a groundbreaking work in the history of geriatric medicine, marking one of the earliest systematic efforts to address the health challenges of the elderly. The treatise explores the physiological changes, diseases and psychological conditions associated with aging.

The text covers a wide range of ailments common among older adults, such as cardiovascular diseases, neurological disorders, respiratory conditions and musculoskeletal issues, while emphasizing the importance of distinguishing between normal aging and pathological states.

[Maxime Durand-Fardel](#) also highlights the impact of social and environmental factors on elderly health, advocating for compassionate, tailored care and preventive measures to improve quality of life.

This book preceded Charcot's similar work by more than a decade and is considered by a number of observers including Freeman to be the first very good attempt at physiology of old age. Freeman writes: "A typical example is the work of Durand-Fardel published in 1954. For the medical world, his book was far superior as a study of geriatrics than the study of Charcot in 1867 that needed the additional chapters by the American A. L. Loomis in 1881 to enhance its value. Charcot's work took precedence because of the immense influence he exerted on the subject derived from his eminence in medicine."

References: Freeman p. 49; Grmek p. 70; Martin, Histoire de la gériatrie, p. 105; Stambler p. 231.



Maxime Durand-Fardel, 1854. *Traité clinique et pratique des maladies des vieillards*. Paris: Baillière, 1849, From the Medical Library of Tim C. Opler.

1855: Pierre Flourens

Flourens argued that the natural limit of human lifespan was about 100 years. He noted that certain animal species had longer lives than humans while others were shorter. He related longevity of a species to its period of gestation.

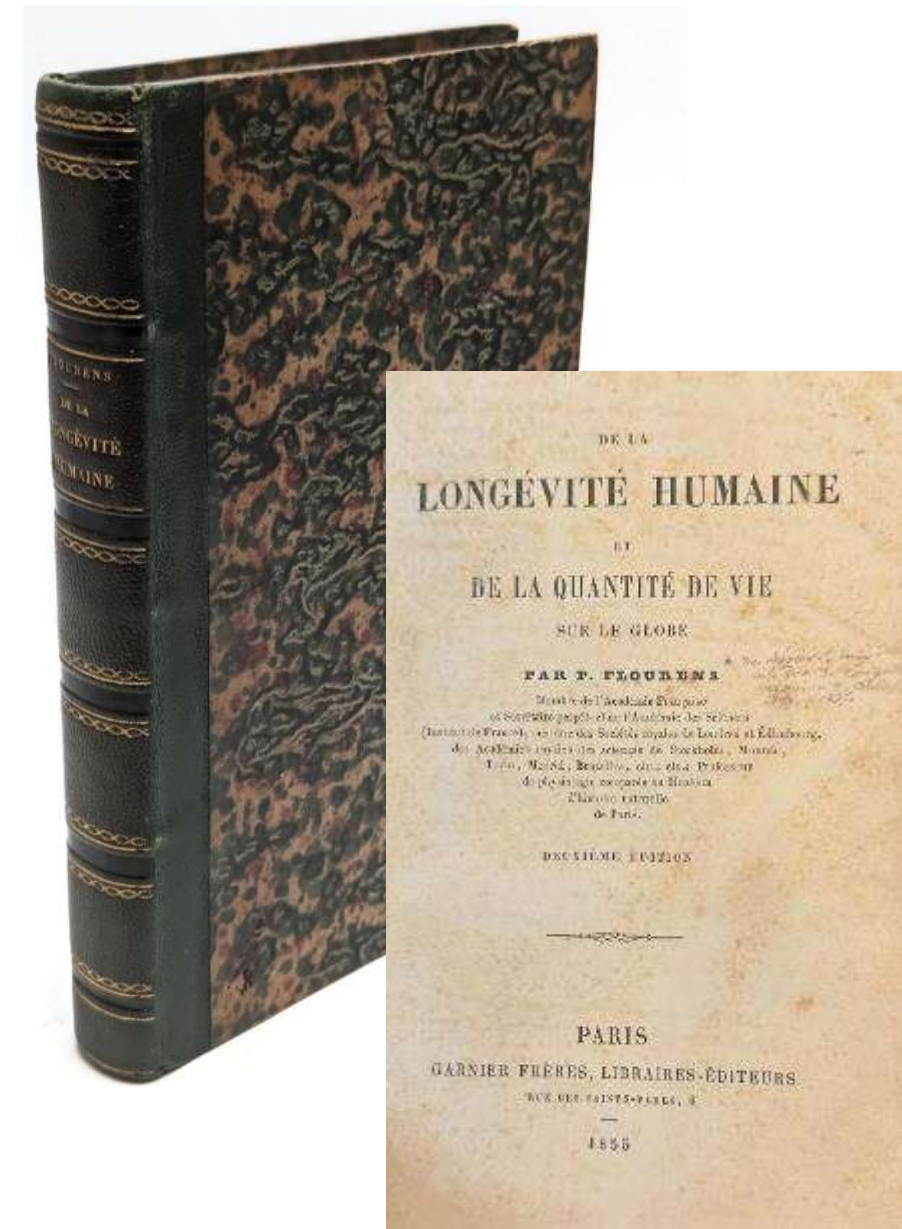
Pierre Flourens' *De la longévité humaine et de la quantité de vie sur le globe* (On Human Longevity and the Quantity of Life on the Globe), published in 1855, is a pioneering scientific exploration of human aging and lifespan. [Pierre Flourens](#), a respected French physiologist, sought to bring a systematic and empirical approach to the study of longevity, challenging the exaggerated claims of extreme old age that were prevalent in myths and anecdotal accounts.

By analyzing historical records and applying biological principles, he argued that human lifespan has a natural limit, which he estimated to be around 100 years. His work emphasized the importance of physiological processes in determining aging and rejected unscientific assertions about immortality or extreme longevity.

Flourens' approach was groundbreaking for its time, as it marked a shift from speculative or anecdotal discussions of aging to a more rigorous, evidence-based analysis. He carefully examined the biological mechanisms underlying aging and concluded that lifespan is governed by inherent physiological constraints rather than external factors alone.

This perspective laid the foundation for modern gerontology, the scientific study of aging, by focusing on the natural limits of human life and the biological processes that influence it. Flourens' work also highlighted the need for critical evaluation of claims about aging, a principle that remains central to aging research today.

References: Bailey 56; Grmek p. 92; Haycock p. 138; Medawar p. 24; NYAM p. 124; Osler 2609; Stambler p. 232.



Pierre Flourens, *Longevite Humaine*, Paris, 1855 (second edition). From the Medical Library of Tim C. Opler.

1860: Michael Faraday

Following Boyle, Faraday likened the burning of a candle to aging.

Michael Faraday's *The Chemical History of a Candle* (1861) is not a book on aging, but it offers a fascinating metaphor that can be applied to human longevity.

Best known as the father of the electric motor, [Michael Faraday](#) compared life with the flame of a candle, and aging of the organism with the burning of the candle.

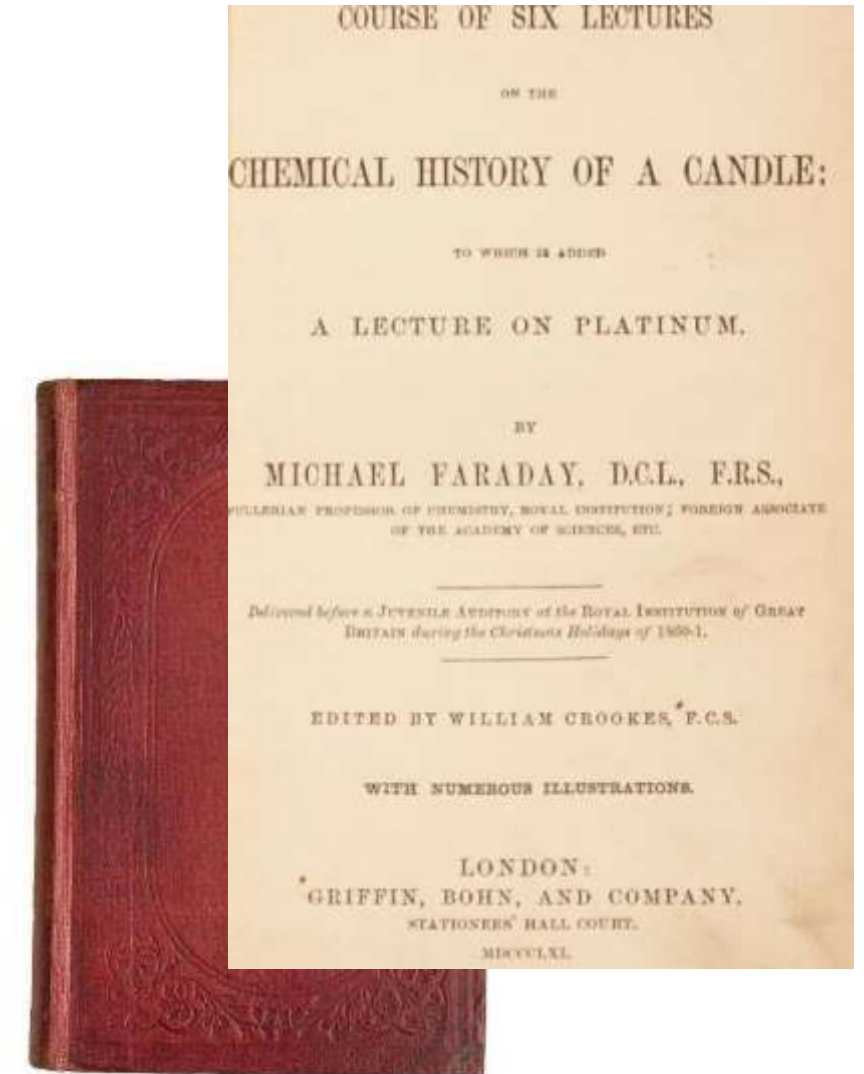
In this work, Faraday principally explores the combustion of a candle as a way to explain fundamental principles of chemistry and physics, illustrating how energy, matter and transformation are interconnected.

While his primary focus was scientific education, the metaphor of the burning candle has often been used to describe the human lifespan—how life, like a flame, depends on the careful balance of fuel (nutrition and energy), oxygen (environment and habits), and structure (bodily integrity).

In today's parlance, the rate of a candle's burning might be likened to metabolism, where factors such as genetics, lifestyle, and environmental influences determine the pace at which the body expends its vitality.

Moreover, the concept of oxidation—central to both combustion and human cellular aging—parallels modern discussions on oxidative stress and its role in senescence.

References: Grmek p. 65.



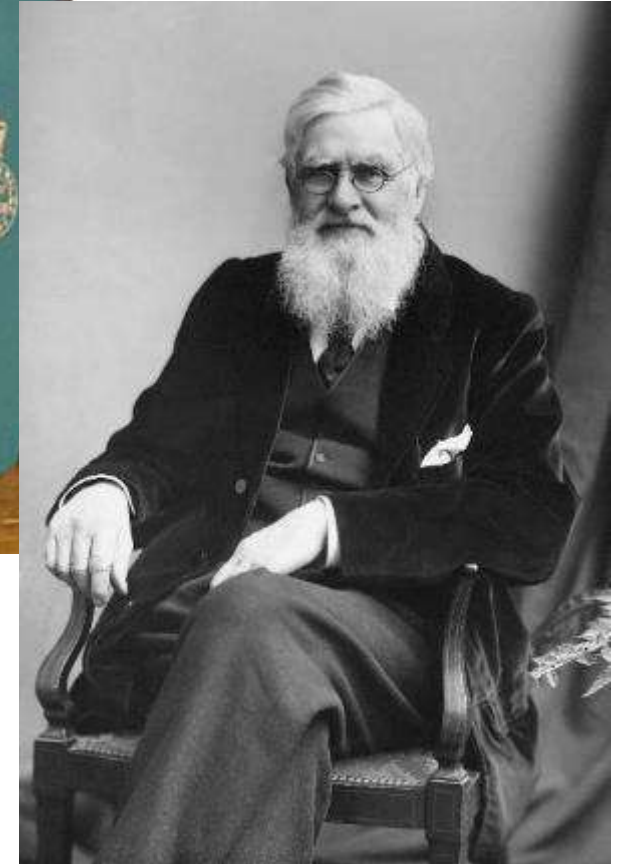
Michael Faraday, *A Course of Six Lectures on the Chemical History of a Candle; to which is Added a Lecture on Platinum*, London: Griffin, Bohn & Co, 1861 (first edition). From the Medical Library of Tim C. Opler.

1865: Alfred Russel Wallace

Proposed the first evolutionary theory of aging. If too many people lived for a long time, they could compete for resources needed for other members of the species of reproduction age. Evolution favors death after reproduction.

[Alfred Russel Wallace](#) wrote: “Any organism therefore that, by accidental or spontaneous fission, could become two organisms, and thus multiply itself indefinitely without increasing in size beyond the limits most favourable for nourishment and existence, could not be thus exterminated: since the individual only could be accidentally destroyed,—the race would survive. But if individuals did not die they would soon multiply inordinately and would interfere with each other's healthy existence. Food would become scarce, and hence the larger individuals would probably decompose or diminish in size. The deficiency of nourishment would lead to parts of the organism not being renewed; they would become fixed, and liable to more or less slow decomposition as dead parts within a living body. The smaller organisms would have a better chance of finding food, the larger ones less chance. That one which gave off several small portions to form each a new organism would have a better chance of leaving descendants like itself than one which divided equally or gave off a large part of itself. Hence it would happen that those which gave off very small portions would probably soon after cease to maintain their own existence while they would leave a numerous offspring. This state of things would be in any case for the advantage of the race, and would therefore, by natural selection, soon become established as the regular course of things, and thus we have the origin of old age, decay, and death; for it is evident that when one or more individuals have provided a sufficient number of successors they themselves, as consumers of nourishment in a constantly increasing degree, are an injury to those successors. Natural selection therefore weeds them out, and in many cases favours such races as die almost immediately after they have left successors.”

References: GMN 234 (citing first German ed).



Alfred Russel Wallace's essay written between 1865 and 1870 entitled “The Action of Natural Selection in Producing Old Age, Decay, and Death,” included as a chapter August Weismann's 1889 book *Essays Upon Heredity and Kindred Biological Problems*. Oxford: Clarendon Press. First English edition. From the Medical Library of Tim C. Opler.

1867: Jean-Martin Charcot

Charcot wrote a careful taxonomy of diseases of old age based on what he observe in his work with elderly patients at the Salpêtrière.

[Jean-Martin Charcot](#) was one of the greatest physicians of the 19th Century and is considered the father of neurology.

In his work on diseases of the aging, Charcot differentiates between three groups of diseases: those related to aging, those that appear at any age but take a particular turn in the elderly, and those that are less harmful in the elderly.

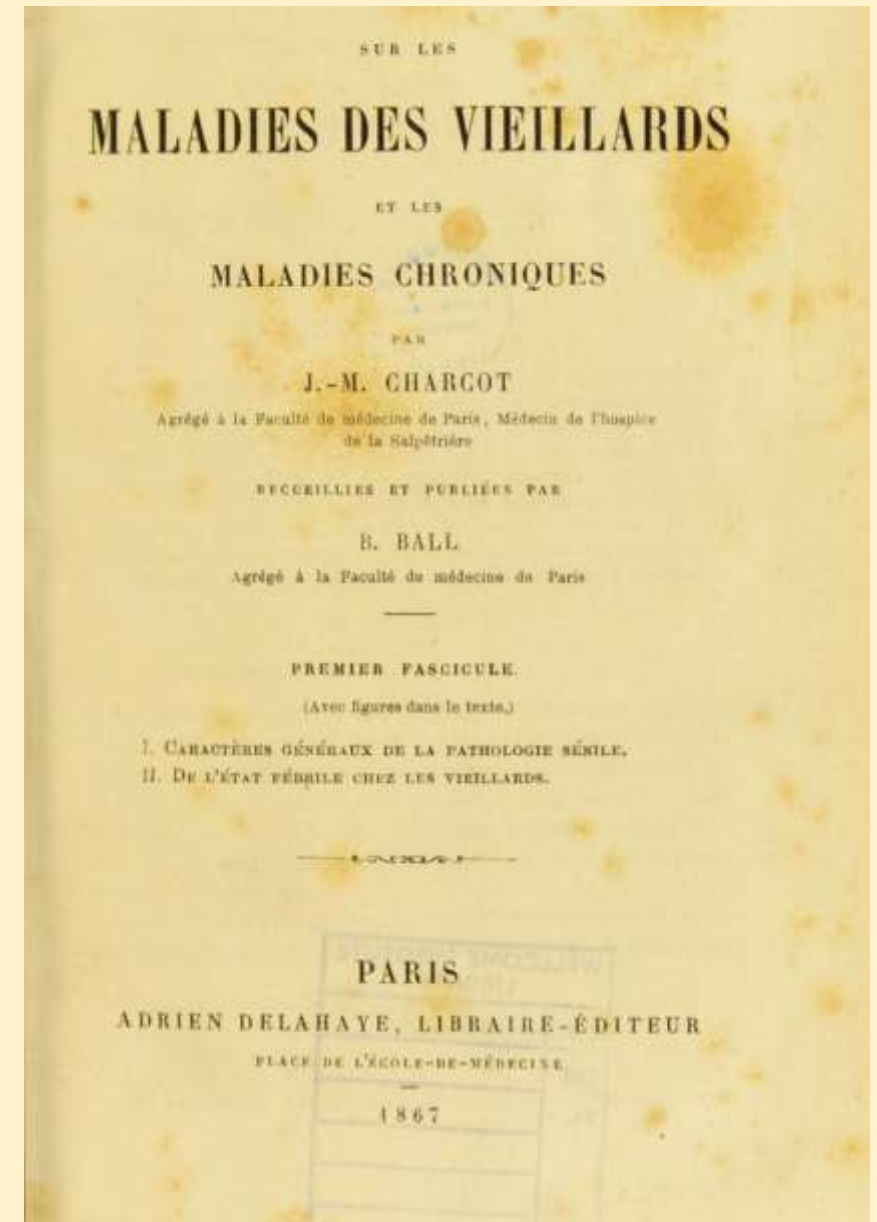
Specifically, Charcot distinguished: 1) diseases specific to old age, e.g. senile marasmus, cerebral atrophy, senile tremor. 2) diseases met at any age but more severe in the aged, e.g. respiratory diseases; 3) diseases to which old people oppose less resistance, e.g. tuberculosis, typhoid fever.

Among many Charcot of studies on diseases of old age, the present paper recalls those devoted to pneumonia, asthma, tuberculosis, arterial, i.e. aneurysmal, embolic, atheromatous diseases, diseases of the liver, digestive and urinary tracts.

The clinical cases he presents come from the [Salpêtrière](#).

The book is considered one of the founding works of modern geriatrics.

References: Achenbaum p. 37; Bailey 34; Freeman p. 91GMN 2222; Grmek p. 84; Haycock p. 142; Morley p. 1133; NYAM p. 125; Schafer p. 8; Stambler p. 231; Waller 1908. Also see J.T. Freeman, "A Centenary Essay: Charcot's Book," *Gerontologist* 7:286-290. 1967.



J-M Charcot, *Sur Les Maladies des Vieillards*, Paris: Adrien Delahaye, 1867. First edition. From the Medical Library of Tim C. Opler.

1873: William Thoms

Debunks claims of very long life and sets up standards for validation.

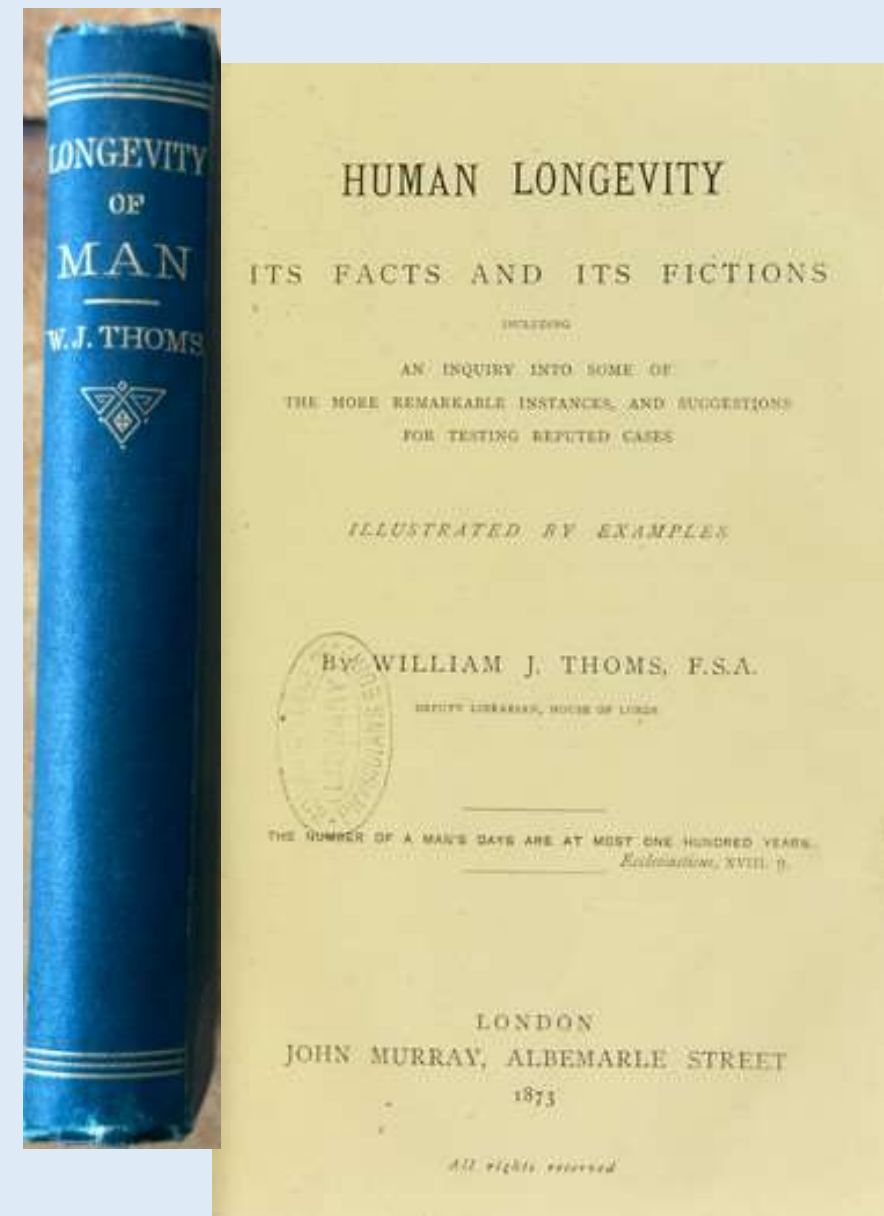
William Thoms' *Human Longevity: Its Facts and Its Fictions* (1873) is a critical examination of historical claims about extreme human lifespans.

[William Thoms](#), a British antiquarian and founder of the term "folklore," challenges widely accepted reports of individuals allegedly living well beyond a hundred years, sometimes even to 150 or more. Using a rigorous analysis of historical records, parish registries, and official documents, he exposes inconsistencies and exaggerations in many of these accounts, arguing that most claims of extraordinary longevity are either mistakes, fabrications, or misinterpretations of records.

A key theme of Thoms' book is the importance of accurate record-keeping in validating longevity claims. Thoms demonstrates that many supposed centenarians were often confused with ancestors of the same name or had unreliable birth records. He also critiques the credulity of earlier historians and biographers who uncritically accepted claims of extreme old age without verification. His work highlights how folklore, social prestige, and even governmental pension incentives may have contributed to exaggerations of age.

Ultimately, *Human Longevity* was a key effort in demystifying myths surrounding extreme old age. Thoms' skeptical and evidence-based approach laid the groundwork for modern demographic and gerontological studies.

References: Bailey 146; Freeman p. 48; Haycock p. 140; Hayflick p. 195; Shock 17485.



William J. Thoms, *Human Longevity: Its Facts and Its Fictions*, London: John Murray, 1873. From the Medical Library of Tim C. Opler.

1882: August Weismann

Weissman promoted the idea that aging has an evolutionary basis and was the first to publish the earlier essay by Alfred Russell Wallace on the same topic.

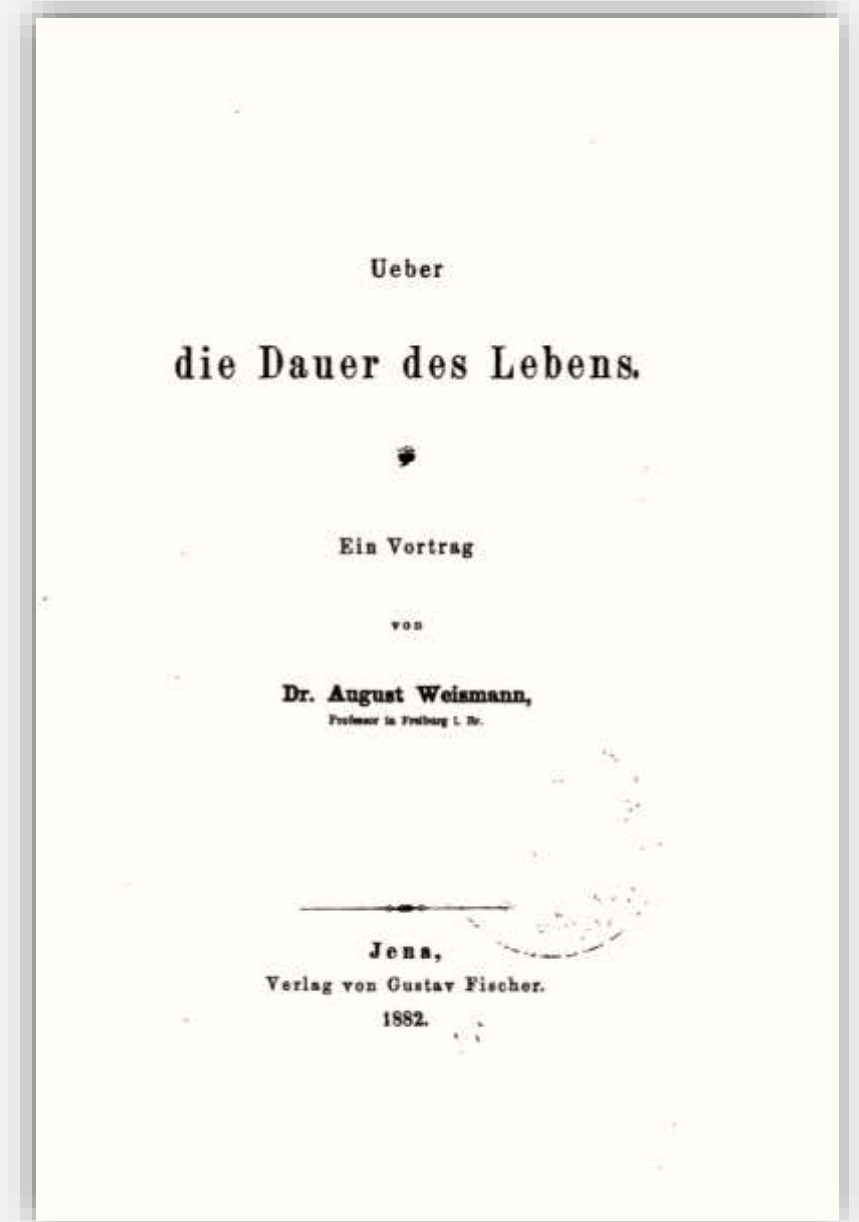
August Weismann's 1882 paper, "Über die Dauer des Lebens" (On the Duration of Life), introduced one of the earliest biological theories to explain why organisms age and die.

[August Weismann](#) proposed that aging is not an inevitable consequence of living but rather an evolved trait shaped by natural selection. He argued that aging serves a purpose in the broader context of evolution: by removing older individuals from the population, aging makes room for younger, more adaptable generations. This idea was revolutionary because it framed aging as a beneficial process for the species, even if it is detrimental to the individual.

Weismann's theory was rooted in the concept of programmed death, suggesting that aging and death are not accidental but are instead genetically determined. Like Alfred Russell Wallace he believed that the lifespan of an organism is regulated by its reproductive potential, with natural selection favoring traits that optimize reproductive success rather than indefinite survival. This perspective laid the groundwork for later evolutionary theories of aging, such as Medawar's mutation accumulation theory and Williams' antagonistic pleiotropy theory.

This idea has continued to gain contemporary adherents. See, for example, André C. R. Martins, "Change and Aging: Senescence as an Adaptation," *PLoS One*, 2011.

References: Freeman p. 76; Grmek p. 148; Haycock p. 159; Hayflick p. 236; Medawar p. 20; Stambler 235; Waller 10193.



August Weismann, *Über die Dauer des Lebens*, Jena, Verlag von Gustav Fischer, 1882. From the Medical Library of Tim C. Opler.

1889: George Murray Humphry

Identifies rural living, active lifestyle and temperate habits as key contributors to longevity.

[George Murray Humphry's](#) book *Old Age* systematically examines the lives of nearly 900 individuals who lived to at least 80 years, including 74 centenarians. Drawing from firsthand accounts and collected data, Humphry sought to analyze the factors contributing to longevity, exploring patterns in health, lifestyle, and hereditary influences.

Humphry's work combined statistical inquiry with anecdotal evidence, aiming to provide a comprehensive picture of aging in the 19th century. Unlike purely theoretical discussions, *Old Age* was rooted in empirical observations, making it one of the more scientific efforts of its time to understand human longevity.

The book identifies common themes among long-lived individuals, such as temperate habits, active lifestyles, and resilience against disease. Humphry noted that many centenarians had lived simple physically active lives, often in rural settings, and generally maintained a balanced diet. He also examined the role of heredity, suggesting that while genetics played a role, environmental and behavioral factors were equally crucial in determining lifespan. His study also explored the decline of physical and mental faculties in old age, emphasizing the importance of maintaining mental engagement.

Humphry's work was an early attempt at data-driven longevity research, predating modern gerontology. While some of his conclusions, particularly about heredity, were speculative by today's standards, his meticulous data collection and focus on lifestyle factors were ahead of his time.

References: Bailey 87; Grmek p. 129; NYAM p. 126; Shock 17394.



George Humphry, *Old Age. The Results of Information Received Respecting Nearly Nine Hundred Persons Who Had Attained the Age of Eighty Years, Including Seventy-Four Centenarians*, Cambridge: Macmillan and Bowes, 1889. From the Medical Library of Tim C. Opler.

1893: Brown-Séquard

Argued that male androgens could increase longevity in a study that was later disproven. Failed to run a clinical trial with an active control.

In 1889, [Charles-Edouard Brown-Séquard](#), aged 72, reported dramatic rejuvenating effects after self-administering testicular extracts of dogs and guinea-pigs.* His report resulted in widespread use of testicular extracts throughout Europe and North America for several decades. More recently, the male ageing process has been attributed to partial androgen deficiency, or "andropause", and testosterone treatment is claimed to improve well-being in middle-aged and elderly men.

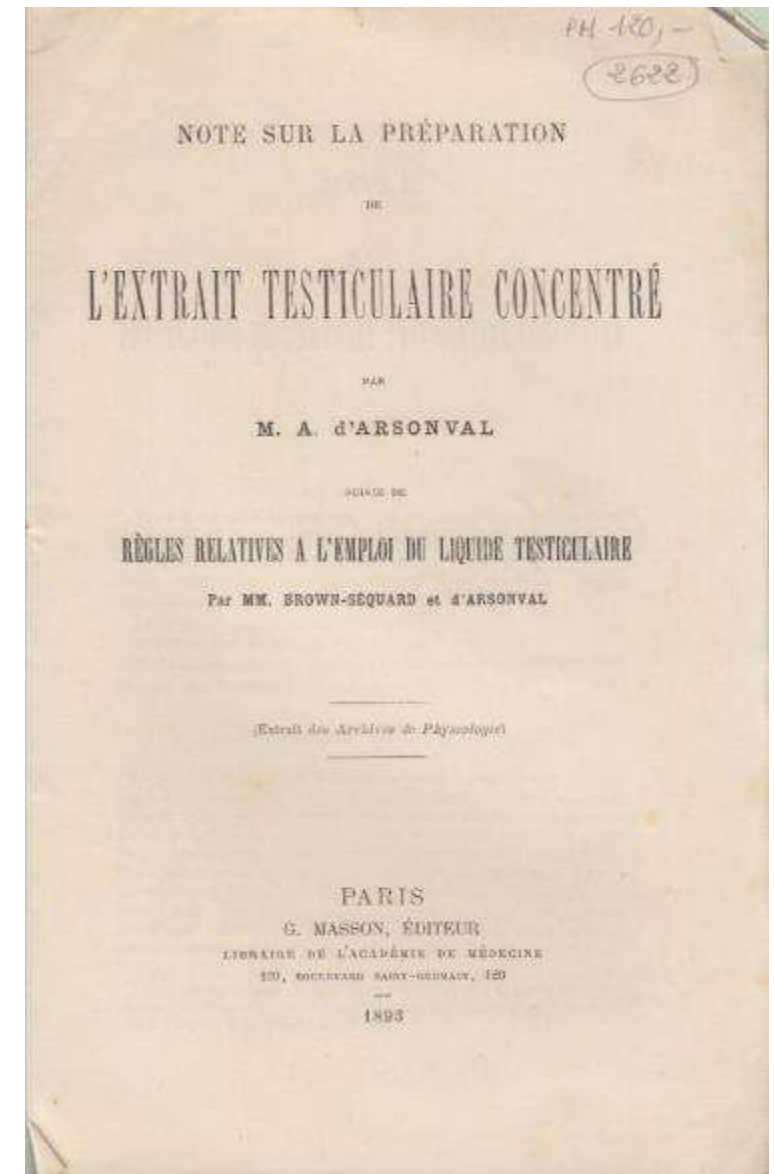
Subsequently, Cussons and colleagues replicated Brown-Séquard's methods and found a negative result. She reports: "We prepared extracts from five dog testes using Brown-Séquard's methods and assayed testosterone concentrations. Testosterone concentrations were four orders of magnitude less than that required for a biological effect. Our study illustrates the marked placebo response that can be evoked by androgen treatment. It cautions against the empirical use of testosterone treatment for older men, unless a diagnosis of hypogonadism has been substantiated. **

At the moment of the experiment Brown-Séquard was 72 years old. After the experiment he claimed he felt as if he became younger by 30 years. However, 5 years later he died. Leonard Hayflick later referred to Brown-Séquard's ideas as "scrotum hokum".

References: Grmek p. 73; Haycock p. 169; Hayflick p. 272; Morley p. 1133; NYAM p. 125; Stambler p. 232.

*Brown-Séquard C.E. (1889) The effects produced on man by subcutaneous injection of a liquid obtained from the testicles of animals. *Lancet* 137: 105–107.

** See Cussons AJ, Bhagat CI, Fletcher SJ, Walsh JP. Brown-Séquard revisited: a lesson from history on the placebo effect of androgen treatment. *Med J Aust.* 2002 Dec 2-16;177(11-12):678-9.



Charles-Edouard Brown-Séquard & Dr. Jacques Arsène d' Arsonval, Note sur la préparation de l'extrait testiculaire concentré par M. A. d'Arsonval, suivie de règles relatives à l'emploi du liquide testiculaire, par MM. Brown-Séquard et d'Arsonval, G. Masson (Paris), 1893. From the Medical Library of Tim C. Opler.

Section 7:

The Early to Mid 20th Century:

Aging Research Picks Up Steam in
the 1900 to 1975 Period



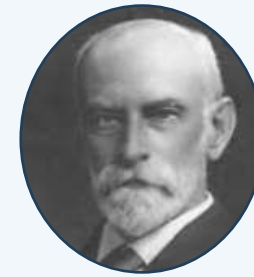
Enormous Progress

We entered the 20th Century with a strong understanding of what human lifespans are, a general view that lifestyle moderation was important to living longer and important hypotheses that linked aging to evolutionary forces and cellular damage.

However, we lacked specific insights from molecular biology as to what specifically explains the theoretical 90- to 100-year lifespan of humans.

Researchers made rapid [progress](#) on this topic between 1900 and 1975, although society continued to see its fair share of quackery and false starts linked to extraordinary tales of long-lived Bulgarian yoghurt eaters, testicular hormone injections and questionable drug candidates like Gerovital-1.

By 1975 we had made remarkable progress in molecular biology. Scientists rigorously applied insights from the genetic revolution, cell biology and caloric restriction theory to emerge with specific theories that had potential to explain human lifespan. We transitioned from an era of often vague philosophical inquiries to one of rigorous biological investigations supported by sound hypothesis generation.



Charles S. Minot
Aging is caused by cell damage.



Clive McCay
Caloric restriction increases lifespan



Peter Medawar
No evolutionary pressure after reproduction

1907
1908



Max Rubner
Basal metabolic rate impact lifespan



Barbara McClintock
Chromosome ends preserved in cell replication

1935
1938

1952
1955



Harman Denham
Oxidative stress theory

Evolutionary Perspectives

Theories of aging in the 20th Century continued to be shaped by insights into the theory of **evolution**, continuing earlier work by the Comte de Buffon, Alfred Russell Wallace and August Weissman.

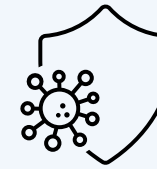
Foundational contributions in the 20th Century were made by J.B.S. Haldane, Peter Medawar and George C. Williams. Medawar was among the first to realize that the strength of natural selection declines with age even in a population of theoretically immortal organisms, because intrinsic immortality does not keep out death from accidents, predation or disease. Therefore, the probability of survival will keep declining with age, rendering late-life events unimportant in evolutionary terms.

Medawar proposed aging results from the declining force of natural selection with age. Harmful mutations that manifest late in life are not strongly selected against, leading to the accumulation of genetic damage over generations. Macfarlane Burnet extended this idea by emphasizing the role of immune system decline in aging, leading to increased susceptibility to disease.

George C. Williams introduced the antagonistic pleiotropy theory, which posits that some genes beneficial in early life may have detrimental effects later on, contributing to aging.



John Gurdon
iPSCs



Immune system
declines with age

Roy Walford



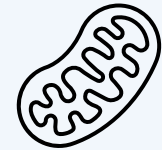
Macfarlane Burnet
Intrinsic mutagenesis

1961
1962



Leonard Hayflick
Cell replication
capacity is finite.

1969



1972

Mitochondria
Oxidation damages
the mitochondria

1974

Cellular Insights

Charles S. Minot was the first to explicitly link changes in the cell as the main causes of aging in 1907.

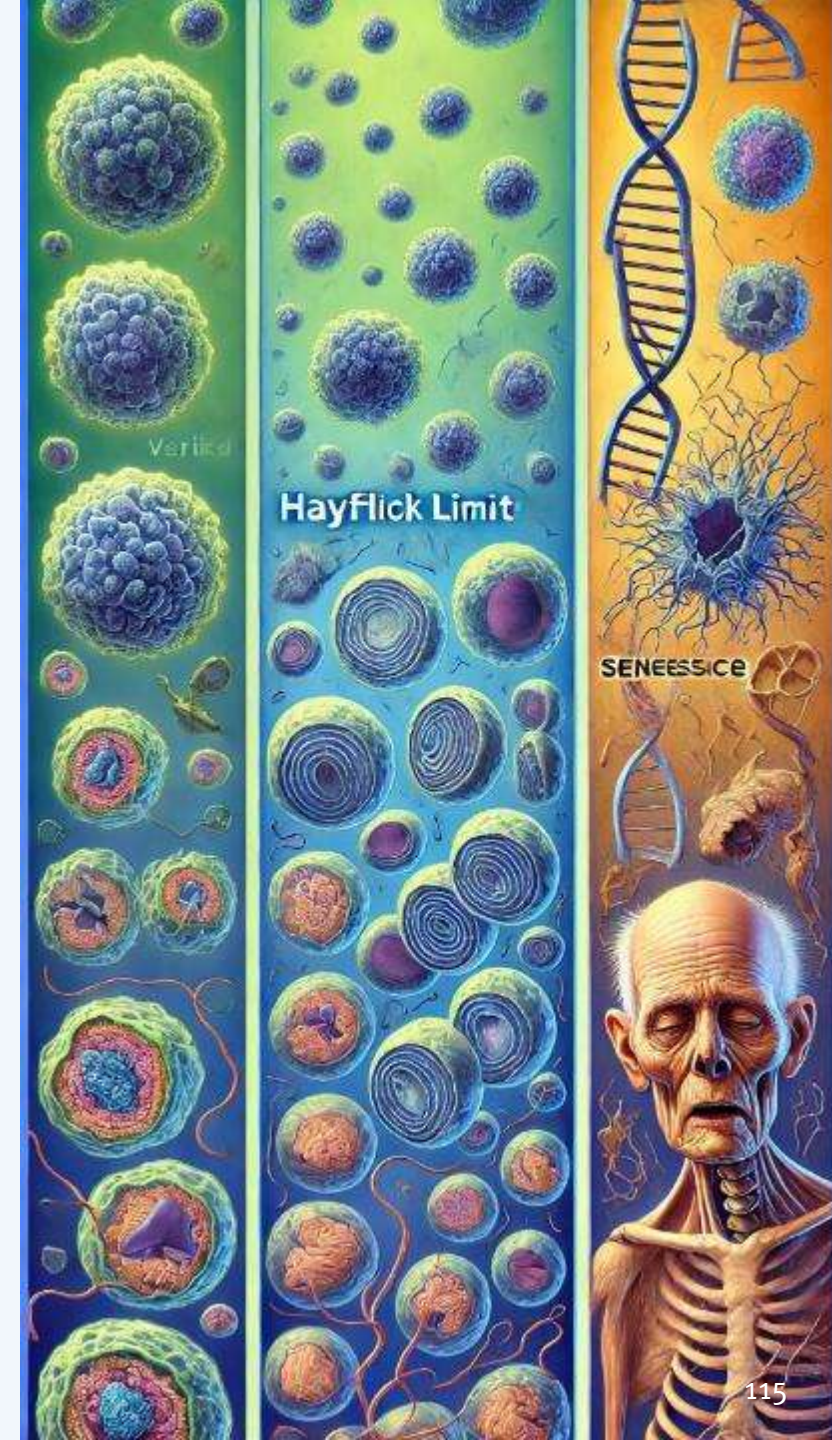
His work expanded on that of Bichat a century earlier who linked aging to changes in the body's tissue. Bichat specifically linked aging to breakdown in the cell cycle.

A major breakthrough in aging research came with the discovery of cellular senescence. Alexis Carrel, a Nobel laureate, initially claimed that cells could divide indefinitely in culture, suggesting that aging was not an intrinsic property of cells.

However, Leonard Hayflick and Paul Moorhead later demonstrated that normal human cells have a finite replicative capacity, a phenomenon now known as the Hayflick limit.

This discovery challenged Carrel's findings and established cellular senescence as a fundamental aspect of aging.

The work in the mid-20th Century set up the next 50 years for a spectacular run in insights into how cell senescence matters in aging.



Genes and Aging

The discovery that genetic factors influence lifespan was another major 20th Century breakthrough in gerontology.

Sir Macfarlane Burnet (1899–1985) was an Australian immunologist whose work on the immune system had significant implications for understanding aging.

Macfarlane Burnet argues that aging is fundamentally driven by the gradual accumulation of genetic mutations within somatic cells. He argues that these mutations occur spontaneously due to inherent errors in DNA replication and repair processes, rather than being primarily caused by external environmental factors.

Burnet builds on evolutionary and immunological perspectives, suggesting that aging is an inevitable consequence of an organism's biological programming, wherein natural selection favors early-life fitness over long-term genomic stability.

Tomas Lindahl is a Swedish biochemist whose work on DNA repair mechanisms earned him the Nobel Prize in Chemistry in 2015. Lindahl discovered the base excision repair (BER) pathway, which corrects small, non-helix-distorting lesions in DNA, such as those caused by oxidative stress and spontaneous hydrolysis. His research demonstrated that DNA is inherently unstable and requires constant repair to maintain genomic integrity.

Lindahl's work has profound implications for aging, as DNA damage accumulates over time and contributes to cellular dysfunction and age-related diseases. By elucidating the mechanisms of base excision repair, Lindahl provided insights into how cells counteract the effects of oxidative stress, a major driver of aging. His research has also highlighted the importance of maintaining DNA repair capacity to prevent genomic instability and promote longevity.

Additional Theories of Aging Enter the Field

Oxidative Stress and the Free Radical Theory

One of the most influential theories of aging is the free radical theory, proposed by Denham Harman in the 1950's. Harman hypothesized that reactive oxygen species (ROS), byproducts of mitochondrial respiration, cause cumulative cellular damage, leading to aging. This concept linked metabolism to oxidative stress, influencing subsequent studies on antioxidants and lifespan extension.

The Role of Metabolism and Caloric Restriction

The connection between metabolism and aging was highlighted by Max Rubner, who formulated the rate-of-living theory, proposing that organisms with higher metabolic rates age faster. Although later challenged, this theory influenced research on caloric restriction, which was further explored by Clive McCay, showing that reduced caloric intake prolongs lifespan in rodents.

Cell Renewal and Reprogramming

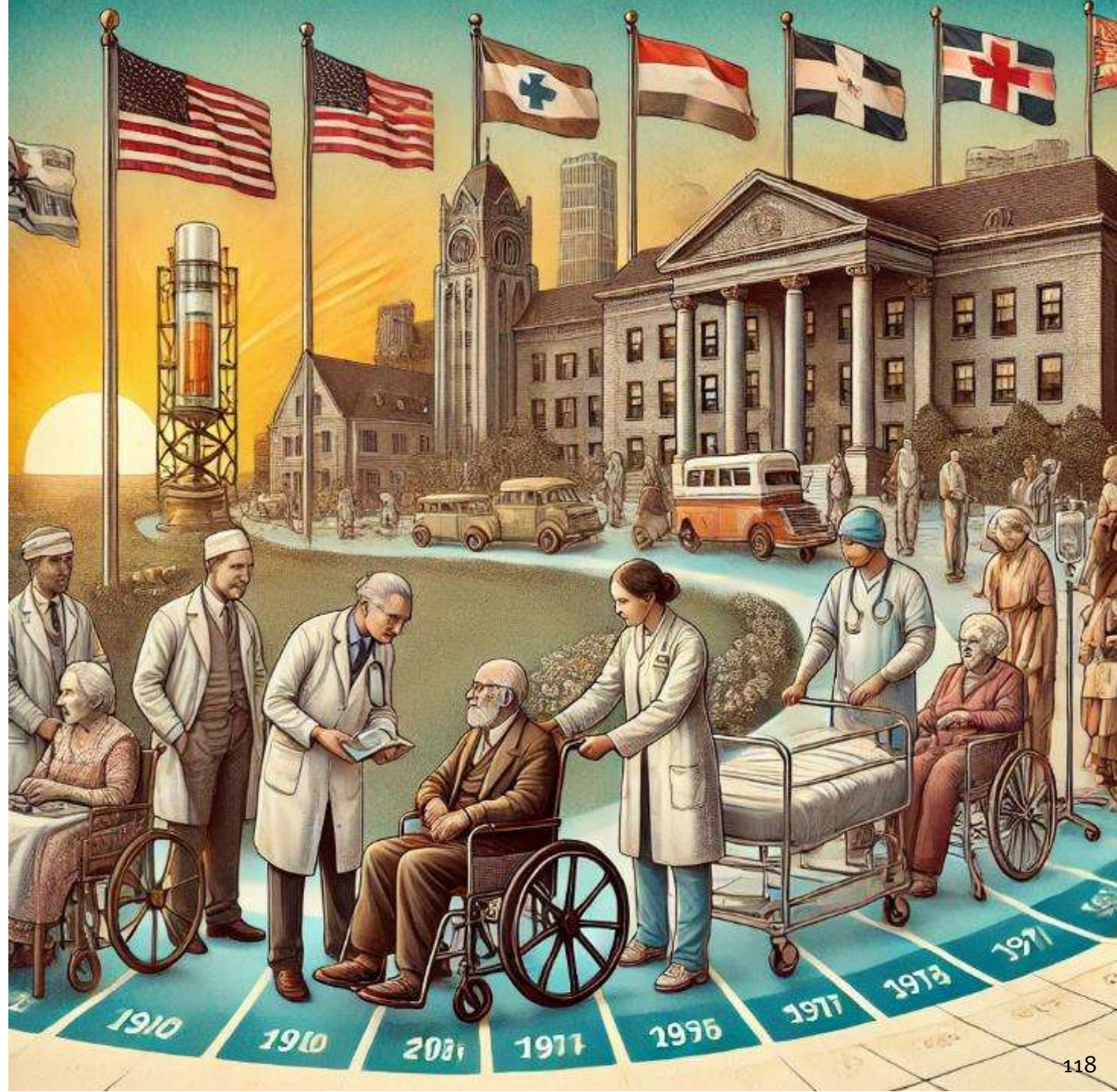
Another major milestone in cell biology was achieved by John Gurdon, whose work in the early 1960's on nuclear reprogramming and cloning demonstrated that cellular age is reversible. His research paved the way for induced pluripotent stem cells (iPSCs), raising hopes for regenerative therapies in aging – which are under development at present. John Gurdon received the Nobel Prize in Medicine in 2012 in acknowledgement for this work.

The Rise of Geriatrics

As lifespans increased throughout the 20th century due to progress in public health and the battle against infectious diseases, elderly populations began to increase substantially, creating societal pressure to better care for the elderly. This triggered the growth of the field of geriatrics, very much in the spirit of what Zerbi outline in his 1489 book *Gerontocomia*.

The word “geriatrics” was invented by Ignatz L Nascher, a Vienna-born immigrant to the United States in 1909.

While the American Geriatrics Society was founded in 1942, the thriving and influential medical specialty of geriatric medicine was essentially a product of the British NHS. While far from perfect, the care of the elderly today is far better than it was in 1900.

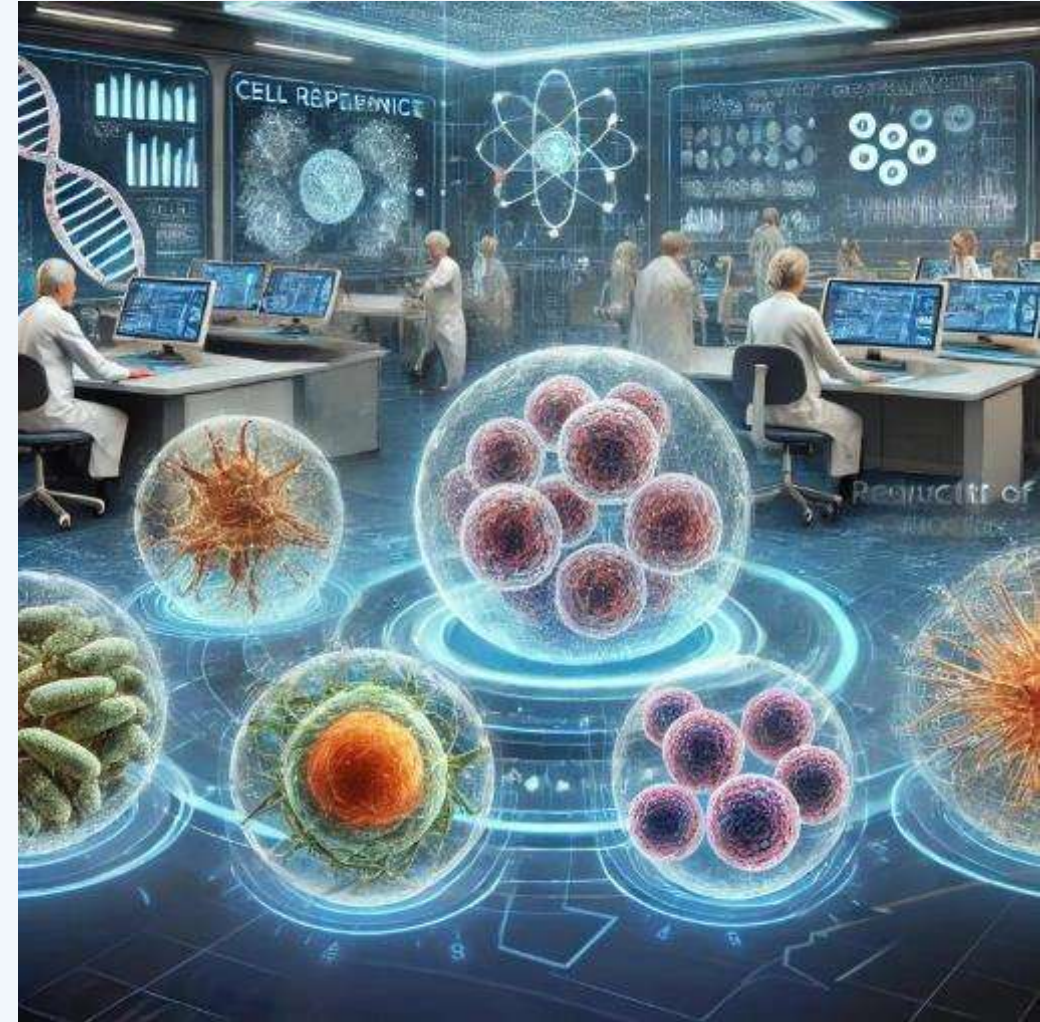


20th Century Scientists Open Door to Aging Research

The 20th century witnessed extraordinary progress in aging research, from evolutionary theories to molecular mechanisms involving telomeres, metabolism, and oxidative stress.

As we now transit to the extraordinary era of 21st century biology, the integration of genetic tools, advances in pharmacologic modalities, advances in computation and progress in regenerative medicine appear to be bringing humanity ever closer to significantly extending lifespan and the associated period of healthy life.

The agenda for the current century was described well by Nathan Shock, a leading geriatrician of the 20th century but also author of the most comprehensive bibliography and study of aging and geriatrics ever written. He wrote the following parting words in 1989, right before his death: “I would remind you that we were formed and nurtured in the firm belief that the biological phenomenon we call ‘aging’ was worthy of scientific pursuit. We have achieved some degree of success. I would caution, however, that our future will be determined only, and only, by the quality of our scientific research on understanding the basic mechanisms of aging processes” (see Baker GT. Nathan’s last words. *Exp Gerontol.* 1990;25:205–209).



1903: Sir Hermann Weber

Argued for short sleep cycles, ample exercise, moderate diet, fresh air and daily baths.

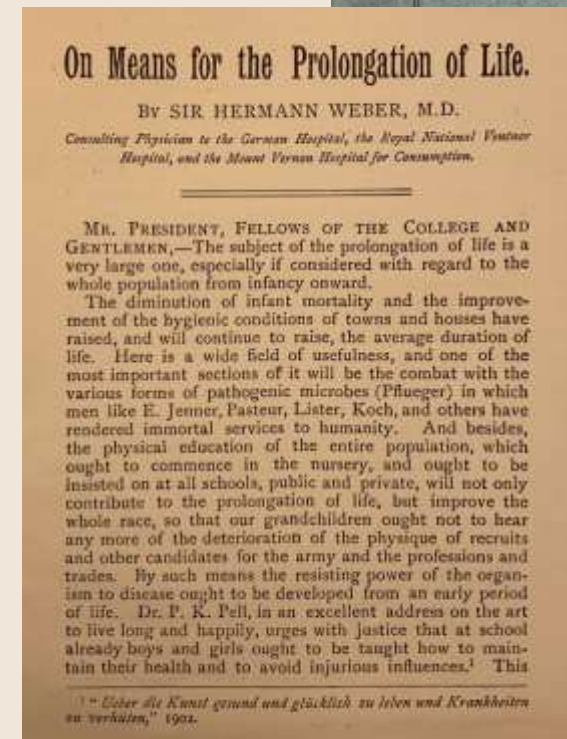
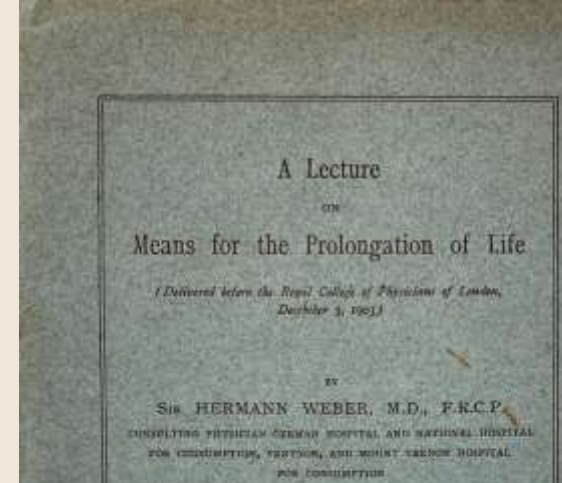
Hermann Weber's 1903 address, "On Means for the Prolongation of Life," was delivered before the Royal College of Physicians in 1903. Weber was 80 years old at the time.

[Hermann Weber](#) emphasized preventive medicine and lifestyle modifications as key to extending human life and improving health. Weber highlighted the importance of hygiene, sanitation, and public health initiatives to combat infectious diseases, which were major causes of mortality at the time. He advocated a balanced diet, moderation in eating, and regular physical activity to maintain vitality and prevent chronic disease. Weber also stressed the significance of mental and emotional well-being and intellectual engagement.

Weber (1823 to 1918) was a German-born physician who earned his medical degree from the University of Heidelberg. Weber was a man of extraordinary charm and quickly obtained a large and distinguished practice, his patients including five Prime Ministers. Weber was the leading advocate of his time for climatotherapy, the use of specific climates to treat chronic diseases, particularly respiratory conditions like tuberculosis.

What is most striking about this lecture is that it has *no real evidence* for any of its recommendations and was delivered relatively recently. Weber's core recommendations differ little from those provided by authors such as Galen 1700 years earlier or Cornaro three hundred years earlier (eat in moderation, exercise and get fresh air). If you will, as recently as 125 years ago there was essentially *no* scientific evidence with which to advise on how to live a long life. The contrast, for example, with Peter Attia's 2024 book *Outlive* is striking. Attia has quite a bit of scientific evidence to go on and uses it to dispense advice on how the reader can live a long life.

References: Bailey 285; Osler 4199



Hermann Weber, *On Means for Prolongation of Life*, London: John Bale, Sons and Danielsson, 1903. A Lecture custom printed in a blue wrapper. Also appeared in the *British Medical Journal* at the time. From the Medical Library of Tim C. Opler.

1903: Elie Metchnikoff

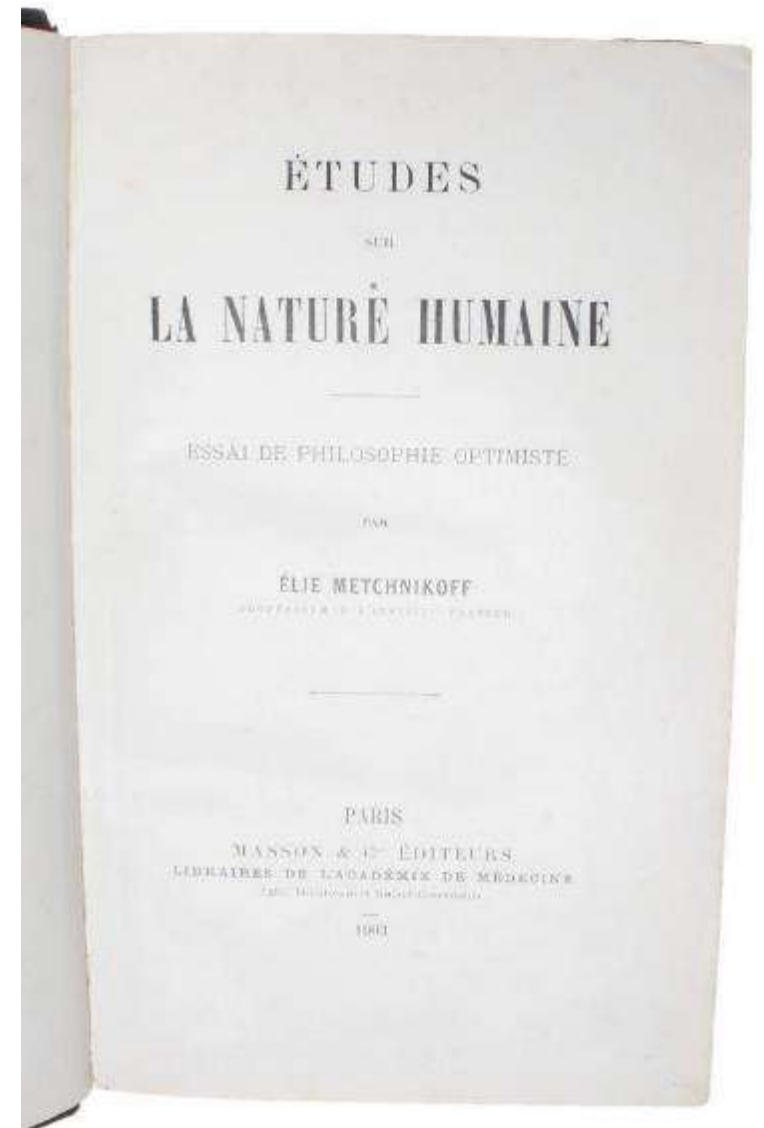
Saw longevity from control of inflammation and a maintaining a healthy microbiome. While plausible, neither idea survived evidential scrutiny.

[Elie Metchnikoff](#), a Russian immunologist and Nobel laureate, developed a unique perspective on aging and longevity, focusing on the role of the immune system and gut microbiome.* He believed that aging was partly due to the accumulation of harmful cellular debris and toxic substances in the body, which phagocytes—immune cells he discovered—could not effectively clear as people aged.

In his 1903 book *Études sur la nature humaine. Essai de philosophie optimiste* (Studies on Human Nature. Essay of Optimistic Philosophy), he argued that toxins produced by harmful gut bacteria played a significant role in aging and chronic diseases. This view contrasted with the prevailing idea that aging was an unavoidable decline, as Metchnikoff believed scientific interventions could improve longevity. Metchnikoff was the first to coin the word gerontology (in this book). A central element of his theory was the impact of intestinal health on aging. He proposed that putrefactive bacteria in the colon produced harmful compounds that contributed to tissue degeneration and aging. To counteract this, he recommended consuming fermented dairy products, particularly yogurt and kefir. "In his *Studies on Human Nature* Metchnikoff argued that when diseases have been suppressed and life has been hygienically regulated, death would come only with extreme old age. Death would then be natural, accepted gratefully, and robbed of its terrors." (D.S.B. IX: 334-35).

References: Achenbaum p. 43.; Bailey 248; DSB IX, 334; Freeman p. 47; Grmek p. 91.; Haycock p. 159; Hayflick p. 275; Medawar p. 17; Morley 1133; NYAM p. 126 (later ed); Stambler p. 232.

* Grmek (1958) wrote of this work: "Metchnikoff defended his attitude very forcibly and produced, by his *Optimistic Studies*, a sensation in the first decades of the 20th century... In the light of modern scientific investigations, both of Metchnikoff's assumptions have proved to be wrong. In senescence neither an exogenous intoxication nor a pathologic phagocytosis plays any part whatsoever."



Elie Metchnikoff, *Études sur la nature humaine. Essai de philosophie optimiste*, Paris. 1903. From the Medical Library of Tim C. Opler.

1907/1908: Charles S. Minot

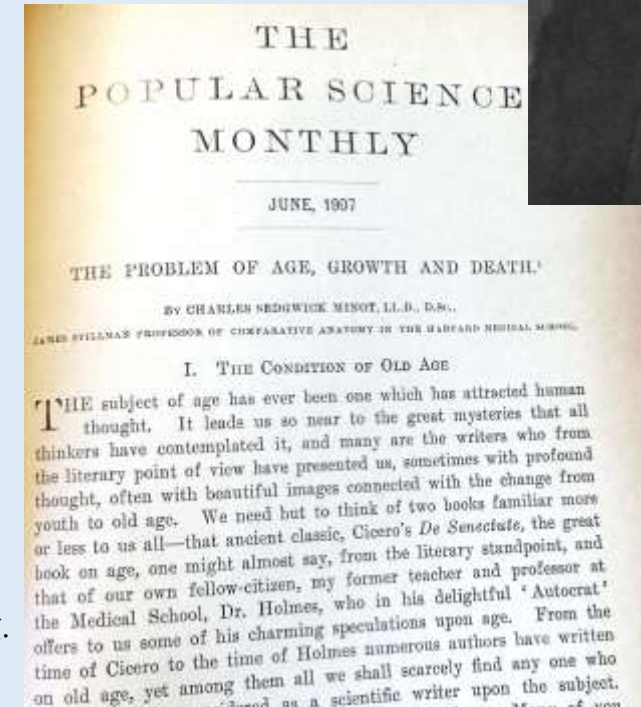
Minot was the first to explicitly link changes in the cell as the main causes of aging. His work expanded on that of Bichat (1800) who linked aging to changes in the body's tissue.

Minot's theory, outlined in his book as *The Problem of Age, Growth, and Death* (1908) and accompanying [article](#) in *Popular Science Monthly* in 1907 links aging to the developmental processes of cells and tissues. He introduced the term cytomorphosis to describe the progressive changes in cellular structure and function that occur over an organism's lifespan.

[Charles Minot](#) saw these changes as following a predictable pattern that ultimately leads to the decline of physiological functions and the onset of aging. Minot argued that the rate of growth plays a central role in determining the lifespan of an organism. He observed that organisms with rapid growth rates tend to have shorter lifespans, while those with slower growth rates live longer.

This inverse relationship between growth rate and longevity is attributed to the idea that rapid growth accelerates cytomorphosis, leading to earlier cellular maturation and subsequent degeneration. Minot's theory suggests that aging is a continuation of developmental processes rather than a separate phenomenon, emphasizing the importance of understanding cellular changes over time. Modern aging research has expanded on these concepts, incorporating genetic, molecular, and environmental factors into the study of aging. Minot's work was pioneering in portraying aging as a biological process tied to cellular development and growth.

References: Achenbaum p. 39; Bailey 978; Freeman p. 47; GMN 132; Grmek p. 70; Hayflick p. 229; Medawar, p. 20; NYAM p. 126 (book); Osler 3412.



Charles S. Minot
James Stillman Professor
of Comparative Anatomy
Harvard Medical School
1880 to 1914

Charles S. Minot, *The Problem of Age, Growth and Death*, an article in *Popular Science Monthly*, Vol 7, 1907. Also, *The problem of age, growth and death*. New York: G. P. Putnam, 1908. Both from the Medical Library of Tim C. Opler.

Charles Minot Links Death to an Ongoing Breakdown in the Cell Cycle Caused by Genetics

Next as regards death. The body begins its development from a single cell, the number of cells rapidly increase, and they go on and on increasing through many years. Their whole succession we may appropriately call a cycle. Each of our bodies represents a cell cycle. When we die, the cycle of cells gives out, and, as I have explained to you in a previous lecture, the death which occurs at the end of the natural period of life is the death which comes from the breaking down of some essential thing—some essential group of members of this cell cycle; and then the cycle is broken up. But the death is the result of changes which have been going on through the successive generations of cells making up this cycle. There are unicellular organisms; these also die; many of them, so far as we can now determine, never have any natural death, but there are probably others in which natural death may occur. It is evident that the death of a unicellular organism is comparable to the death of one cell in our own bodies.

From *Death*, an article in *Popular Science Monthly*, Vol 7, 1907. From the Medical Library of Tim C. Opler.

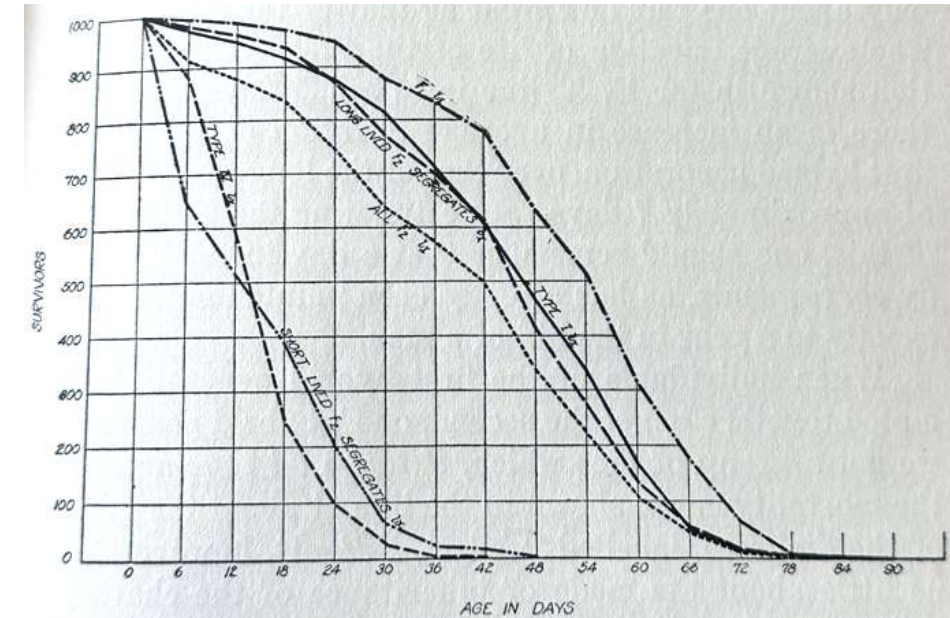


FIG. 49.—Life lines showing the result of Mendelian experiments on the duration of life in *Drosophila*. Explanation in text.



This chart shows the very different life spans that results from Minot's Mendelian experiments with fruit flies.

1908: Max Rubner

Pioneer in basal metabolic rate theory.

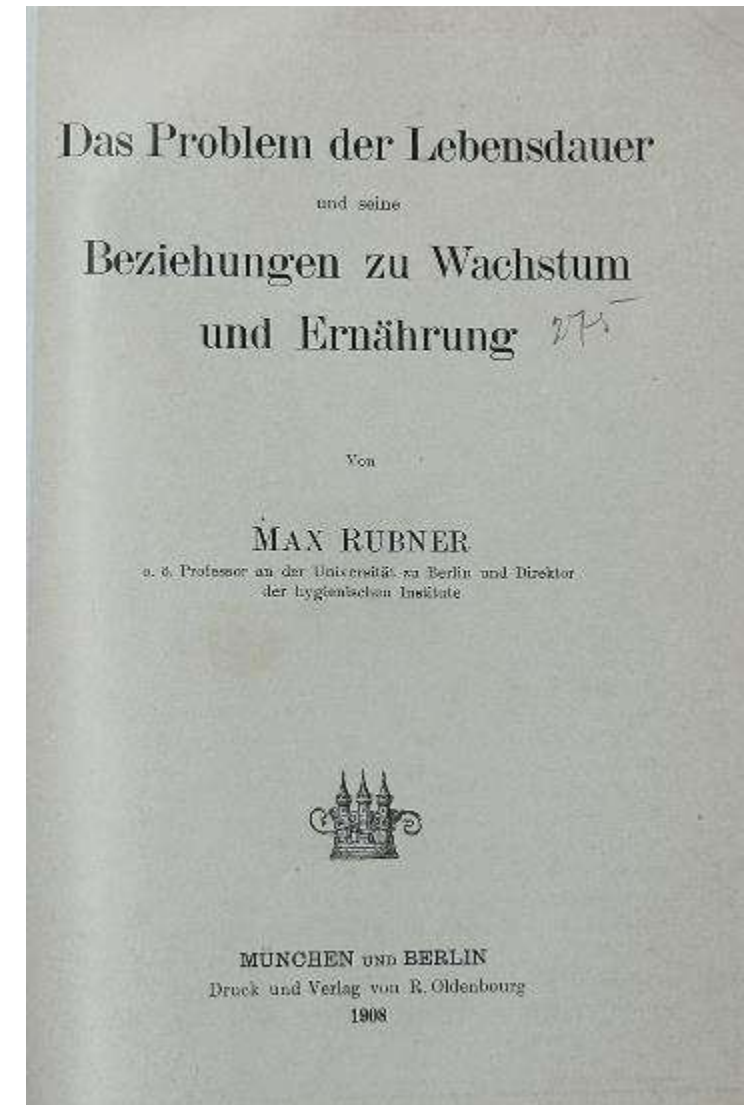
Max Rubner's *Das Problem der Lebensdauer und seine Beziehungen zu Wachstum und Ernährung* (The Problem of Life Span and Its Relationship to Growth and Nutrition) explores the relationship between metabolism, growth, nutrition, and lifespan. As a physiologist, Rubner was particularly interested in the role of energy consumption in aging and developed what later became known as the "rate of living" theory. He argued that the total energy expenditure of an organism over its lifetime is relatively fixed, meaning that species with higher metabolic rates tend to have shorter lifespans.

[Max Rubner's](#) work was among the first to systematically connect metabolic processes to longevity, laying the foundation for later studies on how energy balance affects aging. Rubner supported his theory with empirical data, comparing the metabolic rates and lifespans of different animal species. He observed that smaller, warm-blooded animals with higher metabolic rates, such as rodents, tended to age and die more quickly than larger animals like humans or elephants, which have slower metabolic rates.

He suggested that aging is essentially a process of gradual wear and tear at the cellular level, driven by the consumption of metabolic energy. Furthermore, he explored the role of nutrition, suggesting that caloric intake and energy metabolism influence longevity, ideas that later influenced research on caloric restriction and aging.

Rubner's work was a major contribution to early gerontology, but his conclusions have since been refined. While his rate of living theory provided a compelling framework, later discoveries, particularly in genetics and molecular biology, showed that lifespan is influenced by more than just metabolic rate.

References: Grmek p. 75.



Max Rubner, *Das Problem der Lebensdauer und seine Beziehungen zu Wachstum und Ernährung*, Munich and Berlin. 1908. From the Medical Library of Tim C. Opler.

1912: Arnold Lorand

Argued that aging was caused by deterioration of endocrine glands. This book was quite popular at the time and went through many subsequent printings.

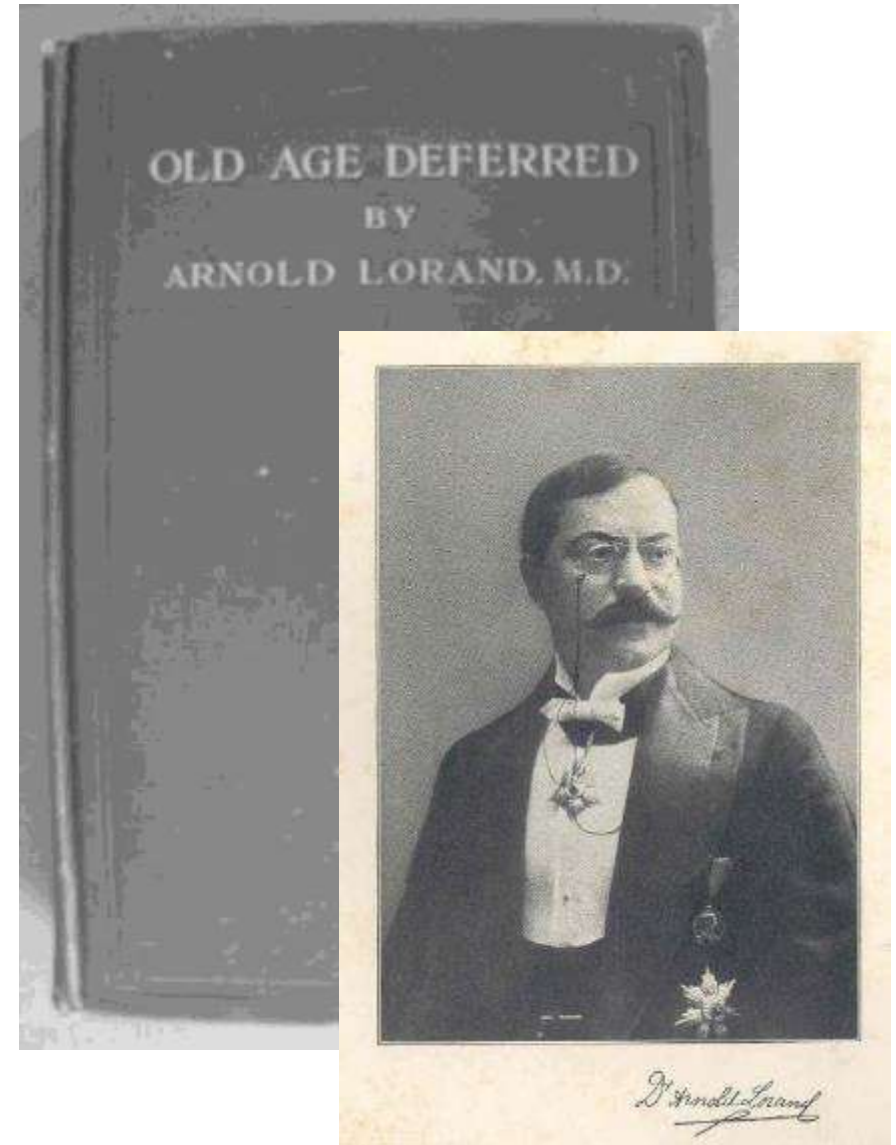
[Arnold Lorand](#), picking up on research of Brown-Sequard, Victor Horsley and others believed that the principal endocrine glands such as the thyroid were the key to longevity. He wrote: "We need no longer grow old at forty or fifty; we may live to the age of ninety or one hundred years, instead of dying at sixty or seventy. All this can be brought about by the observance of certain hygienic measures, and by improving the functions of a certain few of the glandular structures in our body, provided incurable organic disorders have not already too gravely compromised one or more of our main organs."

Lorand believed that glands such as the thyroid, pituitary, adrenal, and sex glands played a critical role in maintaining vitality, metabolism, and overall health.

Lorand emphasized that symptoms of aging, such as fatigue, weight gain, and cognitive decline, were often due to glandular dysfunction rather than chronological age, and he advocated for a holistic approach to delay aging through diet, exercise, and medical interventions.

While Lorand's theories were innovative, they were not without criticism, as some of his ideas, particularly the use of glandular extracts, were based on limited scientific evidence. Subsequent work has failed to validate his ideas regarding the role of the endocrine system as a principal driver of aging.

References: Achenbaum p. 41; Bailey 244 (1910 ed); Grmek p. 102; Morley p. 1144; NYAM p. 127 (later ed); Waller 269 (Swedish version)



Arnold Lorand, *Old Age Deferred*. F.A. Davis (Philadelphia), 1912. Second edition. From the Medical Library of Tim C. Opler.

1912: Alexis Carrel

Argued that fibroblast cells could live and replicate outside of an organism indefinitely. This idea was later challenged by Leonard Hayflick.

[Alexis Carrel](#) (1873 to 1944) was a French surgeon, anatomist and physiologist. He received the Nobel Prize for Physiology or Medicine in 1912. Alexis Carrel concentrated primarily on experimental surgery and the transplantation of tissue and entire organs.

In his 1912 paper on the life of tissues outside of the organism entitled “On the Permanent Life of Tissues Outside of the Organism,” he reported on experiments made at the end of 1911 and at the beginning of 1912. Fragments of connective tissue were kept *in vitro* in a condition of active life for more than two months. He did not observe a limit to how long tissues could grow and replicate in these experiments. For example, fragments of heart pulsated rhythmically at the beginning of the third month of their life *in vitro*. In this paper, Carrel showed that cells could live *in vitro* for quite a long time. The observed rather extended growth of tissue cultures taken from a chick embryo led Carrel to believe that cells could potentially live forever outside of a living organism.

It is important to note that despite later commentaries from adherents of the “Hayflick Limit”, Carrel never claimed that he had proven that tissues could live forever. He simply suggested that it was plausible. This paper became a punching bag of sorts for Hayflick devotees and is attacked rather heavily in Ramakrishnan’s 2024 book *Why We Die* who points to potential experimental errors in his work. Carrel did himself no favor by the selection of the title of this paper which fails to match the data presented within.

References: Achenbaum p. 53; Bailey 1021; Grmek p. 148; Haycock p. 172; Hayflick p. 112; Medawar p. 26; Ramakrishnan p. 69; Stambler p. 232; Waller p. 89.

ON THE PERMANENT LIFE OF TISSUES OUTSIDE OF THE ORGANISM.*

By ALEXIS CARREL, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

PLATES 75 AND 76.

INTRODUCTION.

It is known that the life of a tissue cultivated *in vitro* is very short.¹ Generally, in three to fifteen days after the preparation of the cultures their growth becomes less rapid and eventually stops completely. The tissues then die and the cells disintegrate.

The purpose of the experiments described in this article was to determine the conditions under which the active life of a tissue outside of the organism could be prolonged indefinitely. It might be supposed that senility and death of the cultures, instead of being necessary, resulted merely from preventable occurrences; such as accumulation of catabolic substances and exhaustion of the medium. The suppression of these causes should bring about the regeneration of old cultures and prevent their death. It is even conceivable that the length of the life of a tissue outside of the organism could exceed greatly its normal duration in the body, because elemental death might be postponed indefinitely by a proper artificial nutrition.

In the following experiments I sought not a complete solution of the problem of prolonging indefinitely the life of tissues *in vitro* but the facts that would show in which direction we should proceed in order to secure a complete solution, if such a solution was possible.

In September, 1911, I tried to increase the duration of the life of cultures of connective tissue.² The cultures were washed

* Received for publication, March 15, 1912.

¹ Harrison, Harvey Lectures, Philadelphia and London, 1911-1912; *Exper. Zool.*, 1910, ix, 787; Carrel and Burrows, *Jour. Exper. Med.*, 1911, 387.

² Carrel, *Jour. Am. Med. Assn.*, 1911, lviii, 1611.



Alexis Carrel, “On the Permanent Life of Tissues Outside of the Organism,” *J Exp Med.* May 1 2012;15(5):516-28.

1914: Ignatz Leo Nascher

GMN: “The first modern treatise on the subject. Nascher coined the term ‘geriatrics’ in a paper of that name in N.Y. Med. J., 1909, 90, 358-59.”

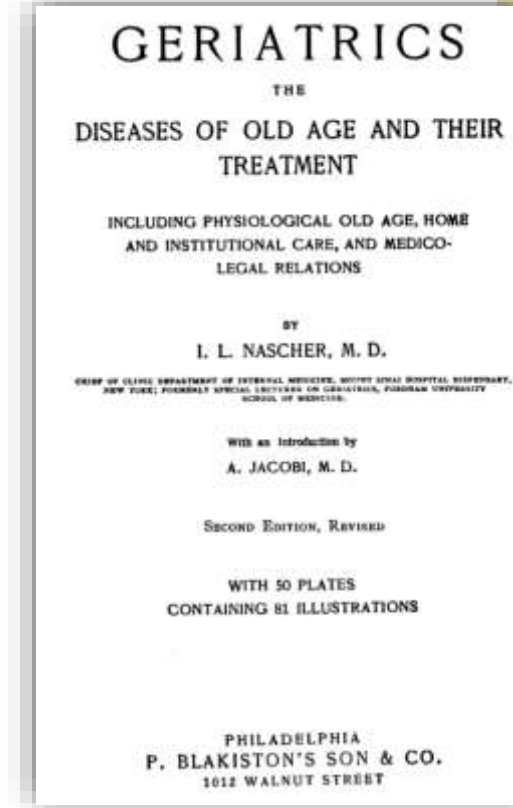
[Ignatz Leo Nascher](#) published *Geriatrics: The diseases of old age and their treatment* in 1914. Nascher wrote that the medical needs of older adults were distinct from those of younger patients and required specialized attention. His work laid the foundation for geriatrics as a medical discipline, emphasizing the importance of understanding the physiological changes of aging and developing tailored treatments for age-related diseases.

His 1914 book comprehensively addressed chronic illnesses, mobility issues, cognitive decline, and the social and legal challenges faced by the elderly. He advocated for a holistic approach to care, integrating medical treatment with considerations for the patient's living environment, social support, and quality of life.

Nascher's work was revolutionary for its time, challenging the prevailing view that aging was an inevitable decline not worthy of medical intervention. He argued that many conditions associated with old age were treatable and that older adults could lead healthier, more fulfilling lives with proper care. He argued that “as a humanitarian it is [the physician’s] duty to prolong life as long as there is life and to relieve distress wherever he may find it.”

Nascher also highlighted the need for specialized training for physicians in geriatric medicine and called for the establishment of institutions dedicated to the care of the elderly. His ideas were ahead of their time and paved the way for the development of modern geriatrics.

References: Achenbaum 50; Bailey 251; Freeman p. 48; GMN 1641.1; Grmek p. 141; Hayflick p. 2; Morley p. 1133; NYAM p. 127; Schafer p. 187; Stambler p. 232.



Ignatz L. Nascher, *Geriatrics: The diseases of Old Age and their Treatment*, 1916 (second edition). Philadelphia: P. Blakiston's Son & Co. Also, signed photograph of Ignatz Leo Nascher. From Ehrman Medical Library Archives at NYU.

1919: Jacques Loeb

Famed molecular biologist Jacques Loeb saw aging as a function of metabolism and heat. The higher the heat of an organism, the faster it ages.

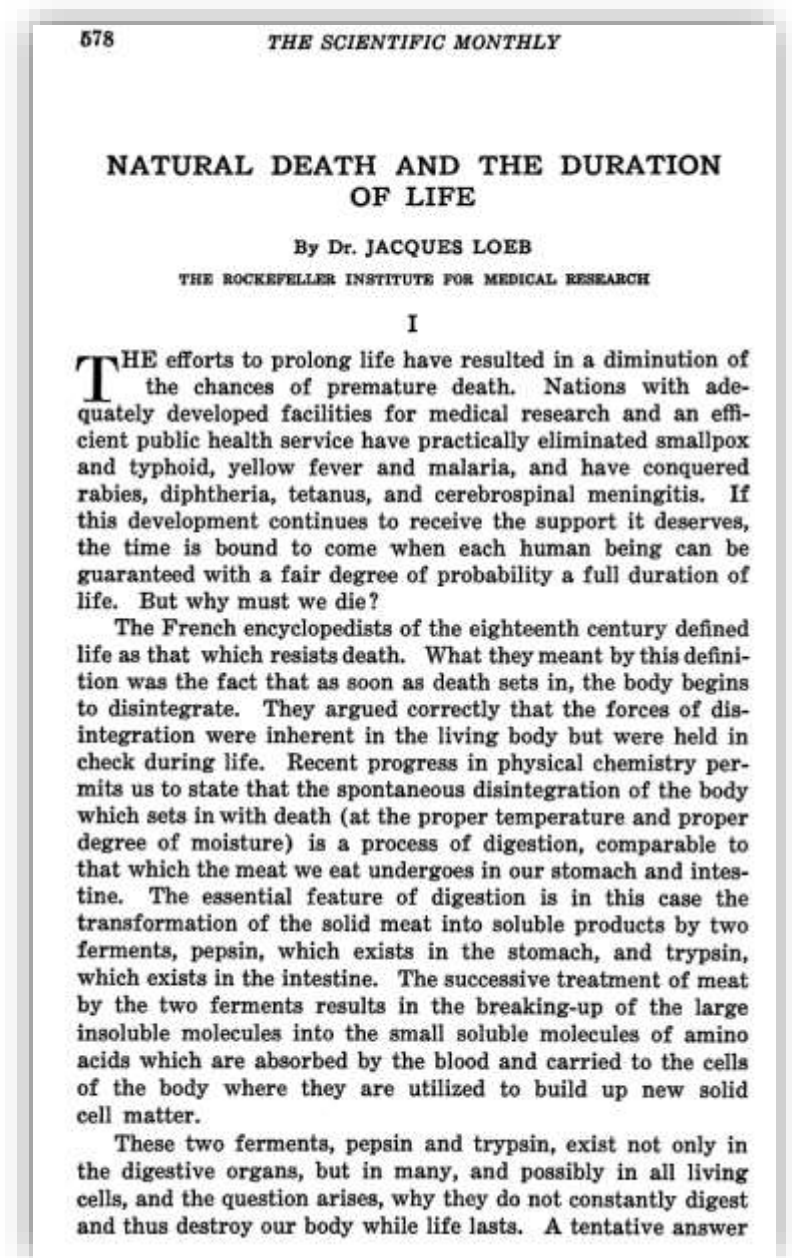
Jacques Loeb's views on aging, as presented in his 1908 paper *Ueber die Ursache des natürlichen Todes* ("On the Cause of Natural Death") and his 1919 paper *Natural Death and the Duration of Life*, emphasize the role of metabolism and temperature in determining lifespan.

[Jacques Loeb](#), a pioneering biologist, argued that the rate of biochemical processes, plays a crucial role in aging and natural death. He proposed that organisms have a finite amount of physiological energy, and their lifespan is largely dictated by how quickly they consume this energy through metabolic activity.

He was not specific in identifying oxidative stress as the cause of aging. A central idea in Loeb's work is that temperature influences the speed of metabolic reactions, thereby affecting the rate of aging. He suggested that higher temperatures accelerate metabolic processes, leading to a faster depletion of an organism's vital resources and an earlier death.

Conversely, lower temperatures slow metabolic activity, potentially extending lifespan. This idea aligned with the broader concept of the "rate of living" theory, which posits that lifespan is inversely related to metabolic rate. Loeb supported his arguments with experimental observations, including studies on cold-blooded animals, whose lifespans could be prolonged by lowering their body temperature.

References: Grmek p. 148; Stambler p. 232.



Jacques Loeb, *Natural Death and the Duration of Life*, *The Scientific Monthly*, 1919.

1920: Raymond Pearl

Pearl proposed that lifespan is inversely related to metabolic rate, suggesting that organisms with slower metabolisms live longer.

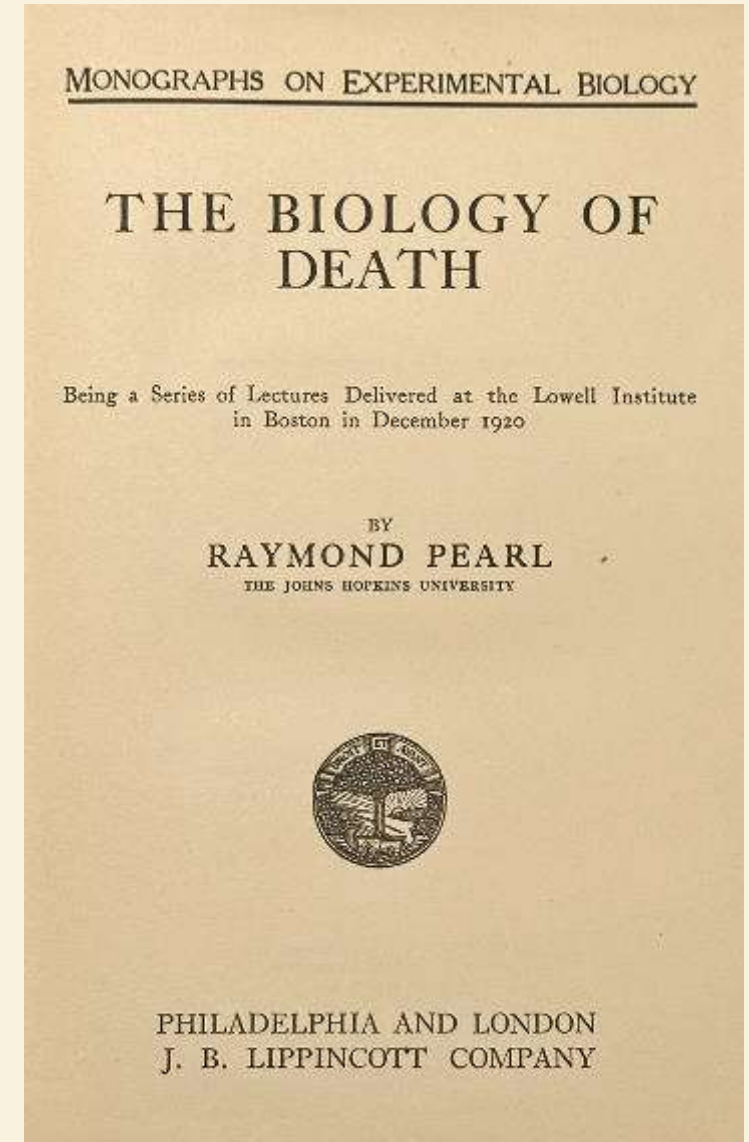
[Raymond Pearl](#) (1879-1940) was an American biologist, regarded as one of the founders of biogerontology. He spent most of his career at Johns Hopkins University in Baltimore.

In 1908 Max Rubner observed that mammals of different size and longevity had equal mass specific metabolic output. Pearl (like Rubner) also asserted that maximum life span is inversely proportional to basal metabolic rate. Pearl accepted Alexis Carrel's erroneous ideas that normal somatic cells don't age, and that aging must therefore be due to dysfunction at the body level. Pearl speculated that lifespan was limited by vital cell components that were depleted or damaged more rapidly in animals with faster metabolisms. Denham Harman's free-radical theory of aging later provided a plausible causal mechanism for Pearl's hypothesis.

The Rate of Living Hypothesis enjoyed prominence as one of the foremost theories of aging for nearly 50 years. However, when modern statistical methods for correcting for the effects of body size and phylogeny are employed, metabolic rate does not correlate with longevity in mammals or birds.

MONOGRAPH CONTENTS: I. The Problem; II. Conditions of Cellular Immortality; III. The Chances of Death; IV. The Causes of Death; V. Embryology and Human Mortality; VI. The Inheritance of Duration of Life in Man; VII. Experimental Studies on the Duration of Life; VIII. Natural Death, Public Health, and the Population Problem.

References: Achenbaum p. 68; Bailey 815; Hayflick p. 64; Medawar p. 28; Stambler p. 232. Also see WC Alvarez, "Raymond Pearl, Student of Longevity," *Geriatrics* 14:56-58, 1959.



Raymond Pearl, *The Biology of Death*, Philadelphia and London: J.B. Lippincott Company (signed by the author), 1922. From the Medical Library of Tim C. Opler.

1920: Serge Voronoff

He claimed that transplanting animal organs could cause human recipients to become younger. This idea, similar to that of Brown-Séquard, was later disproven.

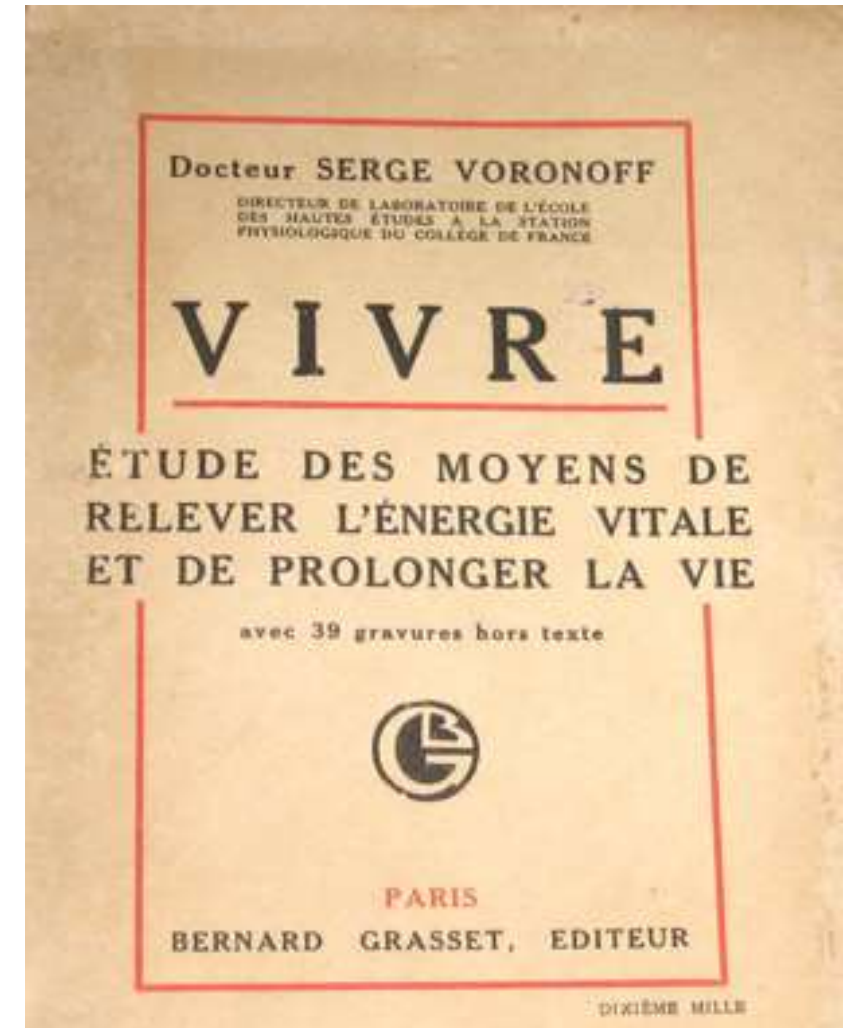
Serge Voronoff's 1920 book "Vivre" (translated as "To Live") is a controversial and ambitious work that reflects the author's fascination with rejuvenation and the extension of human life. Voronoff, a Russian-French surgeon, was infamous for his experiments with glandular transplantation, particularly using animal testicular tissue to restore youth and vitality in aging men.

On October 9, 1919 [Voronoff](#) announced his rejuvenation experiments by graft transplantation. There was much enthusiasm, but Voronoff immediately became notorious for these experiments.

In *Vivre*, Voronoff outlines his theories on the potential of glandular therapy to combat aging, improve physical and mental health, and even enhance human capabilities. The book is a mix of scientific speculation, case studies, and musings on the nature of life.

Voronoff was part of a larger trend that died out in the 1940's. Stambler (2018) writes: "Beside Voronoff, a cohort of French pioneers of endocrine rejuvenation (mainly practicing sex gland tissue transplantations and various other means of endocrine stimulation) included Placide Mauclaire, Lois Dartigues, Raymond Petit, Léopold Lévi, Henri de Rothschild, and others..." Notably, Voronoff's work was later discredited as lacking rigorous scientific evidence.

References: Bailey 478 (English ed.); Grmek p. 103; Haycock p. 177; NYAM p. 127; Stambler p. 232.



Serge Voronoff, *Vivre: Etude des Moyens de Relever L'Energie Vitale et de Plonger La Via*, Paris: Bernard Grasset, 1920. From the Medical Library of Tim C. Opler.

Pseudo-Science in the 20th Century

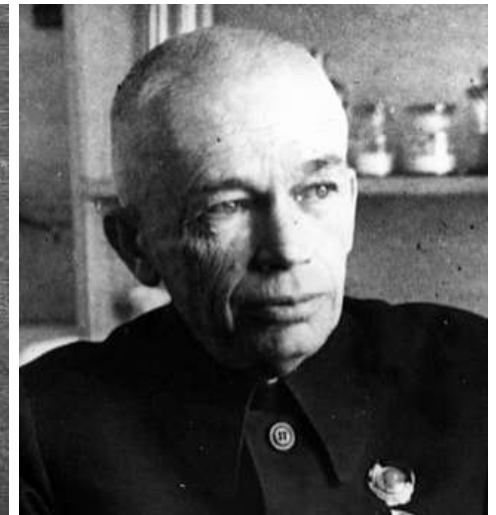
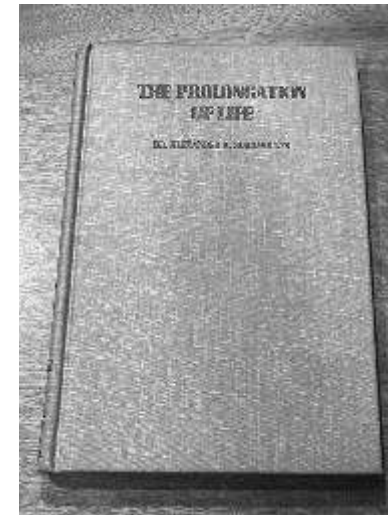
Voronoff's work in the 1920's was but one of many pseudo-scientific efforts that attempted to convince all too gullible audiences to take a shot at immortality.

One can't help be struck by the rise in pseudo-scientific ideas in the late 19th Century and the continuity of these ideas into the 20th and today.

If we live in the era of Enlightenment, someone must have forgot to turn on a few switches.

Ilia Stambler of Bar Ilan University has done quite a good job of illustrating the various anti-aging pseudo-scientific movements of the 20th Century in his detailed 2014 [book](#): *A History of Life-Extensionism In The Twentieth Century*. David Boyd Haycock's 2008 book *Mortal Coil* also reviews the various types of pseudo-science that captured the public imagination in the early 20th Century. Ramakrishnan (2024) picks up where Haycock and Stambler jump off, giving us a full look at today's curious cryopreservation and transhumanist movements. Voronoff and [Steinach](#) were two of many prominent names that pushed poorly validated aging remedies. Key ideas that have been heavily explored in the last 150 years and not validated have included (1) steroid supplementation, (2) cybernetics, (3) organ replacement, (4) hyperbaric oxygen therapy, (5) endocrine rejuvenation, (6) bionics, (7) cryopreservation, (8) cell colloid reversal, (9) busting of cross-linked proteins, (10) light therapy and (11) Anti-Reticular Cytotoxic Serum.

The story of Soviet scientist Alexander Bogomolets' [efforts](#) with anti-reticular cytotoxic serum make for particularly interesting reading. Bogomolets was a legitimate scientist with real credentials in gerontology who wrote the popular book *The Prolongation of Life*. In 1934, he began to promote the idea that a spleen and bone marrow extract from dead humans might be centrifuged and injected into animals. The resulting serum from the animals supposedly had antiaging properties in humans. This idea took off in both the USSR and the US and caught the attention of Josef Stalin who got [regular shots](#) of the stuff. Stalin died at age 75 and Bogomolets died at age 65.



Alexander A. Bogomolets, *The Prolongation of Life*, Second edition of English translation from Russian. Translated by Peter V. Karpovich, M.D., and Sonia Bleeker. Robinson Foundation: New York, 1946. From the Medical Library of Tim C. Opler.

1921: Alexis Carrel

Found that fibroblasts of older animals were less able to replicate and had lower lifespans *in vitro* than those of younger animals.

Alexis Carrel and his coauthor, Albert Ebeling, examined the viability of fibroblasts *in vitro* as a function of the age of their source animal. They find:

“There was, therefore, no doubt that the rate of multiplication of fibroblasts and the duration of their life *in vitro* varied in inverse ratio to the age of the animals from which the plasma or the serum was taken. The graph representing the rate of growth, in function of the age of the animals which have given the plasma, shows that the differences are very large. Another graph expresses the relation between the duration of life and the age. These curves show that there is a definite relation between the rate of proliferation of the fibroblasts, the length of their life *in vitro*, and the age of the animal.” Further: “Under the conditions of the experiments and within the limits of accuracy of the method, there is a definite relation between the rate of growth of a pure culture of fibroblasts, cultivated in plasma, and the age of the animal from which the plasma is obtained, the rate of cell multiplication varying in inverse ratio to the age. A similar relation exists between the duration of life *in vitro* of the fibroblasts, and the age of the animal. The variations in the rate of growth of a pure culture of fibroblasts may be used as a reagent of certain modifications occurring in blood serum under the influence of age.”

While an early work on cell senescence, Carrel and Ebeling fail to cite the very similar Prodomo work of Spallanzani in 1768 or the work of Charles Minot.

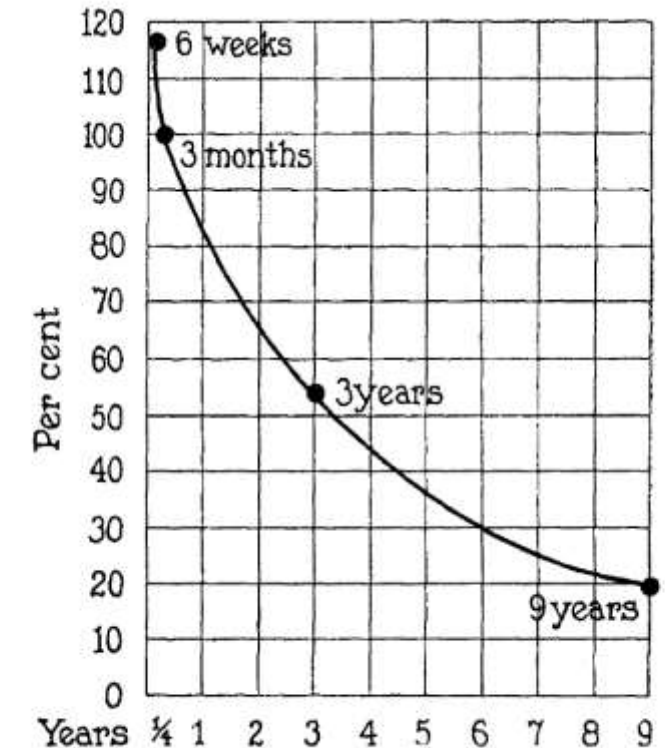
References: Grmek p. 148.

AGE AND MULTIPLICATION OF FIBROBLASTS.

By ALEXIS CARREL, M.D., AND ALBERT H. EBELING, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, June 11, 1921.)



TEXT-FIG. 11. Duration of life *in vitro* of fibroblasts in function of the age of the animals from which the plasma was taken.

Alexis Carrel and Albert Ebeling, *Age and Multiplication of Fibroblasts*, *Journal of Experimental Medicine*, 1921.

1929: Aldred Scott Warthin

Warthin, a Professor of Pathology at the University of Michigan, provided a detailed analysis of aging processes in a 1929 address to the New York Academy of Medicine.

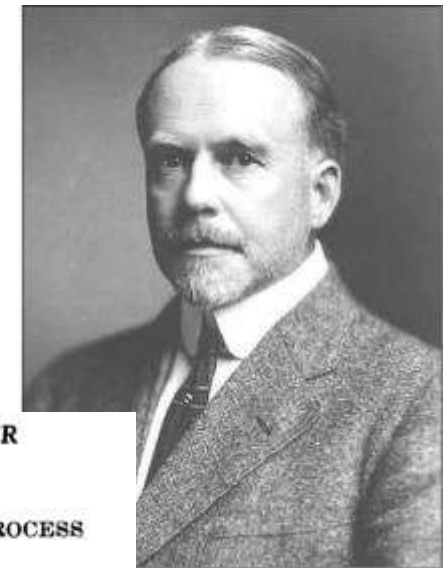
Warthin (1866-1931) was an acclaimed professor of pathology at the University of Michigan and director of the university's pathological library. He worked in a variety of fields and wrote numerous papers and books and made thorough studies on syphilis.

This is a comprehensive work on attitudes towards aging, senility, and death. [Aldred Warthin](#) travels through all stages of human life, beginning with birth, continuing with the different stages of maturity, and concluding with the stages of old age, resulting in death.

He describes many of the physiological and pathological changes that take place during the aging process.

Warthin acknowledges the poverty of aging science a century ago, writing: "Not a single one of all the theories of senescence so far offered has a leg to stand upon; for the greater part they are pure hypotheses constructed about some single fact, such as the increased growth-inhibiting power of the senescent's blood serum or changes in the pH, or some other similar uncorrelated observation. Much has been recently made of the cultivation outside of the animal body of tissue and organ cells. A potential immortality has been claimed for the somatic cell as well as for the germ cell—because of this, biologic hints of the possible immortality of the soma are thrown out by over-enthusiastic biologists and journalists. ...but ... there is so little, or no, biologic foundation."

References: Bailey 1096 (book ver.); Freeman p. 48.



THE WESLEY M. CARPENTER LECTURE

THE PATHOLOGY OF THE AGING PROCESS

ALDRED SCOTT WARTHIN, Ph.D., M.D., LL.D.
Professor of Pathology and Director of the Pathological Laboratories,
University of Michigan, Ann Arbor

Aldred Scott Warthin

In the very heyday of man's powers, in the full summer of his creative possibilities, and of his desires and ambitions, the life-drama played by each human actor discloses, quite suddenly and unexpectedly, its real tragicomic motive. It is, as if the player himself, had been enacting a part in a drama, the course and the end of which he did not fully comprehend. It had been a pleasant play of exciting incident, joyous adventure, vital desire and satisfaction, ambition and achievement. The actor has waltzed with his role to his own satisfaction, and now, well past the middle of the second act, he is shocked by a sharp hint from the prompter's box that his interpretation of his role must be changed into another key—one less pleasant, more difficult and unsatisfying. From now on to the end of the third act he must portray the picture of gradually flagging desire and failing powers, of disillusionment, fading pleasures and progressive fatigue, in other words, senescence, until his role ends in ultimate death. The title of this tragicomedy is "The Life of Man"; its three acts are entitled: I, Evolution; II, Maturity; III, Involution. It is the story of a living, multicellular organism—a chemico-physical machine transforming, storing and releasing energy—capable of building and restoring its own substance, and repairing its wear-and-tear.

Alfred Scott Warthin, *The Pathology of Aging Process*, New York Academy of Medicine: New York 1929. From the Medical Library of Tim C. Opler.

1933: Paul Niehans

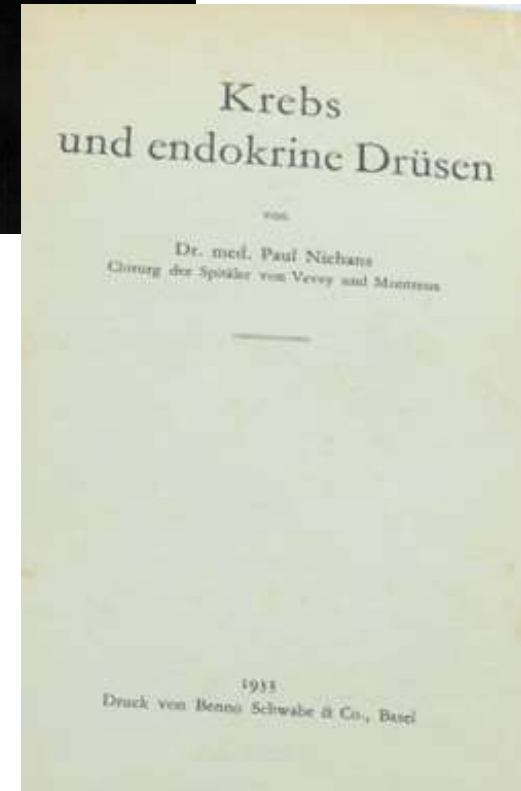
Paul Niehans was one of the first to rigorously employ cell therapy to improve aging. His work was ahead of its time although there is little evidence that his cell therapies were effective.

[Paul Niehans](#) (1882–1971) was a Swiss physician who became one of the most controversial and influential figures in the field of anti-aging medicine. Initially trained in theology and later in medicine, Niehans shifted his focus to endocrinology and rejuvenation therapies. He is best known for developing cellular therapy (commonly referred to as fresh cell therapy), a treatment that involved injecting patients with cells extracted from the organs of sheep fetuses.

Niehans claimed that these fresh cells could rejuvenate aging tissues, repair damaged organs, and restore vitality. His work gained significant attention in the mid-20th century, attracting a wealthy and often celebrity clientele, including Pope Pius XII, who credited Niehans' therapy with improving his health. Despite his popularity among the elite, Niehans' methods were met with skepticism and criticism from the mainstream medical community, which questioned the scientific validity and safety of his treatments.

Niehans' cellular therapy was based on the idea that introducing young, healthy cells into the body could stimulate regeneration and counteract the effects of aging. The therapy typically involved extracting cells from the organs of unborn sheep, which were then processed and injected into the patient. Niehans believed that these cells would migrate to the corresponding organs in the human body and promote healing and rejuvenation. His methods laid the groundwork for research into regenerative medicine.

References: Haycock p. 186; Morley p. 1133.



Paul Niehans, *Krebs und endokrine Drüsen*, Druck: Basel, Benno Schwabe, 1933. From the Medical Library of Tim C. Opler

1935: Clive McCay

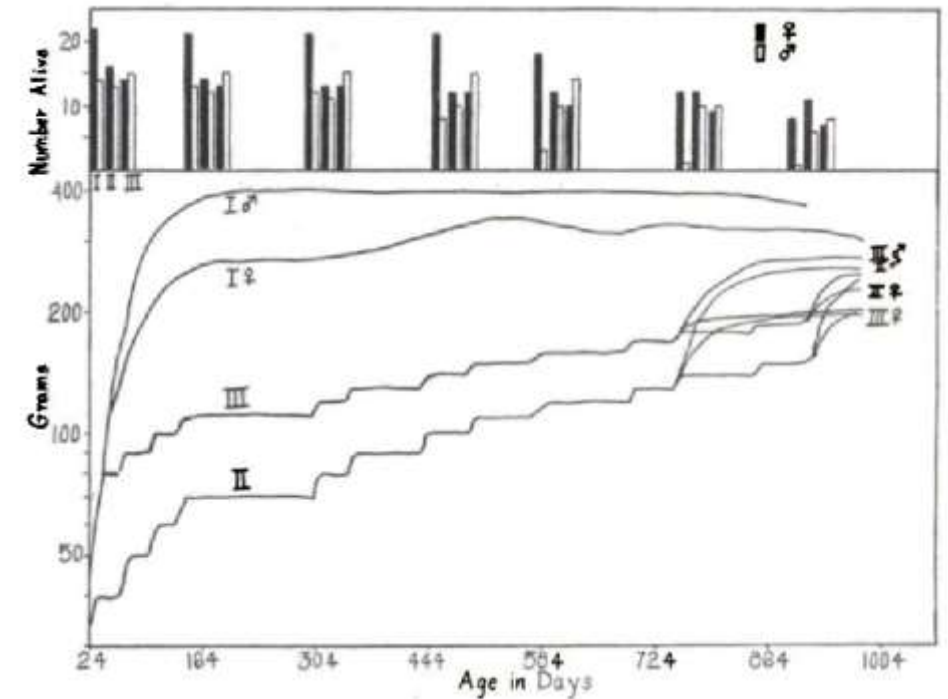
The first to provide data that caloric restriction extends longevity – following the tradition of Cornaro and others.

Clive McCay's 1935 paper, "The Effect of Retarded Growth Upon the Length of Life Span and Upon the Ultimate Body Size," presents groundbreaking research on the relationship between caloric intake, growth rate, and lifespan in rats. McCay and his colleagues conducted experiments where they restricted the food intake of young rats, leading to slower growth rates compared to those fed ad libitum. Despite their delayed development, the calorie-restricted rats eventually reached normal adult body sizes when allowed to eat freely later in life. However, more significantly, the study found that these rats lived considerably longer than their unrestricted counterparts, suggesting that early-life caloric restriction could extend lifespan.

[Clive McCay](#) hypothesized that a slower metabolic rate and reduced physiological stress from lower food intake might be key factors in prolonging life. His findings challenged the prevailing notion that rapid growth and larger body size were necessarily linked to better health and longevity. Instead, the research indicated that prolonged periods of growth, coupled with lower caloric intake, could delay aging and reduce age-related diseases. The study also provided early evidence that longevity was not merely a function of genetics but could be influenced by environmental and dietary factors. The implications of McCay's work extended beyond rodent studies, laying the foundation for future research in aging, caloric restriction, and metabolic health. His findings spurred decades of studies investigating how nutrient intake affects lifespan in various species, including primates and humans.

References: Achenbaum p. 65; Campisi p. 184; Bailey 620; Freeman p. 58; GMN 7245 (1924 article); Haycock p. 120; Hayflick p. 284; Medawar p. 28; Morley 1133; Ramakrishnan 191; Stambler p. 232.

Fig. 1 Growth curves for rats allowed to grow rapidly, no. I, and for those retarded in growth by limiting the calories, nos. II and III. The columns at the top show the numbers alive by sex and group at various periods of the experiment. The top of these columns provides a mortality curve.



Clive McCay, "The Effect of Retarded Growth Upon the Length of Life Span and Upon the Ultimate Body Size," *Nutrition*, 1935. From the Medical Library of Tim C. Opler.

1935: Alfred Worcester

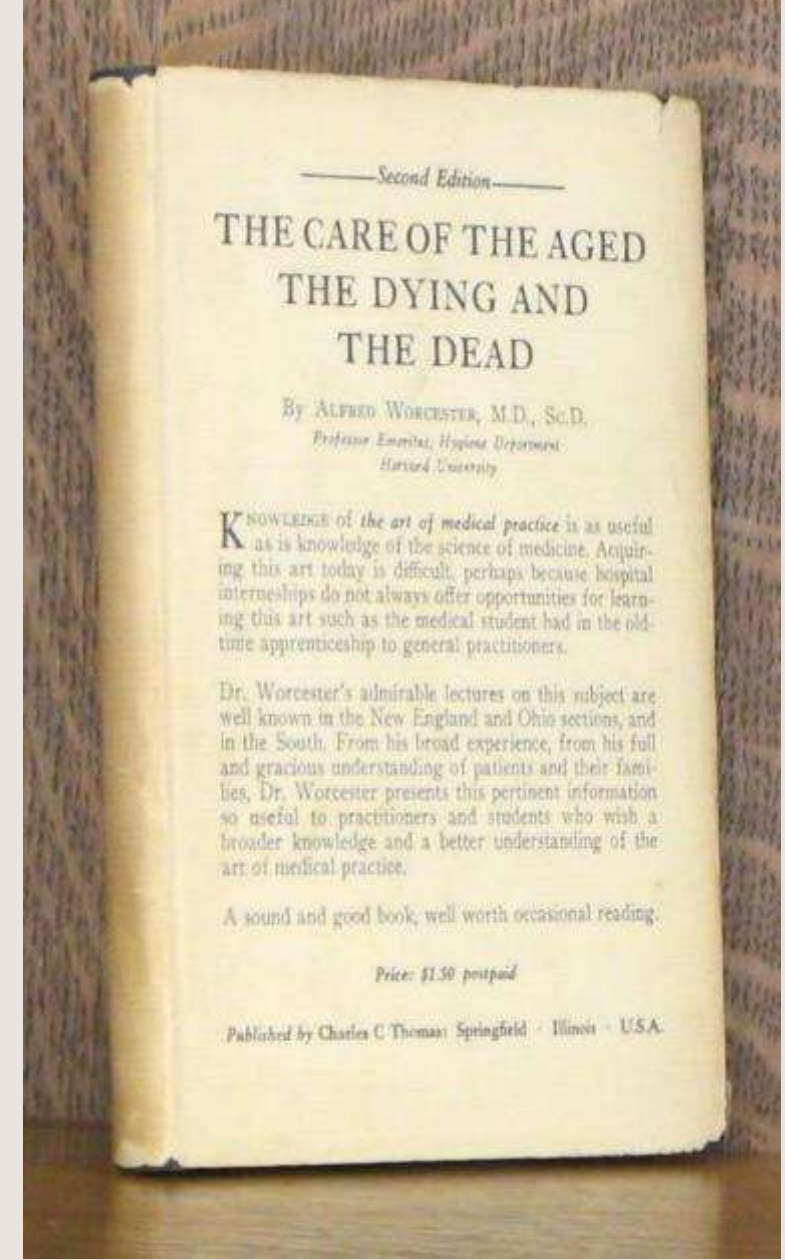
Worcester wrote an early and widely read book on gerontology which focused on the care of the aged. Very much in the spirit of Zerbi's Gerontocomia of 1489.

Alfred Worcester's *The Care of the Aged, the Dying, and the Dead*, published in 1935, is a compassionate and practical guide that addresses the medical, emotional, and ethical aspects of caring for elderly individuals, particularly those nearing the end of life. [Worcester](#), a physician and pioneer in palliative care, emphasizes the importance of dignity, comfort, and respect in the treatment of older adults and the dying.

He provides detailed advice on managing the physical symptoms of aging and terminal illness, such as pain, fatigue, and malnutrition, while also addressing the psychological and spiritual needs of patients. Worcester's approach is holistic, advocating for a balance between medical intervention and empathetic care, and he stresses the role of family and caregivers in creating a supportive environment for the elderly and dying.

In addition to its focus on the care of the aged and dying, Worcester's book also addresses the practical and ethical considerations surrounding death, including the preparation of the body and the emotional support of grieving families. He challenges the medical community's tendency to prioritize curative treatments over comfort care, arguing that the quality of life in one's final years is just as important as its length. Worcester's work is notable for its humane and forward-thinking perspective, which laid the groundwork for modern hospice and palliative care practices. His emphasis on compassion, dignity, and holistic care continues to resonate in contemporary discussions about aging, end-of-life care, and the ethical responsibilities of healthcare providers.

References: Bailey 1238 (later ed.); GMN 10057; Morley p. 1138.



Alfred Worcester, *The care of the aged, the dying and the dead*. Springfield, IL: Charles C Thomas, 1935. From the Medical Library of Tim C. Opler.

1937: David Riesman

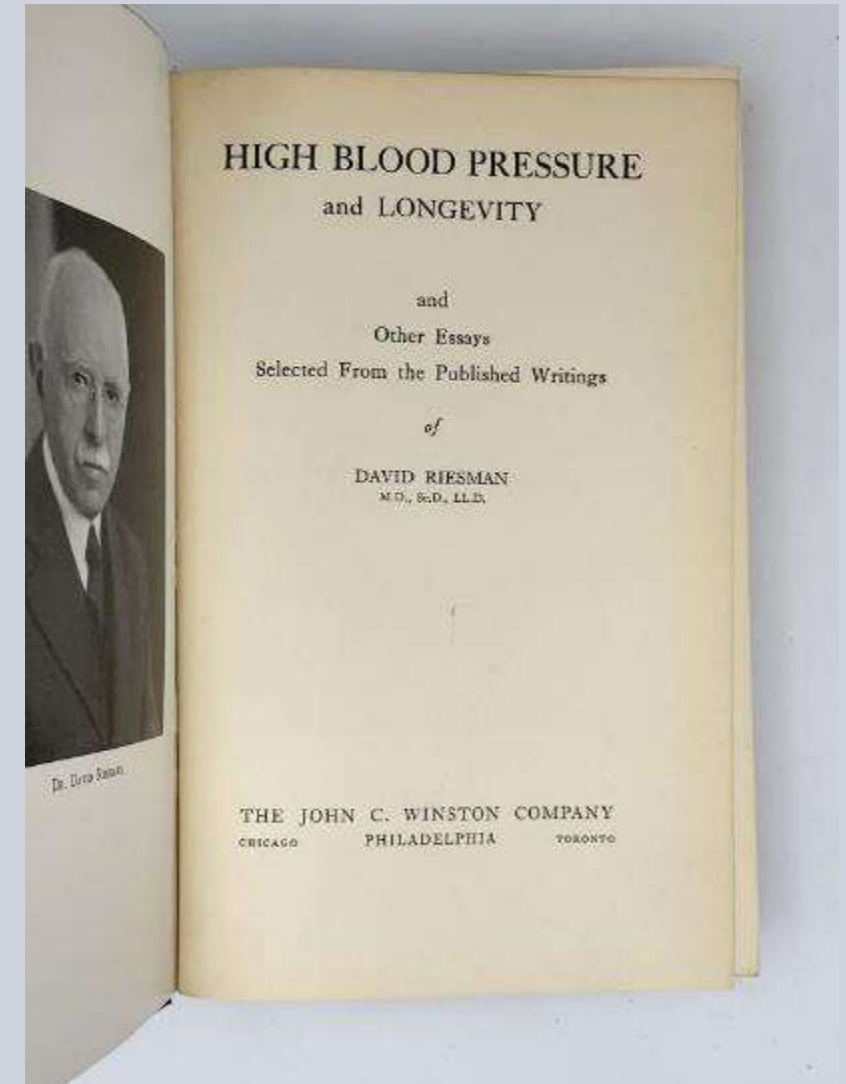
Riesman linked longevity to blood pressure.

David Riesman's *High Blood Pressure and Longevity and Other Essays* (1937) explores the relationship between hypertension (high blood pressure) and lifespan, challenging conventional medical views of the time. Riesman argues that contrary to the widely held belief that high blood pressure is always detrimental, it may, in some cases, be an adaptive physiological response that supports longevity. He suggests that moderate hypertension in older adults could help maintain adequate blood circulation as arterial walls stiffen with age, thus playing a protective role rather than being solely a disease.

Beyond hypertension, the book contains essays on broader medical and social issues, including the evolving understanding of aging, disease prevention, and public health. Riesman integrates medical insights with historical and philosophical reflections, discussing how societal perceptions of health and longevity influence medical practice. His work contributes to a growing discussion in the early 20th century on how chronic conditions should be managed rather than merely treated as pathologies to be eradicated.

Overall, *High Blood Pressure and Longevity and Other Essays* is an early exploration of cardiovascular health and aging. Riesman's nuanced approach to hypertension challenges rigid medical dogma and promotes a more individualized perspective on health and longevity. His ideas foreshadow later debates on whether certain chronic conditions should always be aggressively treated or, in some cases, accepted as part of the body's natural adaptation to aging.

References: Achenbaum p. 104.



David Riesman, *High Blood Pressure and Longevity and Other Essays*, 1937, Chicago: John C. Winston. From the Medical Library of Tim C. Opler.

1938 and 1939: Barbara McClintock

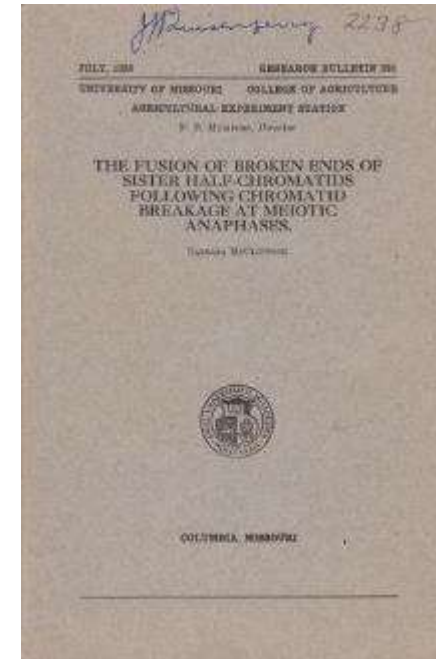
She found important clues about role of telomeres in cell division. At the time of this research her work was obscure. She received the Nobel Prize in Medicine in 1983 for work on genetics and remains the only female to have won the prize solo.

In a 1938 paper, [Barbara McClintock](#) showed that broken ends of a chromosome tend to fuse with each other. This extended findings from an earlier piece of work in 1931 where McClintock found that chromosomes that had been broken after being subject to X-rays saw broken ends fuse to each other.

Published in 1939 in the *Proceedings of the National Academy of Sciences* (PNAS), the second paper shown at right examined the fate of broken chromosomes during meiosis. McClintock observed that broken chromosomes could persist through multiple cell divisions and lead to aberrant chromosomal configurations, providing early evidence of the instability caused by chromosome breakage. Publishing in the 1930's and 1940's McClintock was very much [ahead of her time](#).

Blackburn of UCSF later wrote that this paper was of “seminal importance for the concept of telomere function, which ultimately led to the recognition that telomeres are formed by an active process. She reported that under certain conditions, a broken end lost its tendency to fuse with other broken ends. Thus, she concluded that such a broken end had ‘healed.’ ... it has been critical for our subsequent thinking about the molecular nature of the telomere that she states that “broken ends apparently heal and remain permanently healed”.... The marketed tissue and developmental stage specificity of the capability of a broken to health was the first real hint of a specialized process, under developmental control, that acts to heal broken ends.”*

References: Ramakrishnan p. 73.



Barbara McClintock, “The Fusion of Broken Ends of Sister Half-Chromatids Following Chromatid Breakage at Meiotic Anaphases,” *Mo. Agric. Exp. Res. Stn Res. Bull* 290:1, 1938. From the medical library of Tim C. Opler.



Barbara McClintock, “The Behavior in Successive Nuclear Divisions of a Chromosome Broken at Meiosis,” *PNAS* 25: 405, 1939. From the medical library of Tim C. Opler.

*See Elizabeth Blackburn, “Broken Chromosomes and Telomeres,” in Federoff and Botstein, *The Dynamic Genome*, 1992, pp. 381-388.

1938: Hermann J. Muller

The term "telomere" was first used by the German-American geneticist Hermann Joseph Muller in 1938. Muller coined the term to describe the protective ends of chromosomes, which he observed in his studies on the effects of X-rays on *Drosophila* (fruit fly) genetics.

Hermann J. Muller's "The Remaking of Chromosomes" (1938) was a seminal paper in which he introduced the term "telomere" and explored the mechanisms of chromosome stability.

Published in *The Collecting Net*, the paper built on Muller's earlier research into the effects of X-ray irradiation on *Drosophila* chromosomes. Through his experiments, he observed that broken chromosomes caused by radiation would often fuse at their exposed ends, leading to genetic instability and defects. However, the natural ends of chromosomes did not exhibit this behavior, suggesting that they possessed a unique, protective structure. Muller hypothesized that these terminal structures—which he named telomeres—acted as caps preventing degradation and fusion, ensuring chromosomal integrity during cell division. His work laid the foundation for later research on chromosome aging, replication, and genetic inheritance.

Beyond introducing telomeres, Muller also discussed the broader implications of chromosome manipulation, proposing that radiation-induced chromosomal alterations could serve as a tool for studying genetic changes. He emphasized how chromosome breaks and rearrangements, although often detrimental, could provide insights into the mechanisms of heredity and mutation. This work not only influenced cytogenetics but also foreshadowed later discoveries on DNA repair and telomere function, particularly the roles of enzymes like telomerase (discovered decades later).

References: Ramakrishnan p. 57.



Müller HJ. The remaking of chromosomes. *Collecting Net* 1938; 13:181-198.

1939: Edmund Vincent Cowdry

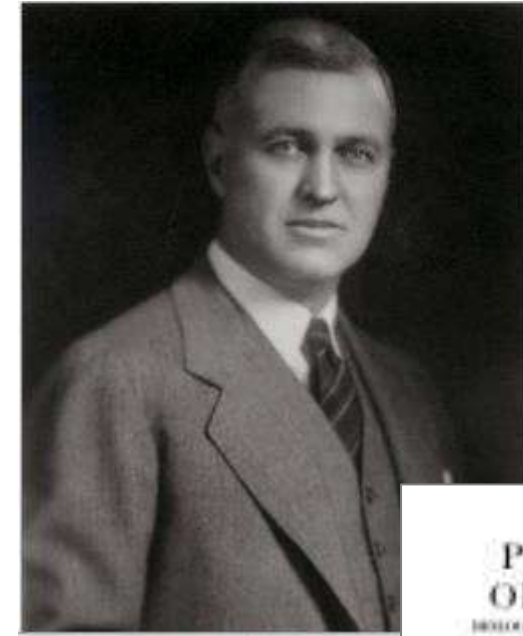
Cowdry' 1939 book marked a watershed moment in organizing intellectual efforts to manage aging, sparking the gerontology movement.

In 1939, [Edmund Cowdry](#) edited *The Problems of Ageing: Biological and Medical Aspects*. This book contains chapters on aging by most of the experts in the U.S. in the 1930's. Cowdry himself was an early gerontologist and ardent advocate for the interests of the aged. Out of the group of chapter authors of this book grew The Gerontological Society of America which was founded in 1945.

This gathering of experts was a watershed moment in aging research. Andrew Achenbaum wrote in 2018: "The 1939 publication of Cowdry's *Problems of Ageing* signals the modern period wherein paradigms have organized questions, hunches, and data ascribed to late life. Theories of aging subsequently have been promulgated, tested, and (if demonstrably robust) then circulated in laboratories, chronicles, and the media... Between 1939 and 1952, Cowdry's *Problems of Ageing* went through three editions. No age-based theory emerged. Nor did contributors to the Cowdry triptych definitively resolve the question of whether aging was pathological or physiological nature. But dedicated researchers and educated formed organizations to promote ideas and collaborations among researchers on aging."

A reading of the book's chapters reveals that the study of aging in 1939 remained descriptive with little theory. Like Warthin's 1929 review it is conspicuous in the absence of conceptualization and evidence for any particular theory. Recent scientific insights from writers like Loeb, Minot, Pearl and Rubner had clearly not yet been absorbed into the medical and academic milieu of the time. This would need to wait until later.

References: Bailey 1025; Freeman p. 48; Morley p. 1137; Stambler p. 235. Also see W. Andrew Achenbaum, *Ever-Emerging Theories of Aging*, *Oxford Research Encyclopaedia*, 2018.



E. Vincent Cowdry, 1939, *Problems of ageing: biological and medical aspects*. Josiah Macy, Jr. Foundation publication. Baltimore: Williams and Wilkins, 1939.

1952: Peter Medawar

Mutations with harmful effects in old age are not weeded out because they happen after reproduction.

Peter Medawar, once an Oxford Professor whose works on graft rejection and the discovery of acquired immune tolerance have been fundamental to the medical practice of tissue and organ transplants.

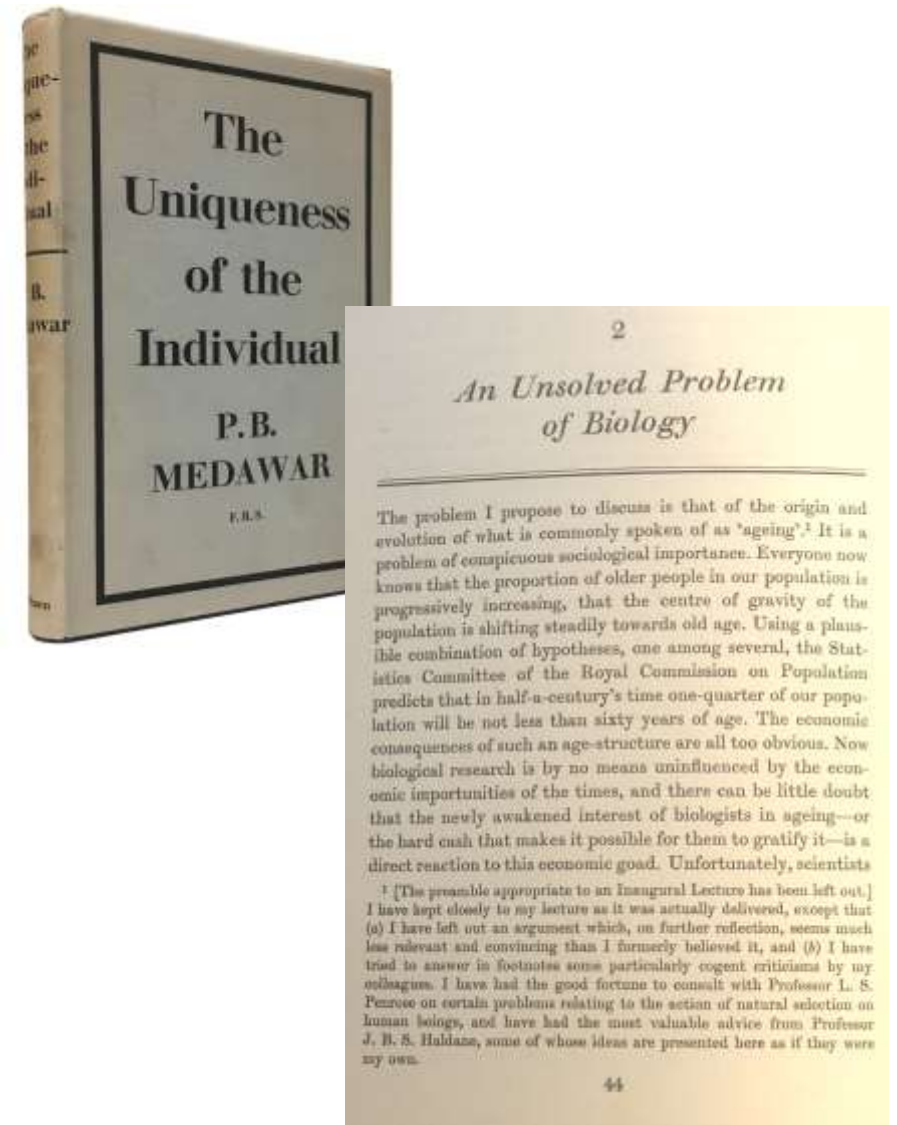
His 1952 essay *An Unsolved Problem of Biology* reprinted in his book *The Uniqueness of the Individual* is a landmark work in aging biology, in which he proposes an evolutionary explanation for why organisms grow old and die.

[Peter Medawar](#) challenges the assumption that aging is an inevitable, programmed process, instead arguing that natural selection weakens its influence on organisms once they have passed their reproductive years.

Medawar suggests that mutations with harmful effects in old age accumulate because they exert little selective pressure, as most organisms in the wild die from predation or disease before experiencing these late-life effects. This idea laid the foundation for what would later be known as the *mutation accumulation theory* of aging.

The implications of Medawar's work were profound, as they shifted the study of aging from a physiological to an evolutionary framework. His ideas influenced later theories, such as Williams' antagonistic pleiotropy hypothesis (1957) and Kirkwood's disposable soma theory (1977), which further explored the trade-offs between reproduction and longevity.

References: Campisi p. 184; Haycock p. 229; Hayflick p. 215; JSG 66; Ramakrishnan p. 20.



Peter Medawar, "An Unsolved Problem in Biology," (1952) in *The Uniqueness of the Individual*. 1957. Inscribed by Medawar to evolutionary biologist Julian Huxley and previously owned by Stephen Jay Gould. From the medical library of Tim C. Opler.

1955: Denham Harman

First to outline free radical theory of aging.

In 1955, [Denham Harman](#) suggested that free radicals produced during aerobic respiration cause cumulative oxidative damage, resulting in aging and death. He noted parallels between the effects of aging and of ionizing radiation, including mutagenesis, cancer and gross cellular damage.

At the time, it had recently been discovered that radiolysis of water generates hydroxyl radical ($\cdot\text{OH}$), and early experiments using paramagnetic resonance spectroscopy had identified the presence of $\cdot\text{OH}$ in living matter.

Harman therefore hypothesized that endogenous oxygen radical generation occurs in vivo, as a by-product of enzymatic redox chemistry. He ventured that the enzymes involved would be those “involved in the direct utilization of molecular oxygen, particularly those containing iron.”

Finally, he hypothesized that traces of iron and other metals would catalyze oxidative reactions in vivo and that peroxidative chain reactions were possible, by analogy to the principles of in vitro polymer chemistry. All of these predictions have been confirmed during the past 40 years. The subsequent realization that energy consumption by mitochondria may result in superoxide anion production linked the free radical theory and the rate of living theory irrevocably: a faster rate of respiration, associated with a greater generation of oxygen radicals, hastens aging. Dr. Harman lived to the ripe age of 98.

References: Bailey 745 (cites 1956 paper) Morley p. 1133 (cites 1957 paper); Ramakrishnan 173; Stambler p. 233.



Denham Harman, *Aging: A Theory Based on Free Radical and Radiation Chemistry*, 1955, US Atomic Energy Commission, University of California Radiation Laboratory.

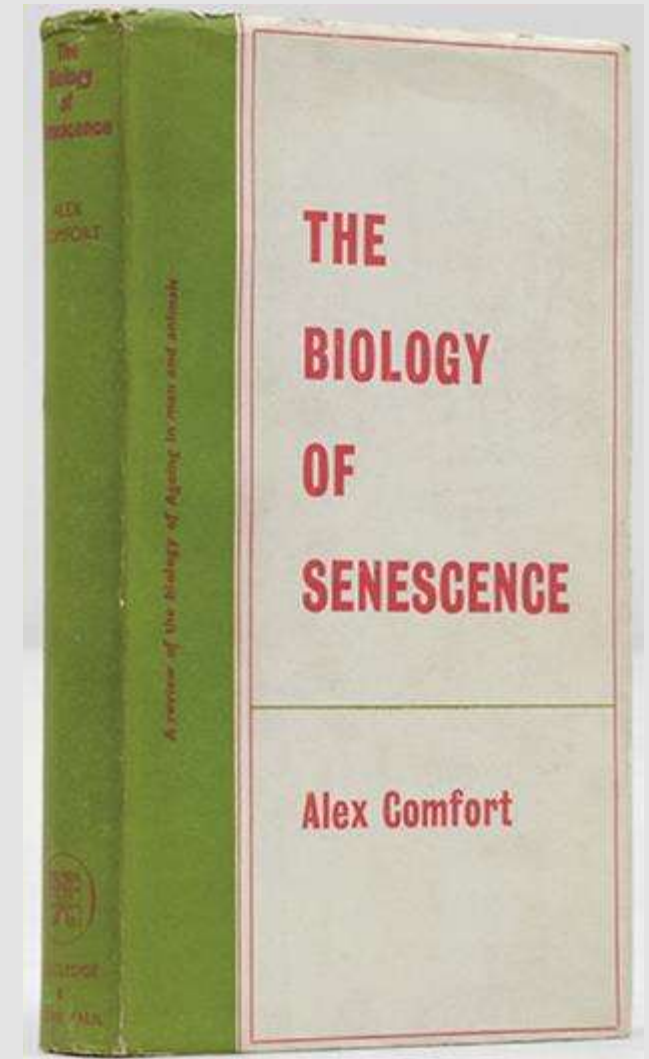
1956: Alex Comfort

Peter Medawar recognized the importance of Comfort's 1956 book entitled *The Biology of Senescence* for summarizing and synthesizing the aging literature to date (very much in the spirit of Venki Ramakrishnan's recent book *Why We Die*). Aimed at the intelligent layman, this book reignited scientific and popular interest in aging – just as scientific interest in gerontology was starting to rise.

Though he is best known as the author of *The Joy of Sex*, [Alex Comfort's](#) career began as director of research in gerontology at University College, London, and he later served as president of the British Society for Research on Ageing, and the first president of the geriatrics and gerontology section of the Royal Society of Medicine.

Comfort explores the mechanisms of aging across various organisms, emphasizing the universality of senescence while also highlighting species-specific differences. The book delves into the cellular and molecular changes associated with aging, such as the decline in cellular repair mechanisms, the accumulation of cellular damage, and the role of genetics. Comfort also critiques the prevailing theories of aging, including the idea that aging is simply the result of wear and tear, and instead proposes a more nuanced understanding that integrates evolutionary biology, physiology, and biochemistry.

In addition to its scientific insights, *The Biology of Senescence* addresses the broader implications of aging research, including its potential applications in medicine and society. Comfort discusses the possibility of extending human lifespan and improving health in later years, while also cautioning against oversimplified solutions.



Alex Comfort, *The Biology of Senescence*, London: Routledge, 1956.
From the medical library of Tim C. Opler.

References: Bailey 720 (later ed.); Grmek p. 77; Hayflick p. 219; Morley p. 1138; Stambler p. 234.

1957: Williams

Williams expanded Medawar's ideas with the antagonistic pleiotropy theory, suggesting that genes beneficial early in life may have harmful effects later, leading to aging. This theory remains a cornerstone of evolutionary biology.

George C. Williams' 1957 paper, "Pleiotropy, Natural Selection, and the Evolution of Senescence," introduces the antagonistic pleiotropy theory of aging, which posits that aging evolves as a byproduct of natural selection favoring genes that provide benefits early in life but have detrimental effects later in life.

[George Williams](#) argued that genes with such dual effects—beneficial in youth but harmful in old age—can be positively selected because their early-life advantages enhance reproductive success, outweighing the costs they impose later. This theory provided a compelling evolutionary explanation for why aging exists, suggesting that it is not an adaptive trait but rather a consequence of trade-offs inherent in the evolutionary process.

Williams' antagonistic pleiotropy theory complemented and expanded upon Peter Medawar's earlier mutation accumulation theory, which proposed that aging results from the accumulation of deleterious mutations that are not eliminated by natural selection because they exert their effects after reproduction. Williams' work emphasized the role of pleiotropic genes—genes that affect multiple traits—in shaping the aging process. His theory has had a profound and lasting impact on the study of aging, influencing research into the genetic and evolutionary basis of senescence.

References: Campisi p. 184; Ramakrishnan p. 21.

PLEIOTROPY, NATURAL SELECTION, AND THE EVOLUTION OF SENESCENCE¹

GEORGE C. WILLIAMS
Michigan State University

Received February 26, 1957

Senescence is a widespread phenomenon, but it has been largely neglected by non-medical biologists. This neglect may be attributed to a number of causes. One is that the process seldom presents itself to students of natural populations, since recognizably senile individuals are not often found in the wild. Another, perhaps, is an emotional difficulty associated with aging, a situation that was hardly helped by the early clinical association of senescence with sex hormones. Another is the existence of theories that have gained tacit acceptance, despite their conceptual obsolescence and poverty of factual support. The most injurious of these is the identification of senescence with the "wearing out" that is shown by human artifacts. A moment of serious consideration should convince a biologist of the fundamental dissimilarity between these two processes. The breakdown of human artifacts is strictly mechanical and is readily cured by mechanical repairs. The system is a static one, since the same material is continuously present, and there is no endogenous change with the passage of time. An organism, on the other hand, is an open system in a state of material flux. Even such structures as bones maintain constant exchanges with the environment. Moreover, an organism produces itself by a morphogenetic process. It is indeed remarkable that after a seemingly miraculous feat of morphogenesis a complex metazoan should be unable to per-

form the much simpler task of merely maintaining what is already formed.

It is true, of course, that some parts of organisms do literally wear out. Human teeth, for instance, show wear similar to that of any tool subjected to friction, but this wear is no more a part of senescence than is the wearing away of replaceable epidermal cells. The senescence of human teeth consists not of their wearing out but of their lack of replacement when worn out.

August Weismann (1891) was the first biologist of the evolutionary era to advance a theory of senescence. He believed that organisms must inevitably show a decline analogous to that of mechanical devices, but that, in addition, there was a specific death-mechanism designed by natural selection to eliminate the old, and therefore wornout, members of a population. He did not clearly indicate how such a mechanism could be produced by natural selection. He was likewise dubious about the exact nature of the death-mechanism, but indicated that it might involve a specific limitation on the number of divisions that somatic cells might undergo.

Weismann's theory is subject to a number of criticisms, the most forceful of which are: 1) The fallacy of identifying senescence with mechanical wear, 2) the extreme rarity, in natural populations, of individuals that would be old enough to die of the postulated death-mechanism, 3) the failure of several decades of gerontological research to uncover any death-mechanism, and 4) the difficulties involved in visualizing how such a feature could be produced by natural selection.

¹ Contribution number 92 from the Department of Natural Science, Michigan State University. The author wishes to thank Dr. Hugh N. Mozingo, Dr. Dennis W. Strawbridge, and his wife, Doris C. Williams, for their help in the preparation of this paper.

1958: Gioacchino Failla

Failla, a radiation physicist who worked on medical aspects of radiation, was the first to argue that accumulation of somatic mutations caused by DNA damage is the main cause of human aging. This paper was quickly followed by a related work by Leo Szilard.

In his 1958 paper, physicist [Gioacchino Failla](#) proposed that the accumulation of DNA damage over time is a fundamental driver of the aging process. Building upon earlier research, Failla suggested that exposure to radiation leads to mutations and other forms of DNA damage, which accumulate in cells and tissues, impairing their function and contributing to age-related decline.

This hypothesis laid the groundwork for the DNA damage theory of aging, which posits that the integrity of the genome is maintained by cellular mechanisms such as DNA repair, damage tolerance, and checkpoint pathways. Over time, the efficiency of these mechanisms diminishes, leading to the accumulation of DNA damage.

Subsequent studies have supported this theory, showing that organisms with enhanced DNA repair capabilities often exhibit increased lifespans, while deficiencies in these mechanisms are associated with accelerated aging. Failla's work has thus been instrumental in linking environmental factors, such as radiation, to genetic mutations and the broader aging process.

References: Stambler p. 234.

By G. Failla

College of Physicians and Surgeons, Columbia University, New York, N. Y.

The Aging Process

The solid curve of FIGURE 1 represents the age-specific death rate of the male population of New York, N. Y., in 1950 as a function of age. The ordinate represents, on a logarithmic scale, the fraction of the individuals of a given age dying per year and the abscissa the age. It will be seen that beyond age 30 the curve approximates a straight line, the equation of which is of the form

$$r = r_0 e^{\alpha t}$$

where r_0 = mortality rate at age 0, from all causes

e = base of natural logarithms

α = slope of straight line

t = age in years

This was first pointed out by Gompertz¹ in 1825, and the relation is now generally called the Gompertz function. From the slope of this curve it may be calculated that the mortality rate doubles about every 7.2 years.

initial portion of the curve (before age 30) is different from the straight portion and in the same country the straight portion remains approximately the same.

It has been found, also, that similar curves have been obtained for the survival data of Sacher² and that of Berg³.

These curves are obtained by subtracting from the total mortality rate the mortality rate caused by accident, homicide, or suicide. The effect of these causes (which account for a small fraction of the total mortality rate before age 30) has the effect of making the curve deviate from the straight line at the early ages as shown.

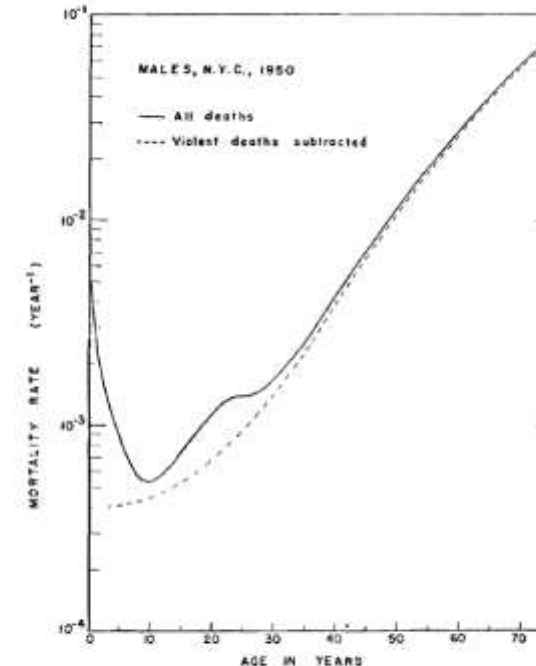


FIGURE 1. The solid curve represents the mortality rate of the male population of New York, N. Y., in 1950 as a function of age. The dashed curve was obtained by subtracting violent deaths, that is, those caused by accident, homicide, or suicide.



Gioacchino Failla, "The aging process and cancerogenesis. Ann N Y Acad Sci. 1958 Sep 30;71(6):1124-40.

1959: Leo Szilard

Written well before MacFarlane Burnet's work on intrinsic mutagenesis and aging, Szilard, the famous physicist, argues that aging is the result of accumulating damage to the molecular machinery of cells, especially DNA.

[Leo Szilard](#) suggested that aging is the result of accumulating damage to the molecular machinery of cells, especially the genetic material. Szilard introduced the idea that this damage was due to "errors" that accumulate over time, gradually compromising the ability of cells to function and divide properly. He thought that the aging process could be modeled and understood using principles from physics and information theory, particularly through the lens of "entropy" and the degradation of systems in time.

Like the 1944 work by physicist Erwin Schrödinger's *What is Life?*, this book used physics concepts to shed light on a problem in biology. Szilard's paper anticipated much that was to come in aging research.* Yet, while self-contained, it strikes us that Szilard's work suffered from lack of contextualization relative to contemporary biology research such as that of Denham Harman or related work such as Claude Shannon's 1948 paper on information theory and entropy (Szilard's paper has zero citations to external work including to Failla who had published a remarkably similar paper just a year before). Szilard was a key collaborator with Albert Einstein and was known for his groundbreaking work in nuclear physics who was best known for conceiving the concept of a nuclear chain reaction in the 1930's.

References: Achenbaum p. 260; Bailey 1000; JSG 102; Stambler p. 234.

* Cutler and Vijg (2023) write: "The difficulty in making sense of the process that most of us will sooner or later fall victim to, makes the paper by Leo Szilard, published more than six decades ago, all the more remarkable. In his "On the nature of the aging process", Szilard recognised DNA, whose molecular structure had been described only six years earlier, as the ultimate template for all cellular functions. Szilard's model of the inevitable and irreversible accumulation of genetic damage remains a simple, yet elegant, explanation of ageing that is applicable at the cellular and demographic levels." ("Genetic wrinkles in time," *Nat Rev Mol Cell Biol* 24, 856 (2023)).

ON THE NATURE OF THE AGING PROCESS

BY LEO SZILARD*

ENRICO FERMI INSTITUTE OF NUCLEAR STUDIES, UNIVERSITY OF CHICAGO

Communicated by Theodore Shedlosky, November 24, 1958

Introduction.—This paper represents an attempt to describe a hypothetical biological process that could conceivably account for the phenomenon of aging. Aging manifests itself in much the same general manner in all mammals, and we are in a position to learn enough about the aging of mammals to be able to test the validity of a theory that leads to predictions of a quantitative kind—as does the theory here presented.

We know that a gene can be responsible for the synthesis of a specific protein molecule, which in many cases has a known enzymatic activity. When we speak later of a mutant, or "incompetent," form of a gene, we mean an altered form of the gene, which cannot synthesize the specific protein molecule in its chemically active form.

Our theory assumes that the elementary step in the process of aging is an "aging hit," which "destroys" a chromosome of the somatic cell, in the sense that it renders all genes carried by that chromosome inactive. The "hit" need not destroy the chromosome in a physical sense. (See note 1 added in proof.)

We assume that the "aging hits" are random events and that the probability that a chromosome of a somatic cell suffers such a "hit" per unit time remains constant throughout life. We further assume that the rate at which chromosomes of a somatic cell suffer such "hits" is a characteristic of the species and does not vary appreciably from individual to individual.

As a result of an aging process of this nature, the number of the somatic cells of an individual organism which have "survived" up to a given age (in the sense of remaining able to fulfil their function in the organism) decreases with age. On the basis of our assumptions, spelled out below, the "surviving" fraction of the somatic cells decreases with age at an accelerating rate.

Our theory postulates that when f , the surviving fraction of the somatic cells of an individual, approaches a certain critical value, f^* , then the probability that that individual may die within a period of one year will come close to 1. On this basis, the theory establishes a relationship between the surviving fraction of the somatic cells and the age of death of the individual.

Because the young mammalian organism may be assumed to have a large functional reserve, we shall assume that the surviving fraction of the somatic cells of an individual may fall substantially before the organism loses its capacity to live, perhaps to a value somewhere between $1/8$ and $1/12$.

Leo Szilard, "On the nature of the Aging Process," PNAS Jan 15, 1959, 45(1), pp. 30-45.



1961: Leonard Hayflick

Discovers that cells cannot replicate indefinitely, eventually hitting the “Hayflick Limit”.

Leonard Hayflick’s groundbreaking 1961 paper, "The Serial Cultivation of Human Diploid Cell Strains", fundamentally changed the understanding of cellular aging and replication limits. Hayflick and co-author Paul Moorhead demonstrated that normal human diploid cells have a finite capacity for replication, contradicting the long-standing belief that cultured cells could divide indefinitely under optimal conditions.

Through meticulous experimentation, [Leonard Hayflick](#) cultured human fibroblasts (WI-38 strain) and observed that they underwent a limited number of cell divisions (approximately 40–60) before entering a senescent state, now known as the Hayflick Limit.

This discovery disproved the earlier theory proposed by Alexis Carrel, who claimed that cells could proliferate indefinitely in culture. Hayflick suggested that cellular aging was an intrinsic property of normal cells and was regulated by an internal biological clock, rather than being solely influenced by external factors.

The implications of Hayflick’s research were profound. It laid the foundation for modern aging biology, cancer research, and regenerative medicine by establishing that cellular senescence is a natural process linked to aging and disease.

References: Achenbaum 122; Bailey 776; GMN 8381, Haycock p. 210; Morley p. 1133bla. Ramakrishnan 71.

THE SERIAL CULTIVATION OF HUMAN DIPLOID CELL STRAINS¹

L. HAYFLICK and P. S. MOORHEAD

Wistar Institute of Anatomy and Biology, Philadelphia, Pa., U.S.A.

Received May 15, 1961

ONLY limited success has been obtained in developing strains of human cells that can be cultivated for long periods of time *in vitro* and that still preserve the diploid chromosomal configuration [41, 47, 48, 58, 59]. Indeed, heteroploidy may be a necessary corollary or even the cause of the alteration of primary or diploid cells *in vitro* to the status of a cell line. Such changes in chromosome number appear to be independent of the type of primary tissue since they have been observed in cells derived from both normal and malignant tissue [4, 22, 23, 31].

These cell lines, of which over two hundred have been reported in the literature, have serious limitations for many kinds of biological studies. Chief among these is the exclusion of their use for the production of human virus vaccines. This limitation is based on the supposition that such heteroploid cell lines, whether of normal or malignant origin, share many of the properties of malignant cells [29, 30, 37]. This objection would be even more important if viruses played a role in human neoplasia. In general, if strains of human cells could be kept continuously under conditions of rapid growth for extended periods of time with the retention of the diploid configuration these objections would not apply.

Furthermore, diploid cell strains would parallel more closely the biology of cells *in vivo*. Although characterizations of heteroploid cell lines are often stated in terms of a modal chromosomal number, this should not obscure the fact that extensive pleiomorphism is present [21]. The cells comprising the modal class in heteroploid cell lines are found to be heterogeneous if chromosomal analysis is extended beyond a simple enumeration [49]. This genomic variability constitutes an important consideration in experiments using cell lines for the study of metabolic or other phenotypic cell markers. The use of cloning as a means of reducing this variability in heteroploid cell lines is unfortunately limited by the rapid re-emergence of a range of chromosomal types among the progeny of the clone [5, 49, 50].

¹ This investigation was supported by a research grant (G-4584) from the National Cancer Institute, National Institutes of Health, United States Public Health Service.

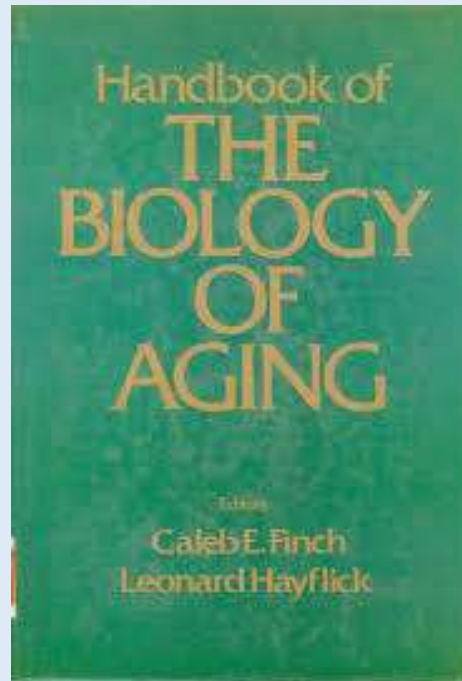
Leonard Hayflick, “*The Serial cultivation of Human Diploid Cell Strains*,” *Experimental Cell Research* 1961, 25, pp. 595-621. From the Medical Library of Tim C. Opler (Hayflick’s personal copy in a bound set of his offprints).

Various Later Books by Leonard Hayflick

Leonard Hayflick (May 20, 1928 – August 1, 2024) was an American anatomist who was Professor of Anatomy at the UCSF School of Medicine and was Professor of Medical Microbiology at Stanford University School of Medicine.

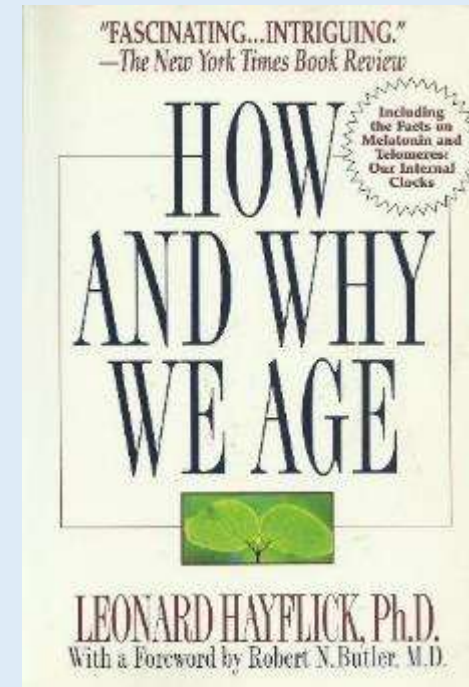
The recipient of a number of research prizes and awards, including the 1991 Sandoz Prize for Gerontological Research, he studied the ageing process for more than fifty years. He is known for discovering that normal human cells divide for a limited number of times in vitro in this 1961 paper (refuting the contention by Alexis Carrel that normal body cells are immortal). This is known as the Hayflick limit. His discoveries overturned a 60-year-old dogma that all cultured cells are immortal.

Hayflick demonstrated that normal cells have a memory and can remember what doubling level they have reached.



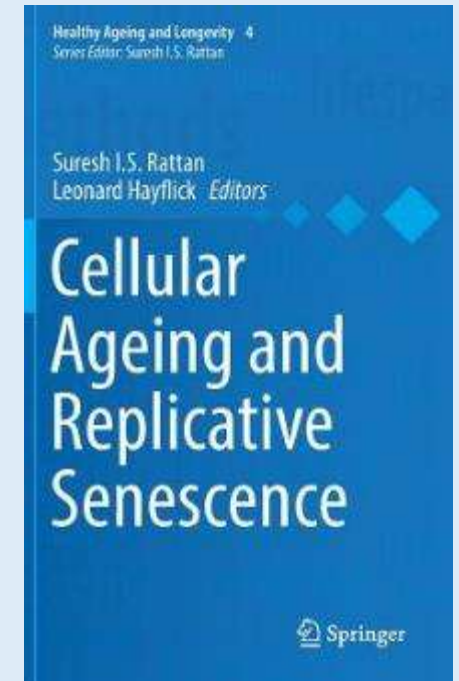
1977

Comprehensive overview of the most important topics in biomedical gerontology including cellular senescence, immunology and loss of cognition.



1994

The book delves into the implications of cellular senescence for human health and longevity, offering insights into the processes that contribute to aging and age-related diseases.



2018

Cellular Ageing and Replicative Senescence revisits more than fifty-five years of research based on the discovery that cultured normal cells are mortal and the interpretation that this phenomenon is associated with the origins of ageing

1962: John Gurdon

The first to clone an animal cell, showing that it was possible to take a mature human cell back to its state at birth. In theory, aging might be reversible.

John B. Gurdon's 1962 paper, "The Developmental Capacity of Nuclei Taken from Intestinal Epithelium Cells of Feeding Tadpoles," is a pioneering study in the field of developmental biology and cloning. Gurdon demonstrated that nuclei from differentiated intestinal cells of *Xenopus laevis* tadpoles could be transplanted into enucleated frog eggs and still support the development of normal, fertile adult frogs.

Gurdon's groundbreaking experiment showed that the nuclei of differentiated cells retain the genetic information necessary to direct the development of an entire organism, challenging the prevailing belief that cell differentiation involves irreversible genetic changes.

In theory, this implies that human aging might be reversible in some way.

The study provided critical evidence for the concept of nuclear equivalence, which states that all cells in an organism contain the same genetic material, regardless of their specialization. [John Gurdon's](#) work laid the foundation for later advancements in cloning and stem cell research, including the cloning of mammals such as Dolly the sheep. It also contributed to the understanding of cellular reprogramming and the potential for reversing cell differentiation, which has profound implications for regenerative medicine and developmental biology. Gurdon's experiments were a major milestone in demonstrating the plasticity of cell nuclei and their potential to regain totipotency.

References: Ramakrishnan p. 88.

The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles

by J. B. GURDON¹

From the Embryology Laboratory, Department of Zoology, Oxford

WITH ONE PLATE

INTRODUCTION

AN important problem in embryology is whether the differentiation of cells depends upon a stable restriction of the genetic information contained in their nuclei. The technique of nuclear transplantation has shown to what extent the nuclei of differentiating cells can promote the formation of different cell types (e.g. King & Briggs, 1956; Gurdon, 1960c). Yet no experiments have so far been published on the transplantation of nuclei from fully differentiated normal cells. This is partly because it is difficult to obtain meaningful results from such experiments. The small amount of cytoplasm in differentiated cells renders their nuclei susceptible to damage through exposure to the saline medium, and this makes it difficult to assess the significance of the abnormalities resulting from their transplantation. It is, however, very desirable to know the developmental capacity of such nuclei, since any nuclear changes which are necessarily involved in cellular differentiation must have already taken place in cells of this kind.

The experiments described below are some attempts to transplant nuclei from fully differentiated cells. Many of these nuclei gave abnormal results after transplantation, and several different kinds of experiments have been carried out to determine the cause and significance of these abnormalities.

The donor cells used for these experiments were intestinal epithelium cells of feeding tadpoles. This is the final stage of differentiation of many of the endoderm cells whose nuclei have already been studied by means of nuclear transplantation experiments in *Xenopus*. The results to be described here may therefore be regarded as an extension of those previously obtained from differentiating endoderm cells (Gurdon, 1960c).

MATERIAL AND METHODS

The animals used for these experiments belong to the subspecies *Xenopus laevis laevis*. The transplantation technique has been carried out as described previously (Elsdale *et al.*, 1960), except that the donor tissue was exposed to

¹ Author's Address: Department of Zoology, Parks Road, Oxford, U.K.
[J. Embryol. exp. Morph., Vol. 10, Part 4, pp. 622-640 December 1962]

1962: Bernard Strehler

Carried out a comprehensive review of the causes of aging, concluding that the DNA damage theory had strong support.

[Bernard Strehler](#) was a Johns Hopkins biology graduate from 1947 who wrote extensively on aging and was one of the first gerontologists to join the NIH.

Strehler's 1962 book, *Time, Cells, and Aging*, explores the biological and biochemical mechanisms underlying aging, positioning it as a fundamental process linked to cellular function and molecular changes over time. Strehler examines how cells experience time-dependent deterioration, proposing that aging is governed by cumulative molecular damage, genetic factors, and environmental influences. He discusses the role of oxidative stress, mutation accumulation, and metabolic inefficiencies in cellular aging, laying an early foundation for modern biogerontology.

The book integrates concepts from genetics, physiology, and molecular biology to explain why organisms age at different rates and suggests that understanding these processes could lead to interventions that slow aging and extend lifespan. His highly influential book [inspired](#) a generation of scientists to better understand aging processes as he framed aging as a problem that could be studied systematically.

In a 1981 interview he said: "My view is that aging is those things that go wrong when cells lose their ability to divide. If we could replace our cells as rapidly as they deteriorate, we could probably live very long, if not indefinitely."

References: Bailey 869 (later ed.); Stambler p. 235.



Bernard Strehler, *Time, Cells and Aging*, Academic Press: New York, 1962. From the Medical Book Collection of Tim C. Opler.

1963: Christian de Duve

This paper is one of the earliest to describe autophagy in mammalian cells. Christian de Duve, who coined the term "autophagy" (meaning "self-eating"), observed the presence of cytoplasmic components within lysosomes, suggesting a process by which cells degrade and recycle their own contents.

In 1963, [Christian de Duve](#) published a seminal paper that significantly advanced the understanding of lysosomes and their role in autophagy, the process by which cells degrade and recycle their own components.

Building on his earlier work in identifying lysosomes as organelles responsible for intracellular digestion, De Duve proposed that these vesicles were not only involved in breaking down extracellular materials (such as those taken in by endocytosis) but also played a crucial role in the degradation of intracellular components.

He introduced the concept of autophagy (meaning “self-eating”), describing how portions of the cytoplasm, including organelles like mitochondria and peroxisomes, could be enclosed by double-membrane vesicles and delivered to lysosomes for degradation. His paper provided biochemical evidence that lysosomal enzymes actively degrade these cellular components, ensuring turnover and cellular homeostasis.

One of the key insights from this publication was that autophagy is a highly regulated process, essential for maintaining cellular integrity under conditions of stress, starvation, or damage.

De Duve demonstrated that lysosomes contain hydrolytic enzymes that function optimally at acidic pH, making them uniquely suited for controlled degradation without harming the rest of the cell.

LYSOSOMES
Edited by A. V. S. de REUCK and MARGARET P. CAMERON
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GENERAL PROPERTIES OF LYSOSOMES

THE LYSOSOME CONCEPT

CHRISTIAN DE DUVE

*Laboratory of Physiological Chemistry,
University of Louvain, Belgium
and Rockefeller Institute, New York*

THE BIOCHEMICAL CONCEPT

RAT-LIVER LYSOSOMES AS BASIS OF THE CONCEPT

THE name “lysosome” was proposed when it was found that, in rat liver, as many as five distinct acid hydrolases with widely differing specificities appeared to be associated together within a special group of cytoplasmic particles (de Duve *et al.*, 1955). As is obvious from the etymology of the word, this choice reflected the belief that the observed association is biologically meaningful. Subsequent work has greatly strengthened this belief, since rat-liver lysosomes are now known to contain at least twelve separate hydrolytic activities, some of which may be due to more than one enzyme species, all showing an acid pH optimum.

Rat-liver lysosomes are characterized by a second property which, though not explicit in their name, has always been considered of fundamental importance: the structure-linked latency of their enzymes. This property is a general one, extending to all the enzymes which have been identified so far as belonging to the particles. It has been attributed to the existence around the lysosomes of a membrane-like barrier of lipoprotein nature restricting the accessibility of their internal hydrolases to external substrates.

Christian de Duve, chapter entitled “General Properties of Lysosome,” in *Ciba Foundation Symposium: Lysosome*, De Reuck A, Cameron MP, eds. Little, Brown, 1963

1963: Leslie Orgel

Orgel formulated the protein-translation error-catastrophe theory of aging in 1963. The theory suggests that errors in protein synthesis, particularly errors in the translation machinery itself, can lead to a cascade of increasingly inaccurate protein synthesis, ultimately causing cellular dysfunction and death.

Leslie Eleazer Orgel (1927 to 2007) was a British chemist and member of the National Academy of Sciences, known for suggesting that human life may have originated from outer space.

[Leslie Orgel's](#) protein-translation error-catastrophe theory of aging, introduced in his 1963 paper "The Maintenance of the Accuracy of Protein Synthesis and Its Relevance to Ageing", proposes that aging results from the accumulation of errors in protein synthesis. Orgel argued that mistakes in translating genetic information into functional proteins could lead to the production of faulty enzymes and structural proteins, which would then contribute to further cellular dysfunction.

Orgel's hypothesis was one of the first attempts to link molecular errors to the aging process, emphasizing the fragility of the translation machinery. While later research showed that cells have robust error-correction mechanisms to prevent such catastrophic failure, his work sparked interest in molecular fidelity as a key factor in aging.

Although the theory is now largely discredited—since experimental evidence does not support an age-related increase in protein translation errors—Orgel's ideas laid the groundwork for subsequent studies on protein homeostasis, quality control systems like chaperones, and the role of proteostasis in longevity and neurodegenerative diseases. His work remains a significant historical milestone in the molecular biology of aging.

References: Bailey 980; Stambler p. p. 235.



THE MAINTENANCE OF THE ACCURACY OF PROTEIN SYNTHESIS AND ITS RELEVANCE TO AGEING

By L. E. ORGEL

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE, ENGLAND

Communicated by Lord Todd, F. R. S., February 15, 1963

The ways in which the accumulation of mutations might contribute to the process of ageing in higher organisms or in individual clones of cells has been discussed at length.¹ No corresponding treatment of the consequences of transcription errors in the translation of the DNA message into RNA and protein sequences seems to be available. Here I show that a consideration of the rate of accumulation of such errors leads to a paradox, the resolution of which may be relevant to the problem of ageing, and that there are a number of simple experiments which should decide whether this is the case.

The basic idea is a simple one, namely, that the ability of a cell to produce its complement of functional proteins depends not only on the correct genetic specification of the various polypeptide sequences, but also on the competence of the protein-synthetic apparatus. A cell inherits, in addition to its genetic-DNA, the enzymes necessary for the transcription of that material into polypeptide sequences; the inheritance of inadequate protein-synthesizing enzymes can be as disastrous as the inheritance of a mutated gene. Similarly, a cell may deteriorate through a progressive decrease in the adequacy of its transcription mechanism, just as it may through the accumulation of somatic mutations.

As a basis for further discussion we shall suppose:² (a) that the sequence of amino acids in a protein is determined by the sequence of nucleotides in a corresponding region of the DNA; (b) that the immediate functions of DNA include the direction of the synthesis of transfer, microsomal, and messenger RNA but not of proteins; and (c) that the information for peptide sequence determination is carried by messenger RNA, but that other forms of RNA may affect the accuracy of protein synthesis.

There is little direct evidence concerning the accuracy of polypeptide-sequence determination. The accuracy of synthesis of messenger RNA is unlikely to exceed that of DNA replication, for which an error frequency of 10^{-8} per base has been estimated.³ This would contribute an error frequency of about 3×10^{-8}

Leslie, Orgel, The maintenance of the accuracy of protein synthesis and its relevance to ageing. *Proc Natl Acad Sci U S A.* 1963 Apr;49(4):517-21.

1969: Roy Walford

The first to thoroughly develop the idea that the loss of immune function is a major cause of age-related pathologies.

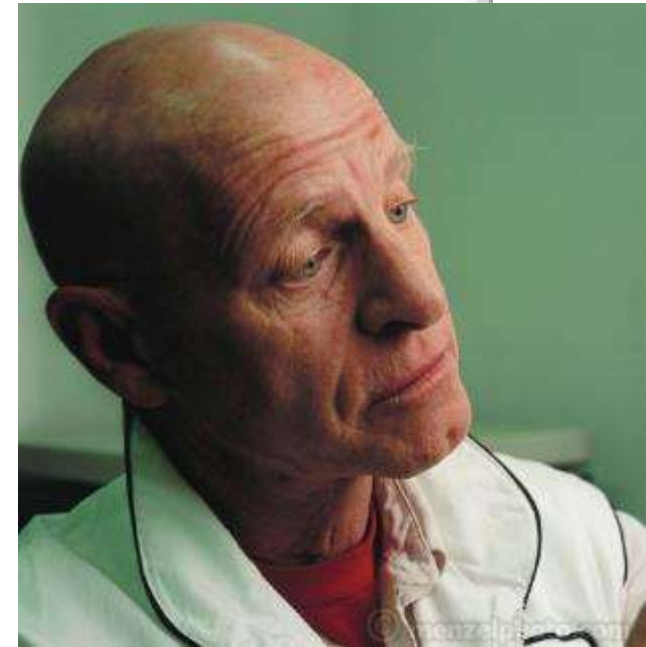
Roy Walford's "The Immunologic Theory of Aging", proposed in the 1960s, is a foundational concept in gerontology that links the decline of immune function to the aging process. Walford hypothesized that aging is driven, in part, by the gradual deterioration of the immune system, a process he termed immunosenescence.

As the immune system weakens with age, it becomes less effective at defending the body against infections, clearing damaged cells, and maintaining tolerance to self-antigens. This decline leads to increased susceptibility to diseases, chronic inflammation, and the accumulation of cellular damage, all of which contribute to the aging process and age-related pathologies.

[Roy Walford](#) also emphasized the role of the immune system in regulating homeostasis and its interaction with other systems, such as the endocrine and nervous systems. He suggested that age-related changes in immune function, such as reduced T-cell diversity and increased autoimmunity, play a central role in the progression of aging. The Immunologic Theory of Aging provided a framework for understanding how immune dysfunction contributes to the aging process and inspired decades of research into the role of inflammation, immune cell senescence, and immune modulation in promoting healthy aging.

Walford's work remains influential in the study of aging and has paved the way for interventions aimed at rejuvenating the immune system to extend healthspan.

References: Bailey 877; Haycock p. 220; Stambler p. 235.



Roy L. Walford, *The Immunologic theory of aging*. Munksgaard, Copenhagen; 1969. From the Medical Library of Tim C. Opler.

1972: Denham Harman

Extends the free radical theory of aging by arguing that the mitochondria are the main culprit in manufacturing free radicals.

After years of frustration over his inability to increase maximum lifespan with antioxidant supplements, Denham Harman concluded that mitochondria were producing as well as being damaged by free radicals, but that exogenous antioxidants don't enter the mitochondria.

And that it is mitochondria that determine lifespan. He published his ideas on what he called the "mitochondrial theory of aging" in the April 1972 issue of the Journal of the American Geriatrics Society.

Denham Harman's 1972 paper, "The Biologic Clock: The Mitochondria?", expanded his earlier Free Radical Theory of Aging by focusing on the central role of mitochondria in the aging process. Harman proposed that mitochondria, as the primary sites of energy production in cells, are also the main source of free radicals. During aerobic respiration, electrons can leak from the electron transport chain and react with oxygen, generating reactive oxygen species (ROS) like superoxide anions. These free radicals, Harman argued, cause cumulative damage to cellular components, particularly mitochondrial DNA (mtDNA), which is especially vulnerable due to its proximity to ROS production and lack of protective histones. This damage impairs mitochondrial function, leading to a decline in energy production and further increases in ROS, creating a vicious cycle that drives cellular aging. This paper had profound effects on aging research.

References: Bailey 756; Ramakrishnan 173.



The Biologic Clock: The Mitochondria?

DENHAM HARMAN, M.D., Ph.D.*

*Departments of Biochemistry and Medicine, University of Nebraska
College of Medicine, Omaha, Nebraska*

ABSTRACT: The author suggests that the maximal life span of a given mammalian species is largely an expression of genetic control over the rate of oxygen utilization. The latter determines the rate of accumulation of mitochondrial damage produced by free radical reactions, the rate increasing with the rate of oxygen consumption, which ultimately causes death.

The mean life span in the United States has remained virtually constant at around 70 years since the mid 1950's (1), so it is likely that the rate of increase in the future in the United States, as well as in other developed countries, will be low unless new ways are found for inhibiting the rate of biological degradation in man. The changes in an organism leading ultimately to death can be regarded as due to a basic aging process(es) on which disease states are superimposed and intertwined. From this point of view, disease serves merely to hasten the death made inevitable by the aging process(es). The age at death, then, of any given organism (ignoring accidental deaths not associated with aging) is determined by the combined deleterious effects of the aging process(es) and disease states; and the maximal

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life span of the species is the value observed when these effects are minimized.

The degradative changes associated with aging and disease processes may be due in part to free radical reactions (2, 3) — reactions that are ubiquitous in living systems (4). Thus decreasing the level of deleterious free radical reactions in an organism might be expected to result in a decreased rate of biological degradation with an accompanying increase in the years of useful, healthy life. This approach offers the prospect of an increase in the average life expectancy to beyond 85 years (the maximal mean life span to be expected (5) if overt diseases such as cancer and cardiovascular disorders were eliminated as causes of death) and a significant increase in the number of people who will live to well beyond 100 years.

Experiments based on this hypothesis — the free radical theory of aging — have been encouraging (6-13). For example, a number of inhibitors of free radical reactions have been

145

Denham Harman, *The Biologic Clock: The Mitochondria*, 1972, Journal of the American Geriatrics Society, April 1972, p. 145-147.

1973: Alexey Olovnikov

J. theor. Biol. (1973) **41**, 181–190

First to outline the telomere shortening theory of aging, providing more substance to the Hayflick Limit. The word “telomere” had yet to be coined.

In his 1973 paper published in the *Journal of Theoretical Biology*, [Alexey Olovnikov](#) proposed the "marginotomy" theory, which provided a theoretical framework for understanding the role of telomeres in aging. Olovnikov hypothesized that the ends of linear chromosomes (telomeres) cannot be fully replicated during DNA synthesis due to the limitations of DNA polymerase, which requires a primer to initiate replication. This incomplete replication, termed "marginotomy," leads to progressive shortening of chromosomes with each cell division. He suggested that this shortening acts as a molecular clock, eventually triggering cellular senescence or apoptosis when telomeres reach a critically short length. Olovnikov's theory elegantly linked telomere attrition to the aging process at the cellular level.

Olovnikov's marginotomy theory was groundbreaking because it provided a mechanistic explanation for the Hayflick limit, the observed finite replicative capacity of somatic cells.

Although the existence of telomerase, the enzyme that counteracts telomere shortening, was not yet known, Olovnikov's work laid the conceptual foundation for future discoveries in telomere biology.

His theory has had a profound impact on aging research, inspiring studies on telomeres, telomerase, and their roles in cellular aging, cancer, and longevity. The paper remains a cornerstone in the field, highlighting the importance of telomere dynamics in understanding the biology of aging.

References: Haycock p. 211; Ramakrishnan p. 73; Stambler p. 235.

A Theory of Marginotomy The Incomplete Copying of Template Margin in Enzymic Synthesis of Polynucleotides and Biological Significance of the Phenomenon†

A. M. OLOVNIKOV

*Laboratory of Chemistry and Synthesis of Antibodies,
Gamaleya Institute for Epidemiology and Microbiology,
Moscow, U.S.S.R.*

(Received 22 June 1972, and in revised form 27 November 1972)

A theory of marginotomy has been proposed to explain the limitation of the cell doubling potential of clones of normal somatic cells. Marginotomy of DNA is the shortening of the replica with respect to the template. Two possible mechanisms of marginotomy are discussed. The first mechanism is a DNA-polymerase, postulated to have a catalytically inactive zone lying between the catalytic centre and the outer edge of the enzyme molecule. The terminal template segment which is equal to the length of the non-catalytic marginal zone of the DNA-polymerase will not appear in the replica. The second mechanism of marginotomy results from a requirement for an initiating RNA-primer for activity of some DNA-polymerases. In this case the final DNA replica will be shorter than the template by the length of RNA-primer.

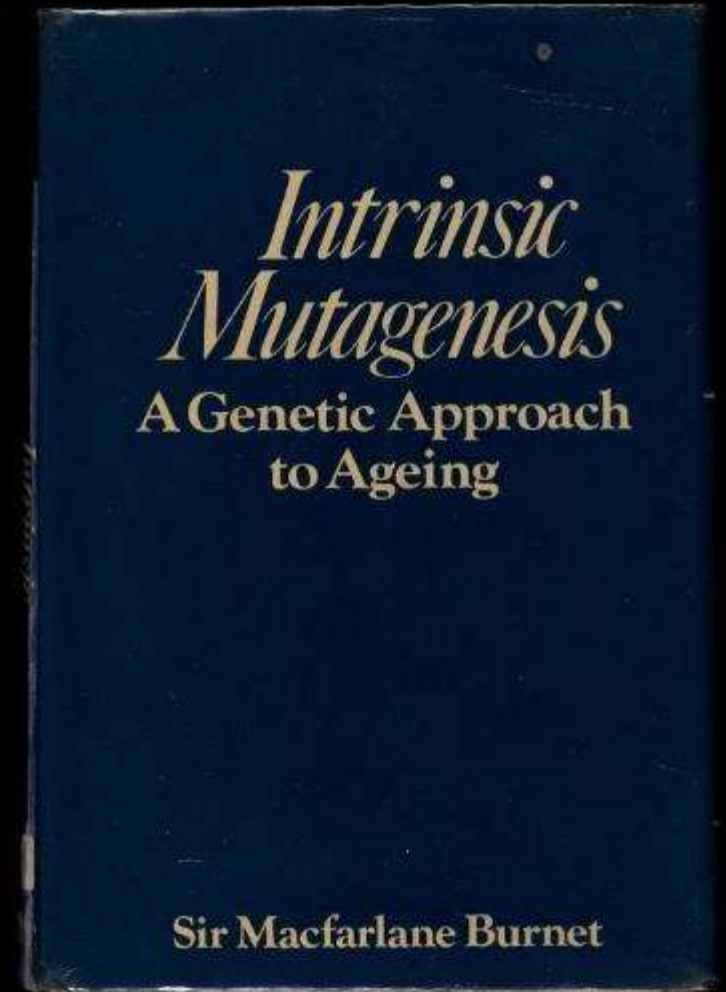
Marginotomy causes the appearance, in the daughters of dividing cells, of more and more shortened end-genes, the so-called telogenes, with every new mitosis. The telogenes function as the starting points of end-replicons in chromosomes and also as “buffers”, being sacrificed during successive mitoses. After the exhaustion of telogenes the cells become aged and are eliminated due to the loss of some vitally important genes localized in end-replicons. Marginotomy is therefore responsible for the loss with age of various cell clones of the body, including some endocrine cell clones. Therefore marginotomy may be the primary cause of various disorders of age of the ageing of multicellular organisms.

A.M. Olovnikov, *A Theory of Marginotomy*, 1972, *Journal of Theoretical Biology*.

1974: MacFarlane Burnet

[Macfarlane Burnet](#) argues that aging is fundamentally driven by the gradual accumulation of genetic mutations within somatic cells. He argues that these mutations occur spontaneously due to inherent errors in DNA replication and repair processes, rather than being primarily caused by external environmental factors. Burnet builds on evolutionary and immunological perspectives, suggesting that aging is an inevitable consequence of an organism's biological programming, wherein natural selection favors early-life fitness over long-term genomic stability. This perspective aligns with the broader framework of evolutionary theories of aging, such as the mutation accumulation and antagonistic pleiotropy hypotheses.

Burnet emphasizes the role of mutagenesis in the decline of cellular function, proposing that as mutations accumulate over time, they disrupt essential cellular processes, leading to age-related degeneration and diseases, including cancer. He discusses how different tissues exhibit varying susceptibilities to mutational damage and explores the implications for organ-specific aging rates. The book integrates experimental findings and theoretical models to support the argument that aging is an intrinsic, mutationally driven process rather than the result of accumulated cellular waste or metabolic damage alone. His approach contrasts with earlier views that attributed aging primarily to oxidative stress or wear-and-tear mechanisms. He received the [Nobel Prize](#) for his lifetime's work.



Macfarlane Burnet, *Intrinsic Mutagenesis*, 1974 (inscribed by the author). From the Medical Library of Tim C. Opler.

Burnet See Two Needs of Any Species



MacFarlane Burnet

CHAPTER 1

THE TWO IMPERATIVES

The two basic evolutionary needs of all species of higher animal are survival to reproductive age, and death when survival offers no reproductive advantages to the species. The thesis of this essay is that both requirements are solved genetically by methods which involve control of mutability in the broad sense. For survival there must be an adequate appearance of mutants to ensure possibilities of adjusting to changes in the ecosystem or occupying any new ecological niches which may become available. It is, however, just as important biologically that the old generation should be discarded as soon as the new one is established. The mechanism is that of senescence, by which, once reproductive performance is completed, ageing develops almost certainly by the progressive acceleration of somatic mutation. Either by way of increasing vulnerability to environmental impacts or by intrinsically developed disease (cancer, etc.), death eventually ensues.

There is no escape from the conclusion that survival and continuing evolution on the one hand and an average lifespan for the individual appropriate to the species on the other are dependent on the occurrence of mutation. It is axiomatic to modern biology that mutation is a wholly random process in so far as the nature of the resulting change in genetic 'information' is concerned. Any control of mutation can only be in the form of modification in one direction or the other of the rate of mutation. It becomes inescapable, then, for us to postulate that the rate of germ-line mutation in the species and of somatic mutation in the individual must have optimal values for each particular species. If those two

Maximizing reproduction of a species requires optimizing survival to reproductive age and avoiding waste of resources once reproduction has occurred. This is not dissimilar to the argument made a century earlier by Alfred Russell Wallace.

1974: Thomas Lindahl

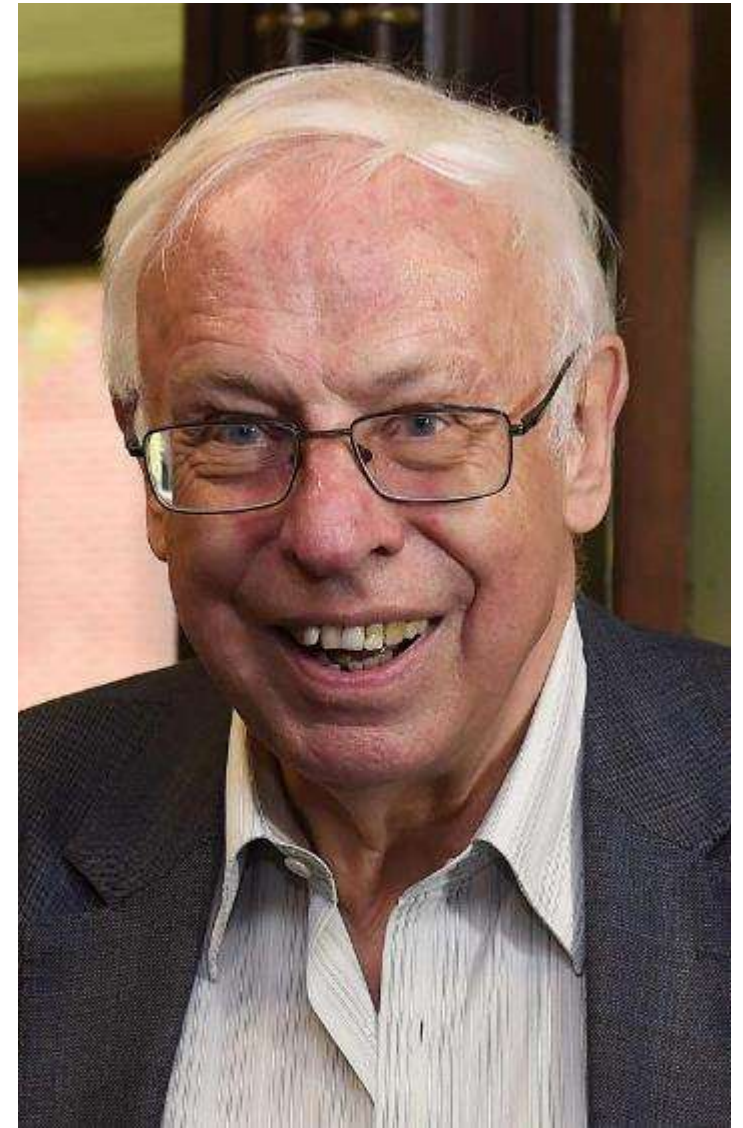
First to explain how cells use enzymes to repair DNA damage. DNA damage repair processes became a central aspect of aging research in the next 50 years. Lindahl shared the 2015 Nobel Prize for this work.

[Thomas Lindahl's](#) 1974 paper, titled "*An N-Glycosidase from Escherichia coli That Releases Free Uracil from DNA Containing Deaminated Cytosine Residues*", marked a groundbreaking discovery in the field of DNA repair.

This study was the first to identify and characterize an N-glycosidase enzyme in *E. coli* capable of excising free uracil from DNA strands containing deaminated cytosine residues. By demonstrating how this enzyme specifically recognizes and removes damaged bases, the work provided critical insights into the mechanism's cells use to counteract endogenous DNA damage.

The discovery of this enzyme also offered a powerful experimental tool for studying the biochemical consequences of base damage in vitro, such as mutagenesis or strand breaks. Furthermore, it highlighted the role of bacterial enzymes in detecting and processing DNA lesions, laying the foundation for understanding how similar repair pathways might operate in higher organisms. This paper thus became a cornerstone for subsequent research into DNA repair, genetic stability, and the molecular origins of mutations.

From both scientific and practical perspectives, Lindahl's findings emphasized the pervasive impact of spontaneous DNA damage in biological systems. By elucidating how deamination—a common hydrolytic process—introduces uracil into DNA (a non-canonical base), the study revealed a fundamental source of genetic errors linked to aging, cancer, and hereditary diseases.



Lindahl, T. (1974) An N-Glycosidase from *Escherichia coli* That Releases Free Uracil from DNA Containing Deaminated Cytosine Residues, *Proc. Nat. Acad. Sci. USA*, 71(9), 3649–3653.

1975: Robert Butler

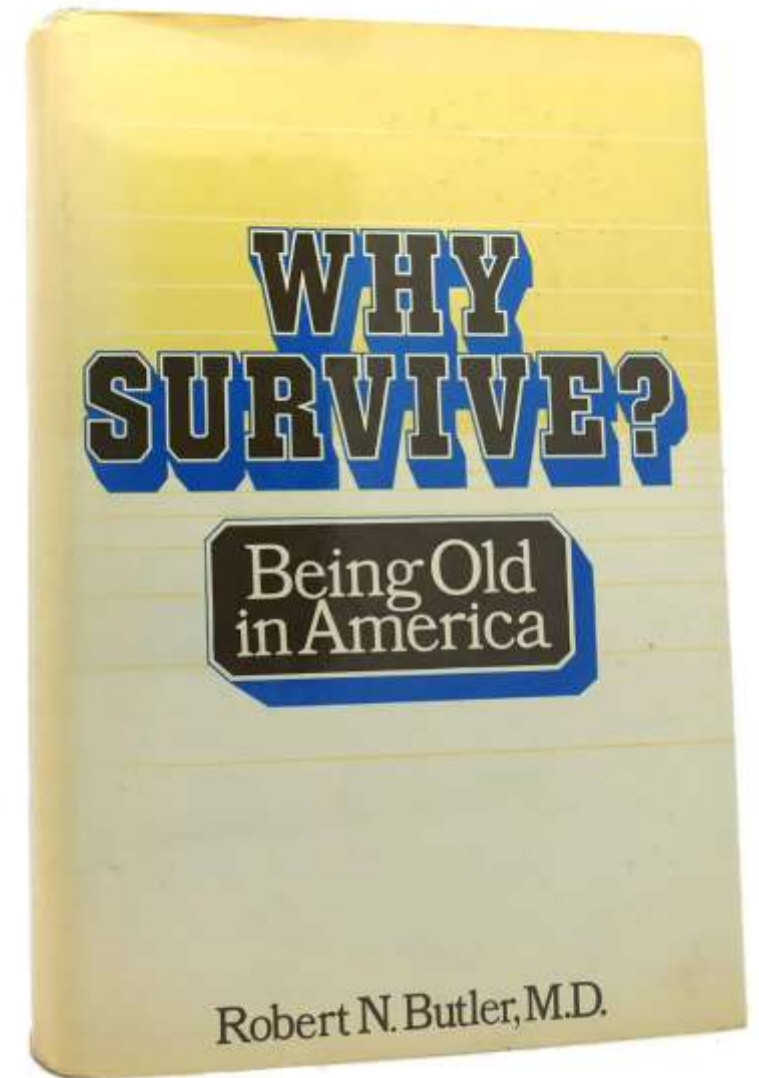
Butler's book *Why Survive?* discussed a range of problems faced by older people in American society stemming from poverty, the failures of the Social Security system, and social isolation. This book led to the foundation of the National Institute of the Aging and multiple steps at reform of policies for treating the aged.

Robert N. Butler's 1975 book *Why Survive? Being Old in America* exposed the harsh realities of aging in the U.S., criticizing societal neglect and systemic ageism as of 1975.

Butler, a gerontologist and psychiatrist, argues that older adults face discrimination, inadequate healthcare, economic insecurity, and social isolation. He coined the term "ageism" to describe the pervasive stereotypes that devalue the elderly, portraying aging as decline rather than a natural life stage. The book highlighted failures in medical care, which often ignored chronic conditions in favor of acute treatments, and economic systems that leave many seniors in poverty due to insufficient pensions and Social Security. Butler also addresses the emotional toll of isolation, as retirees and widowed individuals struggle with loneliness and lack of purpose.

Winning the Pulitzer Prize, *Why Survive?* became a catalyst for change in aging policy and advocacy. Butler's calls for reforms in healthcare, housing, elder abuse prevention, and social support to ensure dignity for older adults led to improvements to Medicare and passage of the Older Americans Act, while inspiring a broader movement to combat ageism. The book remains a foundational text in gerontology, urging society to view aging not as a burden but as a stage of life deserving respect and care. Butler subsequently became the first head of the National Institute of Aging.

References: Acheson p. 256.



Robert Butler, 1975, *Why Survive? Being Old In America*, New York: Harper & Row. From the Medical Library of Tim C. Opler.

Section 8:

The Last 50 Years:

An Explosion in
Understanding of Aging.
Research from 1976 to
2025



An Extraordinary 50 Years

You will recall that Francis Bacon saw the end goal of the Scientific Revolution as achieving meaningful extension of human lifespan.

Were Bacon alive today, we think he would be quite pleased with the progress that has been made in the last half century. However, we suspect he would ask why it has taken so long.

The gradual historical advance of aging research combined with much improved tools, far better funding and advances in our understanding of cell biology and genetics has vastly accelerated the generation of insights into the molecular causes of aging.

Our understanding of the causes of aging has undergone an extraordinary transformation in the last 50 years. The quest to understand the biological mechanisms of aging has led to the identification of key pathways and processes that contribute to cellular and organismal decline.

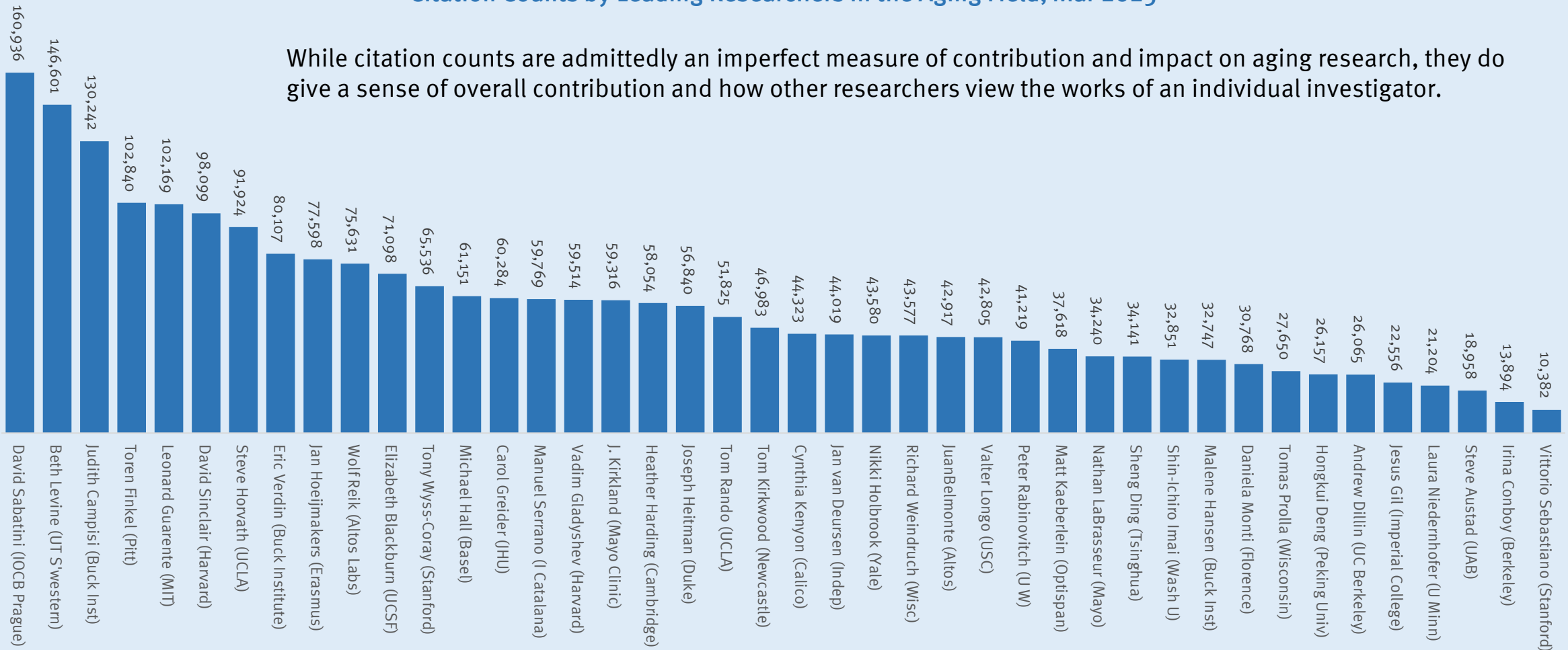
Multiple major scientific milestones in aging research have been hit starting with work in the 1980's but truly accelerating in the 21st century. If there was too little insight emerging into the underlying causes of aging in previous centuries, we now face an embarrassment of scientific riches.



Some Key Contributors to the Recent Aging Literature

Citation Counts by Leading Researchers in the Aging Field, Mar 2025

While citation counts are admittedly an imperfect measure of contribution and impact on aging research, they do give a sense of overall contribution and how other researchers view the works of an individual investigator.

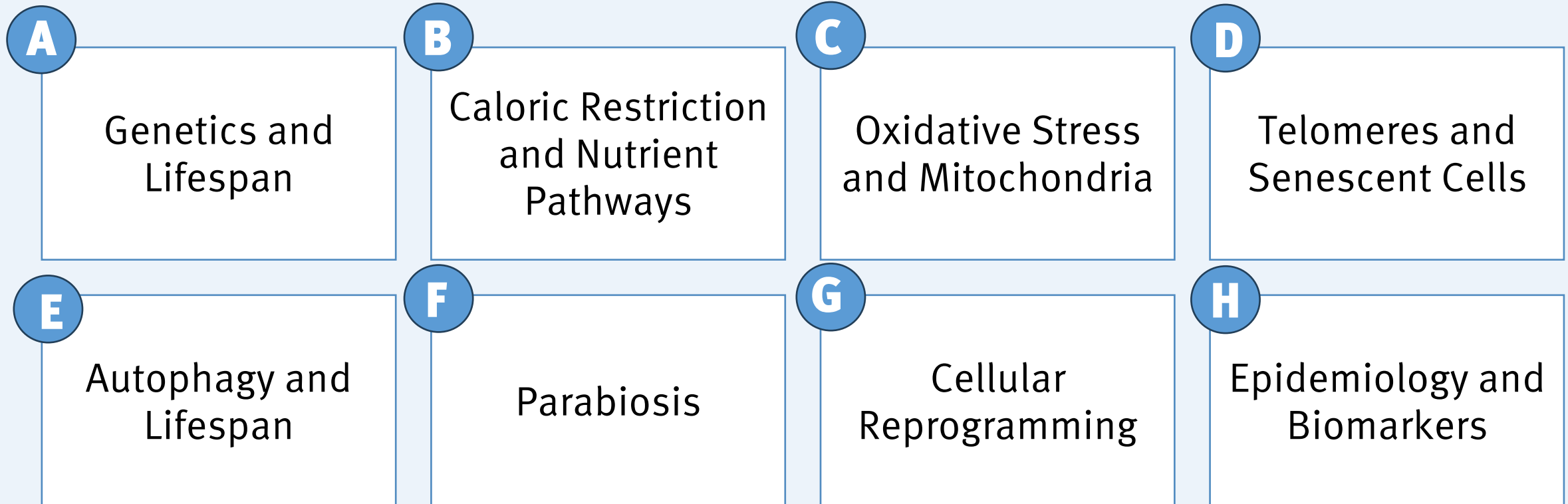


Source: Citation counts taken from Google Scholar in March 2025. When not available we used research.com.

Key Areas of Research Progress in Last 50 Years

The field of aging research has gone from a niche area to one that is very much in the mainstream. To illustrate, over 700,000 new papers covering dozens of topics in aging and longevity have hit the bioscience literature in the last 50 years (versus 41,000 in the previous 190 years).*

The research areas that have been most important scientifically, in our view, are the following:



* Pubmed search for papers listing the words aging, longevity or gerontology in their title or abstract in March 2025.

Section 8.A: Genetics & Lifespan

A persistent idea from antiquity has been that heredity impacts lifespan. Both Aristotle and Pliny the Elder noted that longevity tends to run in families. Carl Linnaeus, father of modern taxonomy, observed that plant and animal species had characteristic lifespans.

Darwin's theory of evolution sharpened interest in aging and heredity. Alfred Russell Wallace noted in 1865 that evolutionary forces might cause our species to select for traits that favor survival through age of reproduction but not long afterwards.

The idea that heredity is likely to play a role in human lifespan was, of course, discussed widely by others including Humphry in 1889 and Minot in his studies at the turn of the 20th Century.

Periodic reviews of aging research reveal the perceived relevance of genetics at the time.

For example, neither Warthin's nor Cowdry's extensive reviews of aging research in the 1920's and 30's emphasized genetics. But, by 1956, Alex Comfort's review identified genes as a central driver of aging.



Rapid Rise of Genetic Insights Into Determinants of Aging

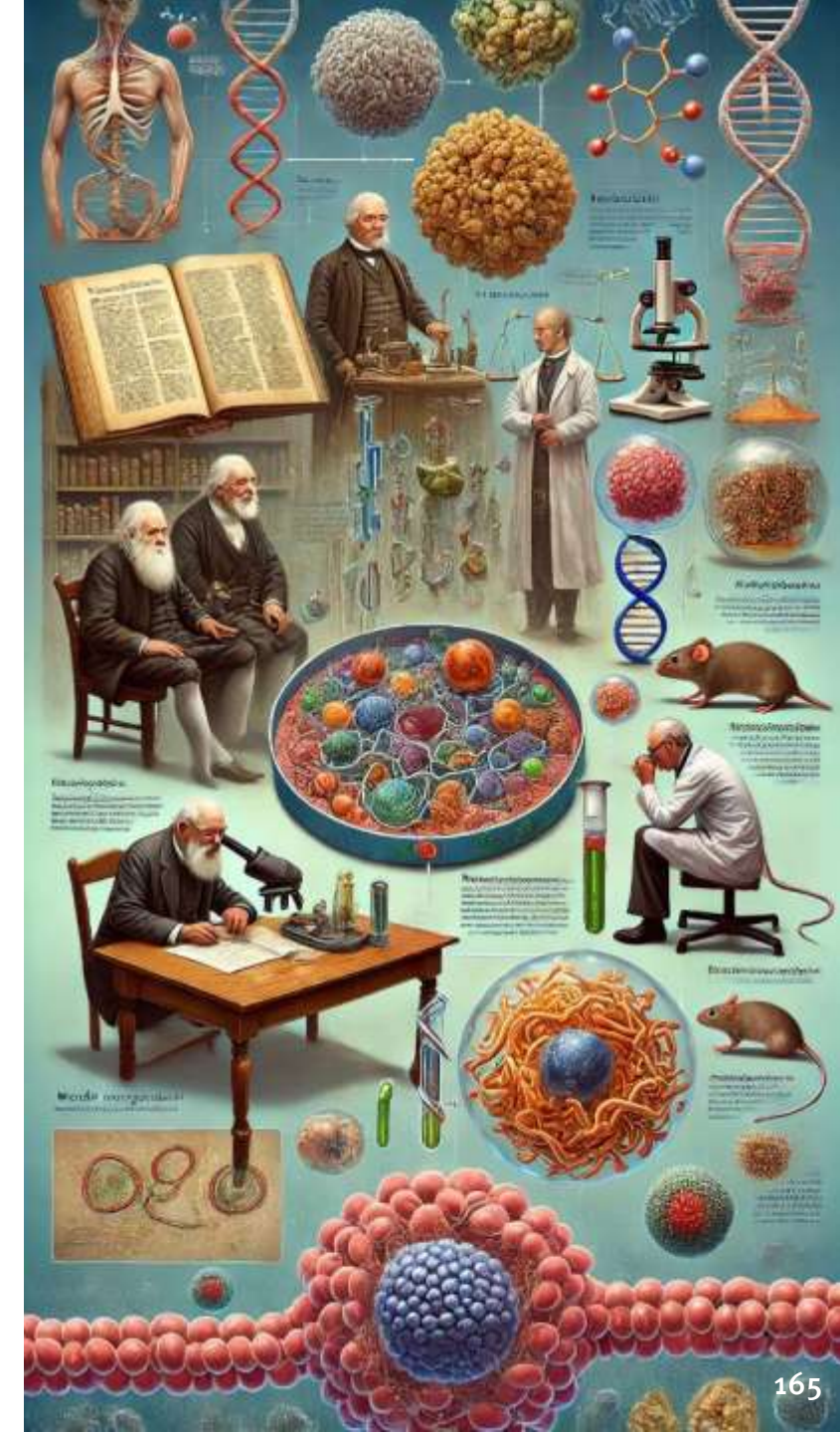
Indeed, from this point on, discussions of the importance of newly discovered DNA to aging were replete in the literature.

Key contributors to aging genetics in the second half of the twentieth century included Medawar, Williams, Failla, Szilard, Strehler, Burnet and Klass.

Reflective of this progress, Venki Ramakrishnan's 2024 book *Why We Die*, the latest thorough review of aging research and literature, integrates a discussion of genes in a balanced discussions of the determinants of mortality.

There is absolutely no question at this point that aging and mortality are, to a significant degree, determined by our genetic makeup. Were this not the case, it would be hard to talk about why butterflies live for two weeks, us humans for 100 years and the Bristlecone Pine, up to 5000 years.

A fruitful area of research has involved investigations of premature aging, called progeria. This is where a mutation occurs that leads to a premature aged phenotype and early death.



Progeria and Model Systems

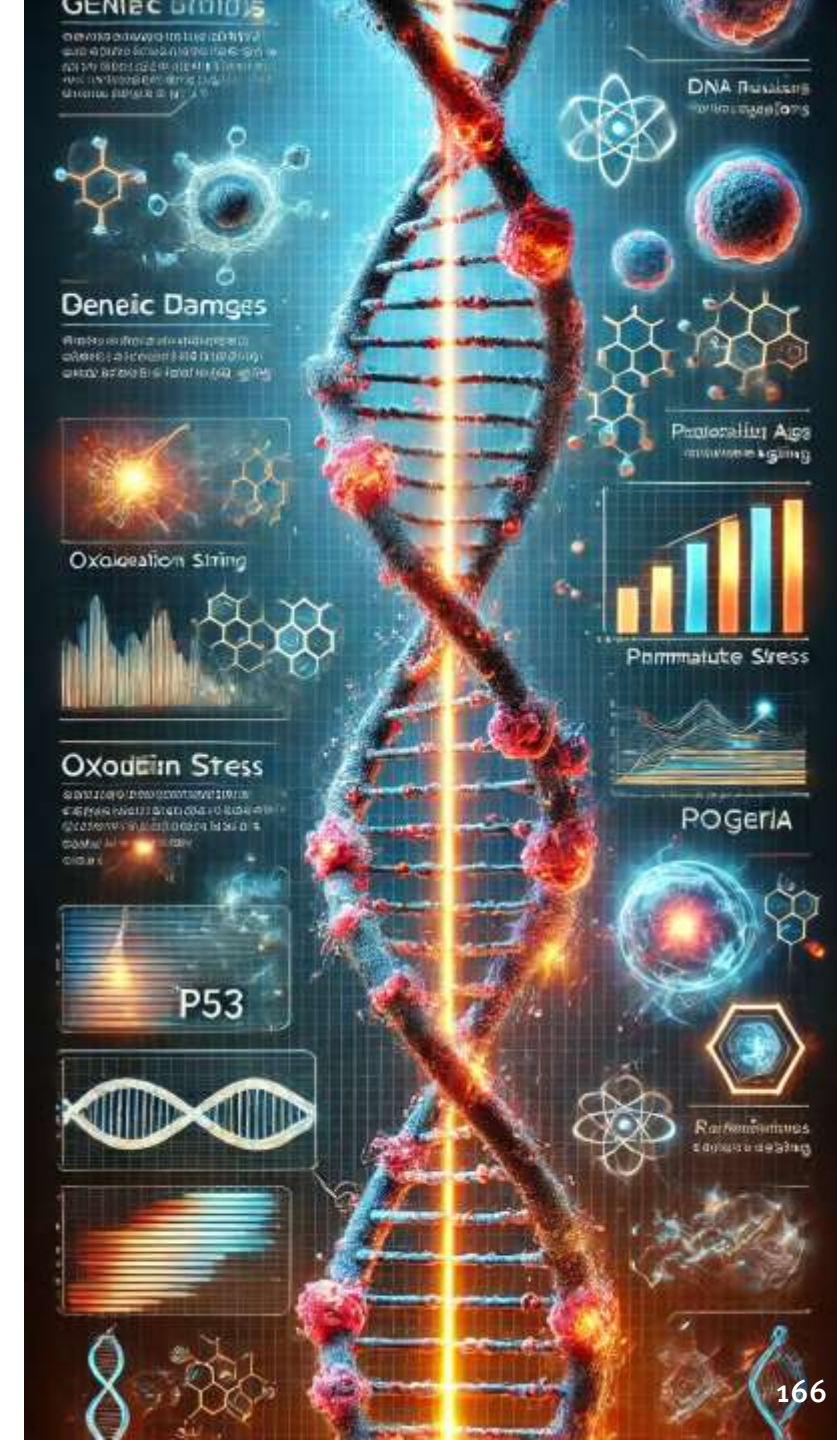
Work by Francis Collins and colleagues has identified a specific gene that leads to Hutchinson-Gilford progeria. A separate group, led by David Orren, has shown that two genetic diseases of early aging (Cockayne and Werner) are due to specific mutations in DNA repair genes

Indeed, a growing literature identifies the inability to repair DNA damage as a crucial determinant of aging.

This, of course, is an idea that has been with us since the work of Leo Szilard and Macfarlane Burnet.

Recent work on the role of P53, an essential DNA repair enzyme by Donehower and others has been illuminating.

Some of the most important work on genetics and aging has come from perturbing model systems such as the fruit fly to see why mutations might impact aging. Both Ross and Chatworth (1980) and Friedman and Johnson (1988) linked genes to longevity in the fruit fly and the worm *C. elegans*, respectively.



Cynthia Kenyon's Work

Cynthia Kenyon is a pioneering geneticist whose work on the nematode *Caenorhabditis elegans* (C. elegans) transformed our understanding of the genetic regulation of aging. In the 1990's, Kenyon discovered that mutations in the daf-2 gene, which encodes an Insulin/IGF-1-like receptor, could double the lifespan of C. elegans. This finding demonstrated that aging is not an immutable process but is subject to genetic control.

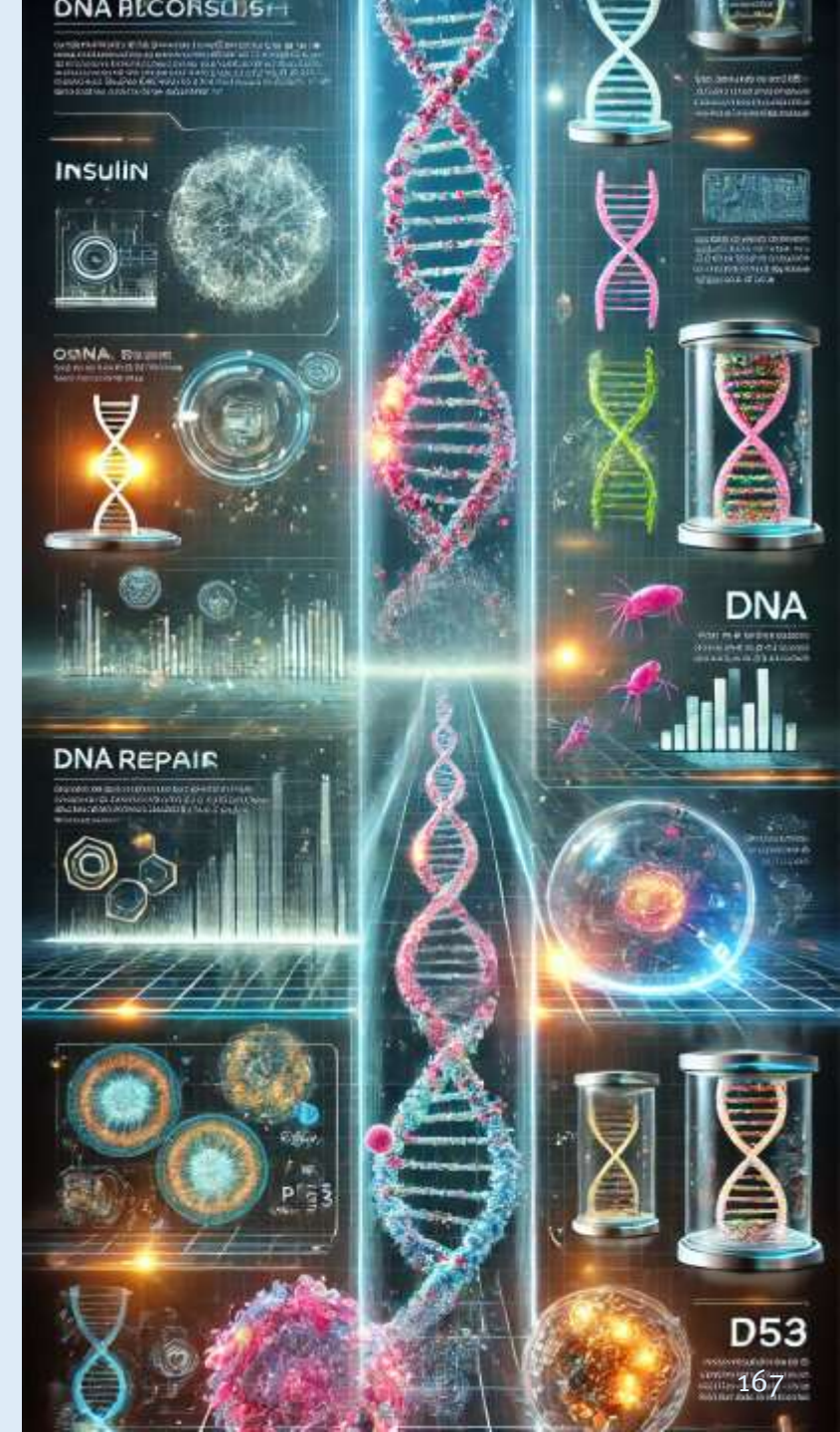
Kenyon's research also identified the role of the daf-16 gene, a transcription factor that promotes longevity by activating stress resistance and repair pathways.

Her work revealed that the Insulin/IGF-1 signaling pathway is a central regulator of aging across species, including mammals.

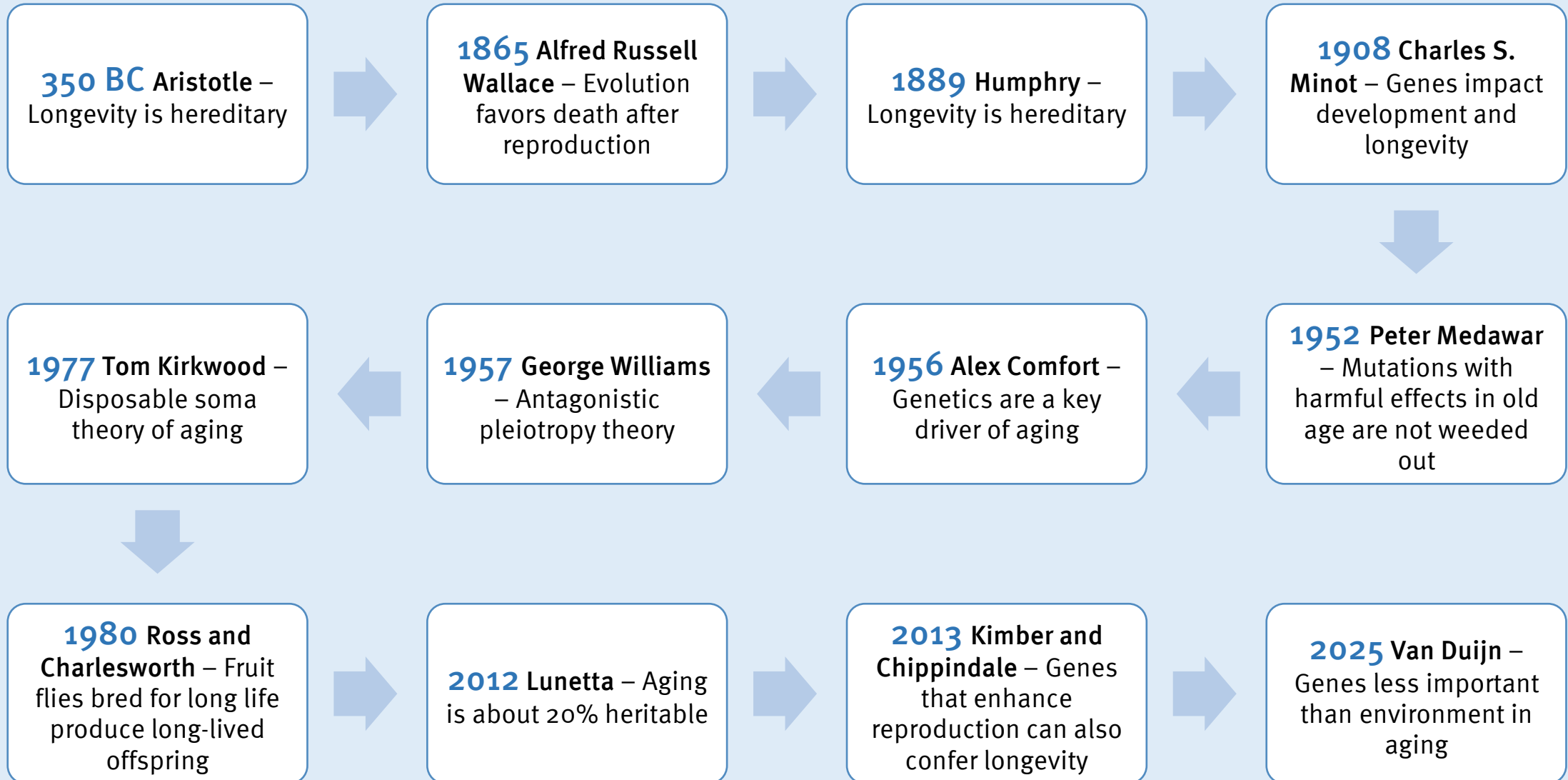
Kenyon's discoveries have inspired research into interventions that mimic these genetic effects, such as caloric restriction and pharmacological agents targeting insulin/IGF-1 signaling, to extend lifespan and healthspan.

Later research has revealed that aging is influenced by complex genetic networks. Genome-wide association studies in humans have identified numerous genetic variants associated with longevity and age-related diseases.

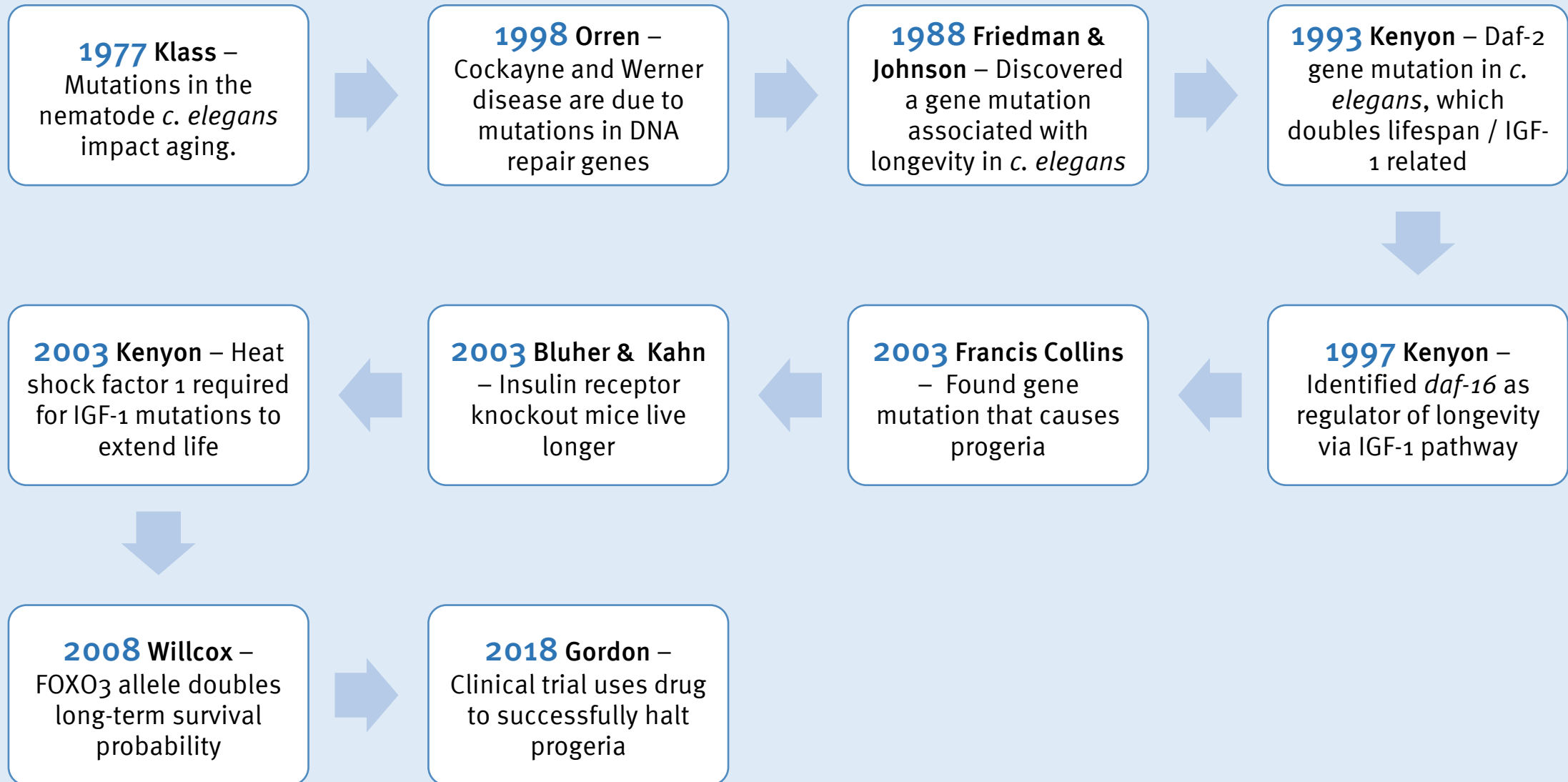
The next few pages provides a timeline of key milestones in genetics and aging research.



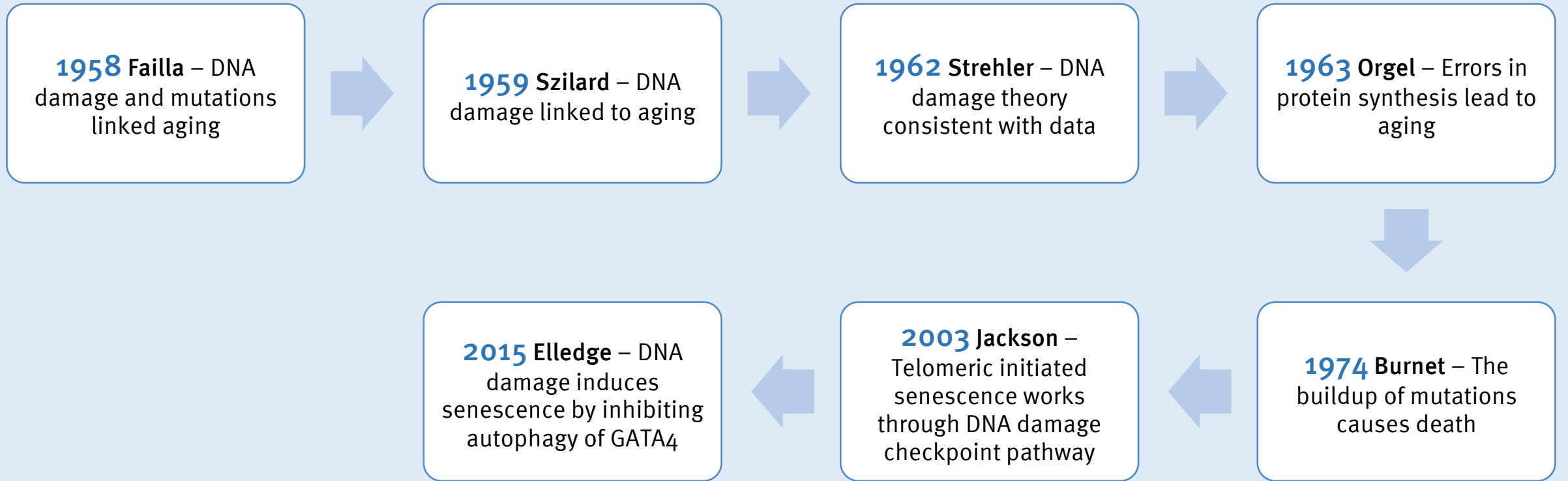
Research Milestones: Aging, Genes & Evolution



Key Insights Into Aging From Genetic Variation



Key Research Milestones: DNA Damage and Aging



1977: Tom Kirkwood

Kirkwood's highly influential "disposable soma theory" posits that aging occurs because evolution favors allocating energy to reproduction over somatic maintenance, leading to gradual cellular damage and senescence.

Tom Kirkwood's "Disposable Soma" theory, introduced in his 1977 paper "Evolution of ageing" in *Nature*, challenges the traditional view that ageing is an inevitable, programmed process. Instead, Kirkwood proposes that ageing results from an evolutionary trade-off between resource allocation for reproduction and maintenance and repair of somatic (non-reproductive) cells.

According to the theory, organisms have limited energy, which must be divided between reproduction (ensuring genetic survival) and somatic maintenance (keeping the body functioning optimally). Natural selection favors strategies that maximize reproductive success, even if it means compromising long-term somatic maintenance. Over time, accumulated cellular damage (e.g., DNA mutations, protein errors, oxidative stress) leads to ageing and decline because the body does not invest enough resources in perfect repair.

The term "disposable soma" reflects the idea that the body is maintained just well enough to support reproduction but is ultimately expendable once its reproductive role is fulfilled. This theory helps explain why species with high extrinsic mortality (e.g., mice) age faster—they invest more in early reproduction than in long-term maintenance—while long-lived species (e.g., humans) allocate more resources to somatic repair, delaying ageing. Kirkwood's model remains foundational in biogerontology, linking evolutionary biology with cellular ageing mechanisms.

References: Haycock p. 215; Ramakrishnan p. 21.

review article

Evolution of ageing

T. B. L. Kirkwood*

An evolutionary view of ageing suggests that mortality may be due to an energy-saving strategy of reduced error regulation in somatic cells. This supports Orgel's 'error catastrophe' hypothesis and offers a new basis for the study of normal and abnormal ageing syndromes and of apparently immortal transformed cell lines.

The question of why we and most other higher animals age is not trivial since many organisms, notably higher plants, live and propagate indefinitely. Perhaps because we are so familiar with ageing it is dangerously easy to fall into circular argument such as that ageing is a mechanism for ridding a population of old and worn-out individuals who would otherwise compete for resources with younger and fitter ones'. This makes sense if, for example, we assume individuals to have a defined reproductive lifespan, but is circular since the cessation of reproduction is itself a phenomenon of ageing. In this paper I show that by adopting an evolutionary point of view it is possible to relate Orgel's theory¹ that ageing is the result of a progressive breakdown in accuracy in protein synthesis to recent theoretical and experimental work on the senescence of diploid human fibroblasts, and I suggest a new reason why we may expect the error theory of ageing to be correct.

In discussing the evolution of error regulatory mechanisms it is appropriate to begin with brief consideration of the emergence of the earliest life-forms. The fate of the first replicating organic molecule would have depended on the speed and accuracy with which it generated 'daughters'. Subject to a continual risk of disruption by chemical and physical agents in the environment the lifetime of any relatively complex molecule must have been strictly limited. In addition primitive replication was probably highly inaccurate so only a relatively small proportion of copies would have been viable. To avoid extinction a replicator needed to produce at least one viable copy before being disrupted and even so there remained a definite chance of extinction which decreased inversely with the expected number of descendants per generation. It is, therefore, highly unlikely that life began all at once in a particular place at a particular time. Much more plausibly large numbers of colonies of different replicators were appearing and disappearing over the same period of time. Gradually, and inevitably, the colonies became larger as the faster and more accurate replicators outlasted the others. The mean levels of accuracy and speed of replication continued to rise while the freely available raw material for biosynthesis was depleted, leading to intensive interspecies competition and strong selection for catalytic activity and hence, for example, to the evolution of organised cells capable of binary fission.

Coping with errors

At a fundamental level evolutionary survival is the preservation of a dynamic balance between information, or

order, and entropy, or disorder. Indeed, given the capacity for replication, neo-Darwinist natural selection may be seen as the direct result of interaction between the information stored in living organisms and the entropy of their environment. The diversity of present-day species testifies to the wide range of evolutionary strategies that are effective for survival, and it is my intent in this paper to demonstrate why ageing should be one of these.

A vital manifestation of entropy is the error inherent in all processes of macromolecular information transfer within living cells. To sustain the prospect of further evolutionary change and so improve its chance of ultimate survival an organism must make occasional copying errors. Too many mistakes would be a serious handicap so what is needed is a compromise, a tolerable level of error sufficient to allow necessary change. Given enough time natural selection ensures that organisms with optimal error levels will dominate. The presence of any error at all, however, poses interesting problems for our understanding of molecular biology.

Orgel¹ pointed out that cellular proteins could be divided roughly into two sets, those involved in the transfer of information from DNA, in replication and in protein synthesis, and those involved in other cell functions. The presence of a low level of error in the synthesis of the second set of proteins would have relatively little effect on cell viability. But erroneous synthesis of some of the first set might have serious consequences since abnormal proteins that retained activity but had reduced specificity could produce further defective proteins and so generate a positive feedback of errors leading to a progressive decline in the accuracy of the protein-synthesising machinery and ultimately to a lethal 'error catastrophe'. Orgel subsequently revised² his original suggestion that the error frequency must increase exponentially and showed that depending on the amount of error feedback allowed, stability of translation may be achieved despite the presence of error. In some circumstances, however, error catastrophe would still occur. Hoffmann³ has proposed a more detailed model of the feedback of errors in the protein-synthesising machinery and concluded that translation is too stable to allow 'error catastrophe' to play any real part in present-day organisms. He supposes the translation apparatus to comprise a set of polypeptide 'adaptors' responsible for inserting amino-acids into a peptide chain according to the sequence specified by the DNA. The average accuracy of the *i*th generation of adaptors (assuming these to be in some sense discrete generations)—that is the probability that an adaptor of the *i*th generation inserts the correct amino-acid—is denoted by *q_i*. Then what matters is the form of the functional relationship between *q_i* and *q_{i+1}*. If *q_{i+1}* > *q_i*, the error level

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Tom Kirkwood, "Evolution of Aging," *Nature*. Nov 24, 1977; 270:301-304.

1977: Michael Klass

The first of a series of brilliant papers to use mutations in the nematode *C. elegans* to study drivers of aging.

Michael Klass's 1977 paper "Aging in the Nematode *Caenorhabditis elegans*: Major Biological and Environmental Factors Influencing Life Span" is a foundational study in aging research.

The paper explores the effects of various biological and environmental factors on the lifespan of the nematode *Caenorhabditis elegans*, establishing it as a model organism for studying aging. Specifically, Klass systematically investigated how factors such as temperature, nutrition and population density influence lifespan, providing some of the first empirical data on the plasticity of aging in a simple organism. His work demonstrated that lifespan in *C. elegans* could be significantly extended or shortened depending on environmental conditions, highlighting the interplay between genetics and the environment in determining longevity.

Klass reported the isolation of eight *Caenorhabditis elegans* long-lived mutant strains. Examining these strains led KLASS to conclude that each strain had a visible defect that led to life span prolongation. The extended life of two strains resulted from the fact that they formed dauer larvae constitutively; one strain was chemotaxis defective; the remaining five strains (MK7, MK31, MK 248, MK 542 and MK546) showed decreased food ingestion which led Klass to conclude that life extension in these five strains resulted from food restriction. Klass concluded that life-extension loci are rare and that there are no loci that display only specific alterations in life span without affecting other characteristics as well.

References: Haycock p. 224; Ramakrishnan p. 145.

AGING IN THE NEMATODE *CAENORHABDITIS ELEGANS*: MAJOR BIOLOGICAL AND ENVIRONMENTAL FACTORS INFLUENCING LIFE SPAN

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SUMMARY

The free-living nematode *Caenorhabditis elegans* is an excellent experimental system for the study of aging. The present study identifies some of the major biological and environmental factors influencing life span as a prelude to more detailed genetic and biochemical analyses. Life span can be altered during any part of the life cycle by a change in either temperature or food concentration. Parental age and parental life span both have relatively small effects on progeny life span. The nematode accumulates fluorescent pigment resembling lipofuscin, and becomes less sensitive to ultra-violet radiation as it ages.

INTRODUCTION

Several species of free-living nematodes are currently being used as experimental systems for the study of aging [1–3]. Nematodes offer the advantages of a relatively simple morphology with a fixed number of somatic cells and a short life span. Most species can be cultured either axenically or monoxenically and large quantities can easily be obtained for biochemical studies. The nematode *Caenorhabditis elegans* is one species whose life cycle has been extensively characterized [4–9]. As an experimental system, *C. elegans* offers the additional advantages of: (a) a genetically manipulable system complete with a large number of characterized markers for genetic analysis [4]; (b) easily obtainable mutants [4, 5]; (c) a simple and extensively mapped nervous system [6, 7]; and (d) a well-characterized gonadogenesis [8, 9].

The present study is an attempt to define the major characteristics of aging in *C. elegans* to provide the basis for more detailed genetic and biochemical analyses. The effects on life span of temperature, nutrition, parental age and life span and u.v. irradiation are described as well as the accumulation of fluorescent pigment.

Michael R. Klass, "Aging in the Nematode *Caenorhabditis elegans*: Major Biological and Environmental Factors Influencing Life Span," *Mechanisms of Aging and Development*. 1977; 6:413-429.

1980: Brian Charlesworth

Tested Medawar's theory that aging is driven by genetics and found that fruit flies bred for long life produced long-lived offspring. This showed that aging has an important genetic component.

Senescence is the post-maturation decline in survivorship and fecundity that accompanies advancing age. Brian Charlesworth tested the two main evolutionary theories have been proposed to account for senescence:

1. *The mutation-accumulation theory.* Deleterious mutations exerting their effects only late in life would tend to accumulate, because of their minimal effects on fitness. More precisely, exclusively late-acting deleterious mutations will attain higher equilibrium frequencies under mutation–selection balance than will mutations that act early, resulting in lower mean values for fitness components late in life. Medawar emphasized the possibility that this effect would be enhanced by selection of modifiers that postpone the age of onset of genetic diseases.
2. *The antagonistic pleiotropy theory.* Williams suggested that many of the genes with beneficial effects on early fitness components have pleiotropic deleterious effects on late fitness components but are nevertheless favored by natural selection.

Charlesworth's experiments in *Drosophila* and *Tribolium* supported the pleiotropy theory, although one such experiment gave results that only bordered on significance, but the mutation–accumulation theory had never previously been tested.

A test of evolutionary theories of senescence

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Senescence is the post-maturation decline in survivorship and fecundity that accompanies advancing age. Two main evolutionary theories have been proposed to account for senescence. (1) *The mutation-accumulation theory.* Deleterious mutations exerting their effects only late in life would tend to accumulate, because of their minimal effects on fitness^{1,2}. More precisely, exclusively late-acting deleterious mutations will attain higher equilibrium frequencies under mutation–selection balance than will mutations that act early, resulting in lower mean values for fitness components late in life (ref. 3, p. 218). Medawar¹ emphasized the possibility that this effect would be enhanced by selection of modifiers that postpone the age of onset of genetic diseases. (2) *The pleiotropy theory.* Williams⁴ suggested that many of the genes with beneficial effects on early fitness components have pleiotropic deleterious effects on late fitness components, but are nevertheless favoured by natural selection. (These theories are based on the decline with age in the effect of age-specific fitness-component changes on total fitness³ (ref. 3, pp. 206–214 and refs 4, 5). Either or both of these theories could apply in any particular population.) Selection experiments in *Drosophila*⁶ and *Tribolium*⁷ support the pleiotropy theory, although one such experiment gave results that only bordered on significance⁸, but the mutation–accumulation theory has never been tested. The present results provide evidence for the pleiotropy theory, but do not support the mutation–accumulation theory.

Tests of evolutionary theories with randomly breeding laboratory populations, as in the present case, require that they be maintained without significant inbreeding after sampling from a long-standing natural population. In August 1975, 400 *Drosophila melanogaster* adults were sampled from the Amherst, Massachusetts, population studies by Ives⁹. There is good evidence that this population has been continuously endemic in the Amherst area since about 1930 (ref. 9). The flies were maintained in the laboratory at 25 °C in 10–16 one-third-pint milk bottles containing Lewis medium and powdered yeast.

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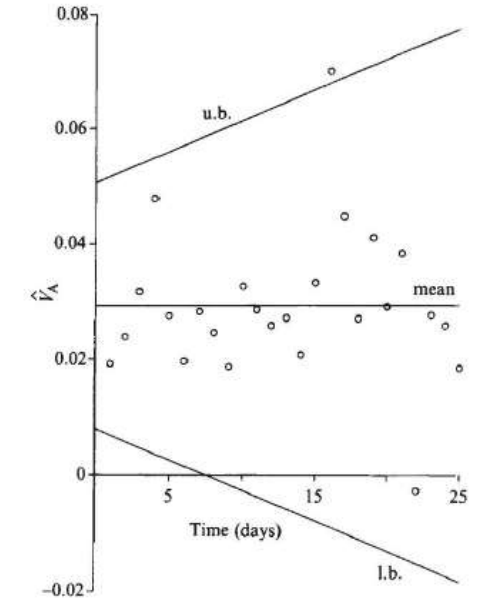


Fig. 1 Estimates of additive genetic variance ($\hat{V}_A$) for egg laying over 24 h. Only data from females which survived the 24 h and laid at least one egg during this period were used, since approximate normality of the data is required for analysis of variance. The data were transformed using the function $x' = \ln(x + 23.81)$, so as to remove the scale effects arising from the fall in egg laying during the 25-day period of assay¹². The estimates were obtained using a sibling analysis of variance with unbalanced cell allocation (ref. 13, p. 459). The total number of individuals scored falls from 1,099 on day 1 to 396 on day 25. The overall mean for $\hat{V}_A$ during the assay period is 0.02925, which is plotted. The least squares linear regression slope for $\hat{V}_A$ on age has a slope of -0.00002 ± 0.00072 , with 95% confidence, which has a t value of -0.0599 , with 23 degrees of freedom. This is not significant at any reasonable level of probability. The best estimate for V_A over the period is its mean value. Because of a confounding design variable, confidence intervals for this estimate had to be estimated on an *ad hoc* basis using the normal equations for the unbalanced design¹⁴, giving the upper and lower bounds (u.b. and l.b.) for daily estimates of V_A shown.

1988: Friedman & Johnson

Friedman and Johnson discovered a specific gene mutation associated with longer life in the nematode *C. elegans*.

The 1988 paper by David Friedman and Tom Johnson, "A Mutation in the age-1 Gene in *Caenorhabditis elegans* Lengthens Life and Reduces Hermaphrodite Fertility," is a landmark study in the field of aging research.

The authors identified the age-1 gene, the first gene ever discovered whose mutation could significantly extend the lifespan of *C. elegans*. This finding provided the first direct evidence that single-gene mutations could influence aging, challenging the prevailing view that aging was a purely stochastic or environmentally determined process. The age-1 mutation increased the mean and maximum lifespan of *C. elegans* by up to 65%, demonstrating that aging is subject to genetic regulation. Additionally, the study observed a trade-off between longevity and fertility, as the age-1 mutants exhibited reduced reproductive output, suggesting a biological cost to extended lifespan.

The discovery of age-1 had profound implications, as it opened the door to the genetic dissection of aging and longevity. Friedman and Johnson's work laid the foundation for subsequent research into the insulin/IGF-1 signaling pathway, which was later shown to play a conserved role in regulating lifespan across species, including mammals. By demonstrating that aging could be modulated by specific genes, this study revolutionized the understanding of aging as a biologically regulated process.

References: Campisi p. 184; Haycock p. 224; JSG 22; Ramakrishnan p. 166.

A Mutation in the *age-1* Gene in *Caenorhabditis elegans* Lengthens Life and Reduces Hermaphrodite Fertility

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ABSTRACT

age-1(hx546) is a recessive mutant allele in *Caenorhabditis elegans* that results in an increase in mean life span averaging 40% and in maximal life span averaging 60% at 20°; at 25° *age-1(hx546)* averages a 65% increase in mean life span (25.3 days vs. 15.0 days) and a 110% increase in maximum life span (46.2 days vs. 22.0 days for wild-type hermaphrodites). Mutant males also show extended life spans. *age-1(hx546)* is associated with a 75% decrease in hermaphrodite self-fertility as compared to the *age-1*⁺ allele at 20°. Using two novel strategies for following the segregation of *age-1*, we present evidence that longer life results from a mutation in a single gene that increases the probability of survival at all chronological ages. The long-life and reduced-fertility phenotypes cosegregate and are tightly linked to *fer-15*, a locus on linkage group II. *age-1(hx546)* does not affect the timing of larval molts, the length of embryogenesis, food uptake, movement, or behavior in any way tested. Although *age-1(hx546)* lowers hermaphrodite self-fertility, it does not markedly affect the length of the reproductive period with all the increase in life expectancy due to an increase in the length of postreproductive life. In so far as we are aware, this mutant in *age-1* is the only instance of a well-characterized genetic locus in which the mutant form results in lengthened life. It is likely that the action of *age-1* in lengthening life results not from eliminating a programmed aging function but rather from reduced hermaphrodite self-fertility or from some other unknown metabolic or physiologic alteration.

HOW genes are involved in specifying the limited life span of metazoans is a major unsolved problem in biology. Although some have argued that genes may not be involved at all, no informed critic can seriously doubt the contribution of the genotype in specifying species-specific life span and other attributes of aging (RUSSELL 1976; LINTS 1978; ROTHSTEIN 1983, 1985, 1987). Although there are many theories suggesting molecular and physiologic processes that may be responsible for this limitation (ADELMAN and ROTH 1982; FINCH and SCHNEIDER 1985), there are few systems in which predictions of these theories can be directly tested with regard to aging and senescence. A problem peculiar to research in aging has been the lack of good experimental systems with which to manipulate the rates of aging. For example, dietary restriction is the only technique currently available that has been reliably shown to lengthen maximum life span (MASORO 1985; WEINDRUCH 1984).

Mutants with altered rates of aging would be quite useful but criteria that can be used to identify such mutants are unclear. The set of genes most directly involved in the specification of life span might be

identified by screening for mutants that have increased life expectancy, especially those with increased maximum life span. By identifying mutants that extend life span we can identify those genes and processes that limit the normal life span. HAYFLECK (1985) has said of this approach "... only by increasing life span or maximum age of death of members of a species will important insight be made into the aging process...."

Mutants that alter the length of life and presumably the rate of aging have been sought in a variety of metazoan species. For example, GOULD and CLARK (1977) and LEFFELAAR and GRIGLIATTI (1984) have isolated adult-onset-lethal mutations in *Drosophila melanogaster*; LEFFELAAR and GRIGLIATTI (1984) have also identified one mutant which they interpret as showing accelerated senescence. In humans several clinical syndromes have been described as examples of accelerated senescence (for reviews see GOLDSTEIN 1971; MARTIN 1978). These human syndromes accelerate only some of the normal characteristics of aging, and it is now widely believed that they only mimic normal aging processes (MARTIN 1978). In contrast, mutants that extend the maximum length of life must have altered those rate-determining processes that limit the life of the wild-type organism.

KLASS (1983) reported the isolation of eight long-lived mutant strains of *Caenorhabditis elegans*. Exami-

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This paper is dedicated to the memory of Dr. Laurence Sandler, a gifted teacher and geneticist, deceased February 7, 1987, at his home in Seattle, Washington.

1993: Cynthia Kenyon

Discovery of the *daf-2* gene mutation in *C. elegans*, which doubles lifespan, highlighting the role of insulin/IGF-1 signaling in aging.

In their groundbreaking 1993 paper published in *Nature*, Cynthia Kenyon and colleagues discovered that mutations in the *daf-2* gene could double the lifespan of the nematode *Caenorhabditis elegans* (*C. elegans*). The *daf-2* gene encodes a receptor in the insulin/IGF-1 signaling (IIS) pathway, which is highly conserved across species, including humans.

The researchers found that reducing the activity of this pathway not only extended lifespan but also maintained the health and vitality of the worms, suggesting that aging is not an inevitable process but is subject to genetic regulation. This study was the first to demonstrate that a single gene mutation could significantly extend lifespan, challenging the prevailing view that aging was solely the result of accumulated damage.

Kenyon's work revolutionized the field of aging research by identifying the IIS pathway as a central regulator of longevity. The discovery that reduced *daf-2* activity led to increased lifespan highlighted the potential for manipulating genetic pathways to delay aging and age-related diseases. Subsequent research showed that the *daf-2* mutation activates another gene, *daf-16*, which encodes a transcription factor that promotes stress resistance and longevity. This paper laid the foundation for understanding how metabolic and environmental signals influence aging and inspired a wave of research into the genetic and molecular mechanisms of lifespan extension, with implications for human health and longevity.

References: Campisi p. 184; Haycock p. 224; JSG 46; Ramakrishnan 146.

A *C. elegans* mutant that lives twice as long as wild type

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We have found that mutations in the gene *daf-2* can cause fertile, active, adult *Caenorhabditis elegans* hermaphrodites to live more than twice as long as wild type. This lifespan extension, the largest yet reported in any organism¹, requires the activity of a second gene, *daf-16*. Both genes also regulate formation of the dauer larva, a developmentally arrested larval form that is induced by crowding and starvation and is very long-lived²⁻⁴. Our findings raise the possibility that the longevity of the dauer is not simply a consequence of its arrested growth, but instead results from a regulated lifespan extension mechanism that can be uncoupled from other aspects of dauer formation. *daf-2* and *daf-16* provide entry points into understanding how lifespan can be extended.

Temperature-sensitive mutations in several genes lead to formation of long-lived dauer larvae in the absence of inducing signals. To learn whether these mutations might also increase lifespans of non-daughters, we examined the lifespans of dauer-constitutive *daf-2*, *daf-11*, *daf-7* and *daf-14* mutants (refs 4, 5 and E. Malone and J. Thomas, personal communication). The decision to enter the dauer stage, an alternative third larval stage (L3) must be made during the first larval stage (L1). Therefore, we shifted the mutant larvae to the non-permissive temperature at later stages, allowed them to grow to adulthood, and measured their lifespans⁶. Because many temperature-sensitive mutations lead to reduced gene activity even at the permissive temperature, we also measured the lifespans of these mutants at lower temperatures. The lifespans of *daf-11*, *daf-7* and *daf-14* adults were not noticeably different from wild type (Fig. 1 legend). In contrast, each of three *daf-2* mutations, *e1370*, *sa189* and *sa193* greatly extended lifespans of adults when shifted to the non-permissive temperature during L4 (Fig. 1a, b legend). *daf-2(e1370)* and *daf-2(sa193)* were also examined at the permissive temperature; the lifespans of both were extended (Fig. 1c, and data not shown).

The magnitude of the lifespan extension was striking. For example, when shifted from 15 °C to 20 °C at the L4 stage, the mean lifespan of wild type was 18 days, whereas that of *daf-2(sa189)* was 42 days (Fig. 1a), 2.3-fold greater than wild type. The difference between ageing rates of *daf-2* and wild-type animals was also readily apparent. When all the wild-type animals had died or become immobile, 90% of the *daf-2(e1370)* animals still moved actively on their plates (Fig. 2c). The long-lived *daf-2* adults resembled wild-type adults much more closely than the dauer. The dauer is developmentally arrested as a small, thin, and sexually immature larval form⁴. In contrast, the *daf-2* non-dauer animals became full-size adults, and appeared to feed and defaecate normally (Fig. 2, and data not shown). Furthermore, at 20 °C, where its lifespan was twice that of wild type, *daf-2(e1370)* animals had nearly normal brood sizes (212 ± 36 for *daf-2(e1370)* (*n* = 29); as compared to 278 ± 35 for wild-type animals grown in parallel (*n* = 19)).

Because one theory of ageing postulates that reproduction decreases lifespan¹, we wondered whether the slight decrease in the brood size of *daf-2* animals could possibly be responsible for their longevity. *spe-26* hermaphrodites, which are defective in spermatogenesis, live longer than the wild type⁷. This is consistent with the model that sperm production decreases the lifespans

Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A *C. elegans* mutant that lives twice as long as wild type. *Nature*. Dec 2, 1993; 366(6454):461-4.

1997: Cynthia Kenyon

Identified *daf-16* as a key downstream effector of the insulin/IGF-1 pathway, regulating longevity.

In their 1997 paper published in *Science*, Cynthia Kenyon and her team identified *daf-16* as a critical transcription factor in the Insulin/IGF-1 signaling (IIS) pathway that regulates lifespan in *Caenorhabditis elegans* (C. elegans). The researchers demonstrated that *daf-16*, a member of the HNF-3/forkhead family of transcription factors, is essential for the dramatic lifespan extension observed in *daf-2* mutants, which have reduced activity in the insulin/IGF-1 receptor. They showed that *daf-16* acts downstream of *daf-2* and is activated when IIS pathway activity is low. Activation of *daf-16* leads to the expression of a wide array of genes involved in stress resistance, metabolism, and longevity, effectively doubling the lifespan of the worms. This study provided the first molecular link between reduced insulin/IGF-1 signaling and increased lifespan, highlighting the role of transcriptional regulation in aging.

The discovery of *daf-16* as a key longevity gene revolutionized the field of aging research by revealing that lifespan extension is an actively regulated process rather than a passive accumulation of damage. Lin et al.'s work established that *daf-16* integrates signals from the environment and metabolism to modulate aging, emphasizing the importance of genetic pathways in determining lifespan. This finding not only advanced our understanding of the molecular mechanisms of aging in *C. elegans* but also had broader implications for aging research in other organisms, including humans. The study paved the way for further exploration of forkhead transcription factors and their role in promoting healthspan and longevity across species.

References: Ramakrishnan p. 149.

daf-16: An HNF-3/forkhead Family Member That Can Function to Double the Life-Span of *Caenorhabditis elegans*

Kui Lin, Jennie B. Dorman,* Aylin Rodan, Cynthia Kenyon

The wild-type *Caenorhabditis elegans* nematode ages rapidly, undergoing development, senescence, and death in less than 3 weeks. In contrast, mutants with reduced activity of the gene *daf-2*, a homolog of the insulin and insulin-like growth factor receptors, age more slowly than normal and live more than twice as long. These mutants are active and fully fertile and have normal metabolic rates. The life-span extension caused by *daf-2* mutations requires the activity of the gene *daf-16*. *daf-16* appears to play a unique role in life-span regulation and encodes a member of the hepatocyte nuclear factor 3 (HNF-3)/forkhead family of transcriptional regulators. In humans, insulin down-regulates the expression of certain genes by antagonizing the activity of HNF-3, raising the possibility that aspects of this regulatory system have been conserved.

The identification of genes that regulate aging (1) is an important breakthrough because it provides a means of investigating this fundamental but poorly understood process. The nematode *Caenorhabditis elegans* has a very rapid rate of aging (2), which is due in part to the activity of the gene *daf-2*. Mutations that reduce the activity of *daf-2*, a homolog of the insulin and insulin-like growth factor (IGF) receptors (3), can slow the rate of aging and more than double the life-span of the animal (4) without substantially affecting its activity or fertility (4-6). The life-span extension caused by *daf-2* mutations requires the gene *daf-16* (4).

In addition to regulating the rate of aging, *daf-2* and *daf-16* also regulate the decision to enter diapause (the dauer phase) (7-10). When food is limited, young animals become dauers instead of developing to adulthood (9). The dauer is a resilient, long-lived, juvenile form that remains small and reproductively immature. Wild-type *daf-2* activity promotes growth to adulthood and prevents dauer formation. Unlike partial loss of *daf-2* function, which specifically affects life-span, more severe loss of *daf-2* function causes the animals to become dauers even in the presence of food (7-11). Thus, *daf-2(+)* has two functions: It promotes growth to adulthood, and it shortens the life-spans of adult animals (12). In addition to its role in life-span extension, the wild-type *daf-16* gene is also required for dauer formation in both wild-type and *daf-2(-)* animals (7-11). Thus, *daf-16* also has two functions: Under dauer-inducing conditions, it promotes dauer formation, and,

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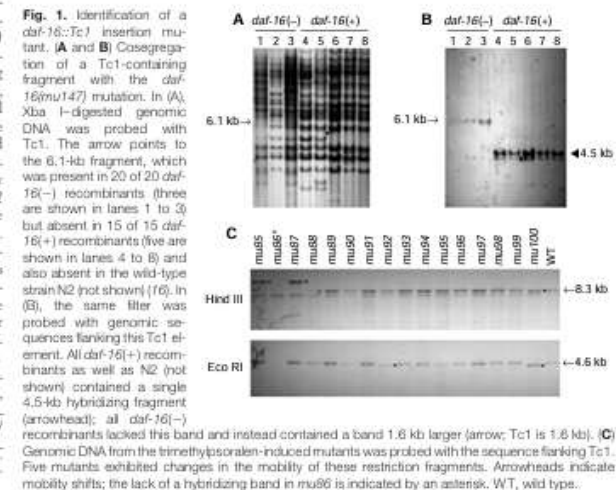
under conditions that do not induce dauer formation, it allows fertile adults carrying weak *daf-2* mutations to remain active for a much longer period and to live twice as long as normal (4).

Because so few genes are known to regulate aging in any organism, we asked how many other genes were likely to have functions similar to that of *daf-16*. To do this, we carried out a genetic screen for additional *daf-16*-like mutants. *daf-16* mutations, as well as mutations in functionally related genes required for both dauer formation and life-span, can be isolated easily as suppressors of *daf-2* mutations (7–9). We mu-

tagenesis. *daf-2(c1370)* hermaphrodites with trimethyloxalene followed by ultraviolet (UV) irradiation, which causes both point mutations and deletions (13), and screened their descendants for rare individuals that did not become dauers but instead grew to adulthood. We found 24 independent *daf-2* suppressors, all of which proved to be new alleles of *daf-16* (14). This result suggests that there are not a large number of genes like *daf-16*. Instead, any genes that act along with, or downstream of, *daf-16* to initiate dauer formation are likely to affect only certain aspects of dauer formation or else are functionally redundant or essential (15). Thus, *daf-16* provides a unique starting point for understanding how aging can be regulated.

We cloned *daf-16* by transposon tagging (16, 17). We isolated a *daf-16::Tc1* insertion mutation, *mut47*, by looking for *daf-2* suppressors in a *daf-2*, *mut-6* strain, in which the transposon Tc1 is active (Fig. 1, A and B) (16). We then cloned the genomic DNA containing this Tc1 element and used sequences flanking Tc1 as a probe in Southern blot analysis of DNA isolated from our trimethylsilanol-induced *daf-16* mutants. In five mutants, we found changes in the mobility of the restriction fragments that hybridized to the probe (Fig. 1C).

The Tc1-tagged DNA was sequenced and found to be present on the cosmid R13H8, which was sequenced by the *C. elegans* sequencing project (18). We obtained the corresponding cDNA sequences by performing



Lin K, Dorman JB, Rodan A, Kenyon C. daf-16: An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science*. Nov 14, 1997 14;278(5341):1319-22.

2003: Cynthia Kenyon

Knocking out insulin receptor on adipocytes increased lifespan. HSF-1 was found to be a key regulatory of longevity for the first time.

This paper by Hsu, Murphy, and Kenyon (2003) investigates the roles of the transcription factor DAF-16 (a FOXO family protein) and heat-shock factor (HSF-1) in regulating aging and age-related diseases in *C. elegans*.

The researchers demonstrate that DAF-16, a key effector of the insulin/IGF-1 signaling pathway, is essential for the longevity observed in animals with reduced insulin signaling (e.g., *daf-2* mutants). They show that DAF-16 activation promotes lifespan extension by regulating stress resistance, metabolism, and detoxification genes.

Additionally, the study identifies HSF-1, a mediator of the heat-shock response, as another critical longevity factor that works in concert with DAF-16. HSF-1 enhances lifespan by maintaining protein homeostasis, particularly through the expression of molecular chaperones like heat-shock proteins, which prevent protein aggregation linked to aging and neurodegeneration.

The paper shows that DAF-16 and HSF-1 jointly regulate a subset of genes critical for longevity and stress resilience. Disruption of either factor abolishes the extended lifespan of *daf-2* mutants,

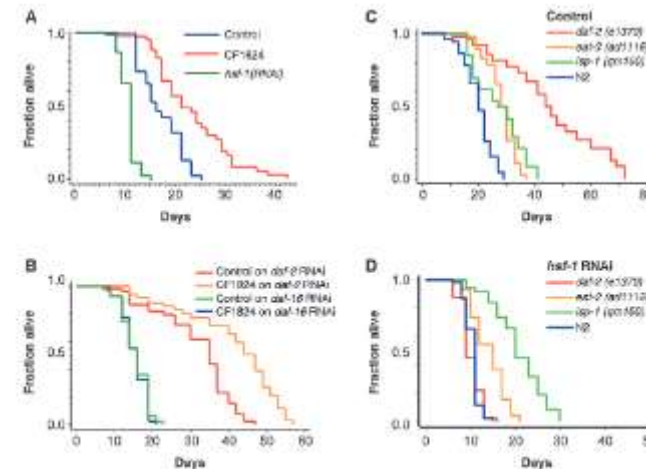
Regulation of Aging and Age-Related Disease by DAF-16 and Heat-Shock Factor

Ao-Lin Hsu, Coleen T. Murphy, Cynthia Kenyon*

The *Caenorhabditis elegans* transcription factor HSF-1, which regulates the heat-shock response, also influences aging. Reducing *hsf-1* activity accelerates tissue aging and shortens life-span, and we show that *hsf-1* overexpression extends life-span. We find that HSF-1, like the transcription factor DAF-16, is required for *daf-2*-insulin/IGF-1 receptor mutations to extend life-span. Our findings suggest this is because HSF-1 and DAF-16 together activate expression of specific genes, including genes encoding small heat-shock proteins, which in turn promote longevity. The small heat-shock proteins also delay the onset of polyglutamine-expansion protein aggregation, suggesting that these proteins couple the normal aging process to this type of age-related disease.

Heat-shock factor activates transcription of heat-shock genes, which encode chaperones and proteases, in response to heat and other forms of

stress. Previous studies have implicated heat-shock proteins (HSPs) in aging. For example, mild heat stress can cause a period of decreased



mortality rate in *Drosophila*, and *hsp70* has been implicated in this effect (1). In addition, expression of genes encoding small heat-shock proteins (sHSPs) is increased in *Drosophila* lines selected for increased life-span (2), and overexpression of *hsp70* increases the life-span of *C. elegans* (3).

Previously, we showed that reducing the activity of *C. elegans* HSF-1 causes a rapid-aging phenotype and shortens life-span (4). Conversely, we found that animals carrying additional *hsf-1* gene copies (5), which were resistant to heat and oxidative stress (Fig. S1, A and B), lived approximately 40% longer than normal (Fig. 1A). Thus HSF-1 activity promotes longevity.

The FOXO transcription factor DAF-16, which functions in the *C. elegans* insulin/

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Fig. 1. HSF-1 promotes longevity. (A) Blue, survival of wild-type animals grown on control bacteria containing vector alone; green, animals grown on bacteria expressing *hsf-1* dsRNA; red, animals carrying additional copies of *hsf-1* (CF1824). Two additional HSF-1 overexpressing lines were obtained and were found to extend life-span (table S1). (B) *hsf-1* overexpression extends life-span in a *daf-16*-dependent manner. Adult life-spans of wild-type and *hsf-1*-overexpressing (CF1824) animals grown on *daf-16* RNAi bacteria (green and blue lines). In addition, *hsf-1* overexpression extends the life-span of animals treated with *daf-2* RNAi (red and orange lines). (C and D) RNAi of *hsf-1* completely prevents the *daf-2*(*e1370*) or *eat-2*(*ad1116*) mutations, from extending life-span. Animals were grown on *hsf-1* RNAi bacteria from the time of hatching. Adult life-spans of wild-type (*hsf-1* RNAi) (blue), *daf-2*(*e1370*) (red), *eat-2*(*ad1116*) (orange), and *hsf-1*(*qm150*) (green) animals grown on (C) control bacteria or (D) *hsf-1* RNAi bacteria. All experiments have been repeated more than once with similar effects. For statistical data, see table S1.

1142

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Hsu AL, Murphy CT, Kenyon C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science*. May 16, 2003; 300(5622):1142-5.

Cynthia Kenyon Interview

Cynthia Kenyon: 'The idea that ageing was subject to control was completely unexpected', *The Guardian*, Mar 17, 2013. (excerpt)

In the old Russian fable of "Little Red Hen", a hard-working hen asks her friends to help her make bread. None of them are interested in her endeavours, until the bread is ready. Suddenly, everybody wants a piece. As Cynthia Kenyon recalls her early enthusiasm for ageing research in the 1980s, as a young professor at the University of California, San Francisco, where she still works. She tells me how one contemporary, now a Harvard professor, told her: "Cynthia, I have friends who work on ageing and it is as if they fall off the edge of the Earth." Yet years later Kenyon's studies "meant that evolutionary biologists had to go back to the drawing board". Suddenly, like little red hen, everyone was interested in what she had been up to.

Eventually, she persuaded a student, Ramon Tabtiang, to help her look for specific genes that might control ageing in her worms. They were "incredibly lucky" and, before long, in 1993, had made their big breakthrough. Partially disabling a single gene – called *daf-2* – caused the worms to live twice as long as normal. Not only that, but the worms appeared to be healthy until the end.

This finding built on her earlier research, but to the rest of the scientific community, "the idea that ageing was subject to control was completely unexpected," Kenyon says, before struggling to find the words to describe how she felt when she realised the magnitude of the discovery. "It was very profound because you look at these worms, and the normal worms are dying, and the worms in this other culture dish are young. And you think: 'Oh my God, they should be dead.' It was like finding something that shouldn't be. It makes your hair stand up." Then came a second realisation: "You just think, 'Wow. Maybe I could be that long-lived worm.'"



The link between diet and ageing makes sense when you consider that the gene *daf-2*, which was partially disabled in Kenyon's worms, activates receptors that are sensitive to two hormones – insulin and a growth hormone called IGF-1. This receptor is normally activated by insulin, and too much sugar in the diet, which may have the opposite effect, overstimulating these receptors, says Kenyon, who warns that "sugar is the new tobacco".

For those who doubt the relevance to humans, Kenyon points to studies showing that people who live to be 100 are more likely to have mutations in the *daf-2* gene. There are also variants in the *FOXO* gene that are more frequent among people who live to be 100.

After more than three decades working with her microscopic worms, Kenyon's last big effort in her career is to "try to move this into people. That's my dream."

1998: David K. Orren

Showed that two genetic diseases of early aging (Cockayne and Werner) are due to specific mutations in DNA repair genes.

David Orren and colleagues' 1998 paper, "DNA Repair and Transcription in Human Premature Aging Disorders," explores the relationship between DNA repair mechanisms, transcription, and premature aging syndromes such as Werner syndrome (WS) and Cockayne syndrome (CS). The study highlights the critical role of transcription-coupled repair (TCR), a subpathway of nucleotide excision repair (NER), in maintaining genomic stability and cellular function. TCR specifically targets and repairs DNA damage on the transcribed strands of active genes, ensuring the integrity of essential transcriptional processes. Bohr's research demonstrates that defects in TCR are a hallmark of premature aging disorders, as patients with WS and CS exhibit impaired repair of transcription-blocking lesions, leading to accelerated aging phenotypes. The paper underscores the importance of understanding how disruptions in DNA repair and transcription contribute to the pathophysiology of these disorders.

Furthermore, Bohr's work emphasizes the broader implications of TCR defects in aging and cancer. The study suggests that the accumulation of DNA damage in actively transcribed genes due to impaired TCR may lead to cellular senescence, apoptosis, or malignant transformation. By linking DNA repair deficiencies to premature aging, the paper provides insights into the molecular mechanisms underlying normal aging processes. Bohr's findings also highlight potential therapeutic targets for mitigating the effects of aging and age-related diseases.

DNA Repair and Transcription in Human Premature Aging Disorders

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The human progeroid disorders Cockayne syndrome and Werner syndrome present with several clinical features that are associated with normal aging. These include distinct changes in the skin. The genes responsible for these conditions have recently been cloned and characterized. They both contain a characteristic helicase sequence, and helicase activity has been demonstrated using the purified Werner protein. Helicases are involved in a number of DNA metabolic transactions, including transcription, replication, and DNA repair. Cockayne cells are deficient

in a special type of DNA repair, transcription coupled DNA repair, but they also appear to be defective in basal transcription. The diverse functions of the Cockayne protein are under intense study. Werner cells may have subtle defects in DNA repair, and possibly also in transcription. The biochemical clarification of the precise role of these gene products is likely to provide very significant clues into the mechanism of aging. **Key words:** Cockayne syndrome/ Werner syndrome. *Journal of Investigative Dermatology Symposium Proceedings* 3:11-13, 1998

DNA REPAIR PROCESSES

Living cells are constantly exposed to environmental agents and endogenous processes that inflict damage in DNA. Several complex enzymatic mechanisms have evolved to repair DNA lesions, and lately there has been a tremendous progress towards a greater understanding of the mechanisms involved. There are several major pathways of DNA repair, and the particular pathway used depends in part upon the type of DNA damage that is being removed. Most types of cellular stress or damage cause the formation of a large spectrum of DNA lesions. Endogenous metabolic processes generate oxygen radicals that are removed from the DNA mainly by the base excision repair pathway. Ultraviolet (UV) exposure generates two major lesions, or adducts, in the DNA: the pyrimidine dimer and the 6-4 photoproduct. Both of these adducts and other bulky lesions are removed by the nucleotide excision repair (NER) pathway.

NER is deficient in the human genetic disorder xeroderma pigmentosum (XP). This condition involves hyperpigmentation of the skin and the continuous development of skin cancer. The significant dermatologic presentation of this disease has established an important link between dermatology and the field of DNA repair. In XP there are seven different complementation groups representing different genes involved in the disorder. These genes have now been cloned and characterized, leading to a clearer understanding of the NER process in humans. There are several recent reviews in which the pathway of NER is discussed in detail (Friedberg, 1994b; Sancar, 1996; Wood, 1996).

DNA REPAIR, HETEROGENEITY AND THE LINK TO TRANSCRIPTION

Another area of very active research in the field of DNA repair concerns the heterogeneity of the DNA repair process. In the last

Reprint requests to Dr. Vilhelm A. Bohr, Laboratory of Molecular Genetics, National Institute on Aging, GRC, NIH, 3600 Nathan Shock Drive, Baltimore, MD 21224-6825.

Abbreviations: CS, Cockayne syndrome; NER, nucleotide excision repair; TCR, transcription coupled DNA repair; WRN, Werner syndrome; XP, xeroderma pigmentosum.

10 years evidence has accumulated that the repair process differs significantly in efficiency between different regions of the genome.

Specifically, DNA repair occurs preferentially in genes, and particularly those that are actively transcribed. The active component of the genome is only about 1% of the total DNA, but it appears to be much more efficiently repaired than the rest of the genome that is largely inactive. A component of this repair is directly linked to the basal transcription process. This pathway is also termed transcription-coupled DNA repair or "strand specific" DNA repair because the transcribed strand of the active genes is preferentially repaired. The molecular link between DNA repair and transcription involves the basal transcription factor TFIIF that contains, among its nine components, at least two DNA repair proteins, XPD and XPB. The multicomponent TFIIH particle participates in both transcription and DNA repair.

CHANGES WITH AGING

Premature aging syndromes In aging research it is important to choose a good model system for the most mechanistic studies. Although there has been much work on cell lines that grow to senescence, there are now human natural mutant syndromes that have signs and symptoms of premature aging. They are segmental progerias, signifying that they have some, but not all of the features that are associated with the aging phenotype. XP has been characterized as such a condition, and others include Cockayne syndrome (CS) and Werner syndrome (WRN). During recent years, the genes for these disorders have been cloned, and this has tremendously aided the research in this area. It is now possible to conduct sophisticated molecular biochemical studies on cells from individuals with these genetic disorders.

With the recent cloning of the WRN gene, and with the information that this gene, the CS complementation group B (CS-B) gene, and some XP genes are putative helicases, gaining a greater understanding of the molecular deficiency in these disorders is a high priority. The patients have many signs and symptoms of normal aging, but they are segmental progeroid diseases, indicating that some features of normal aging are not seen. The function of the CSB and WRN proteins appear to be at the crossroads of aging, DNA repair, DNA replication, and transcription, so these studies nicely combine a mechanistic interest

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Bohr VA, Dianov G, Balajee A, May A, Orren DK. DNA repair and transcription in human premature aging disorders. *J Investig Dermatol Symp Proc.* 1998 Aug;3(1):11-3.

2003: Blüher and Kahn

Knocking out insulin receptor on adipocytes increased lifespan.

The study by Blüher and Kahn (2003) investigated the role of insulin signaling in aging by examining mice with a tissue-specific knockout of the insulin receptor (IR) in adipose tissue, known as FIRKO (Fat-specific Insulin Receptor Knockout) mice. FIRKO mice exhibited a significant increase in lifespan compared to wild-type mice, despite lower body weight and reduced fat mass.

Further, these mice maintained normal glucose levels, displayed enhanced insulin sensitivity in other tissues, and were resistant to age-related obesity and its metabolic complications.

The longevity effect was attributed to altered adipokine secretion (hormones from fat tissue) and improved systemic metabolism, rather than caloric restriction. Reduced oxidative stress and enhanced stress resistance pathways may also contribute.

The study highlighted the critical role of adipose tissue insulin signaling in regulating mammalian aging. It supports the theory that selective modulation of insulin pathways, particularly in fat tissue, can decouple metabolic health from aging, offering insights into longevity mechanisms.

This work underscores the importance of tissue-specific insulin signaling in aging and suggests potential targets for interventions to promote healthy aging.

Extended Longevity in Mice Lacking the Insulin Receptor in Adipose Tissue

Matthias Blüher,¹ Barbara B. Kahn,² C. Ronald Kahn^{1*}

Caloric restriction has been shown to increase longevity in organisms ranging from yeast to mammals. In some organisms, this has been associated with a decreased fat mass and alterations in insulin/insulin-like growth factor 1 (IGF-1) pathways. To further explore these associations with enhanced longevity, we studied mice with a fat-specific insulin receptor knockout (FIRKO). These animals have reduced fat mass and are protected against age-related obesity and its subsequent metabolic abnormalities, although their food intake is normal. Both male and female FIRKO mice were found to have an increase in mean life-span of ~134 days (18%), with parallel increases in median and maximum life-spans. Thus, a reduction of fat mass without caloric restriction can be associated with increased longevity in mice, possibly through effects on insulin signaling.

Longevity is dependent on many factors including genetics (1, 2), hormonal and growth factor signaling (3, 4), body weight (5), body fat content, and environmental factors (4, 6). Food restriction is the most potent environmental variable and has been shown to increase longevity in diverse organisms (6). The effect of restricted feeding on life-span has been studied in rodents for more than 60 years (7–10), and although some studies have suggested that reduced food intake is more important than adiposity (8, 9), it is difficult to separate the beneficial effect of caloric restriction from that of leanness and the various biochemical correlates of leanness.

To investigate this question, we evaluated the life-span of the fat-specific insulin receptor knockout (FIRKO) mouse. These animals were derived by crossing insulin receptor IR (lox⁺/+) mice, in which exon 4 of the insulin receptor is flanked by loxP sites (11), with IR (lox⁺/+) mice that also express the Cre recombinase under the control of the aP2 promoter/enhancer (12). This breeding strategy also generated three littermate control groups: wild-type (WT), IR (lox/lox), and aP2-Cre

mice, which were indistinguishable with regard to physiologic and metabolic parameters and have the same mixed genetic background as the FIRKO mice. For the aging experi-

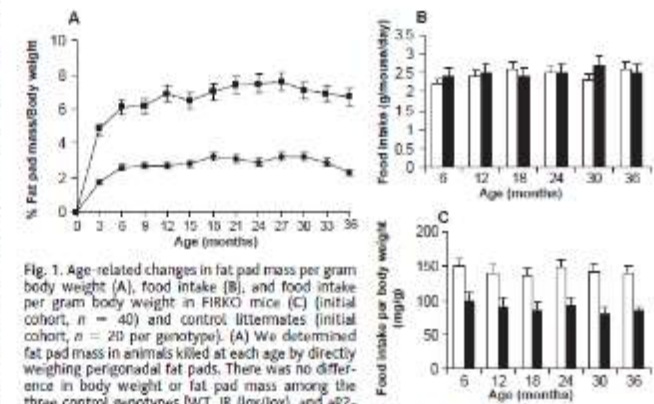


Fig. 1. Age-related changes in fat pad mass per gram body weight (A), food intake (B), and food intake per gram body weight in FIRKO mice (C) (initial cohort, $n = 40$) and control littermates (initial cohort, $n = 20$ per genotype). (A) We determined fat pad mass in animals killed at each age by directly weighing perigonadal fat pads. There was no difference in body weight or fat pad mass among the three control genotypes [WT, IR (lox/lox), and aP2-Cre]. After the mice reached the age of 3 months, the differences in fat pad mass per gram of body weight were significant for all data points between FIRKO mice (diamonds) and all three controls (squares) ($P < 0.05$). (B) In mice caged singly, we determined food intake (gram per mouse per day) daily over 5 days by using at least five FIRKO (white bars) and four control mice (black bars) per genotype ($n = 12$). Data of the control genotypes [WT, IR (lox/lox), and aP2-Cre] are plotted together in the black bars, because there were no differences in daily food intake among them. (C) Food intake per gram body weight—calculated from the food intake and body weight data—was significantly increased in FIRKO mice (white bars) as compared with controls (black bars) ($P < 0.05$).

ments, 250 animals were housed under the same conditions in a virus-free facility on a 12-hour light/dark cycle and were given a standard rodent feed and water ad libitum.

Growth curves were normal in male and female FIRKO mice from birth to 8 weeks of age. Starting at 3 months of age, FIRKO mice maintained 15 to 25% lower body weights and a 50 to 70% reduction in fat mass throughout life (Fig. 1A). The reduction in adiposity was estimated by perigonadal fat pad weight but was apparent in all fat depots and was also reflected by a reduction of ~25% in total-body triglyceride content (13). FIRKO mice are healthy, lack any of the metabolic abnormalities associated with lipodystrophy, and are protected against age-related deterioration in glucose tolerance, which is observed in all control strains (13). FIRKO mice maintained low body fat, despite normal food intake (Fig. 1B). Indeed, because FIRKO mice were leaner, the food intake of FIRKO mice expressed per gram of body weight actually exceeded that of controls by an average of 55% (Fig. 1C).

The median life-span of most laboratory

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2003: Stephen Jackson

Telomeric initiated senescence works through DNA damage checkpoint pathway.

Most human somatic cells can undergo only a limited number of population doublings *in vitro*. This exhaustion of proliferative potential, called senescence, can be triggered when telomeres—the ends of linear chromosomes—cannot fulfil their normal protective functions.

Here the authors show that senescent human fibroblasts display molecular markers characteristic of cells bearing DNA double-strand breaks.

These markers include nuclear foci of phosphorylated histone H2AX and their co-localization with DNA repair and DNA damage checkpoint factors such as 53BP1, MDC1 and NBS1. We also show that senescent cells contain activated forms of the DNA damage checkpoint kinases CHK1 and CHK2.

Furthermore, by chromatin immunoprecipitation and whole-genome scanning approaches, the authors show that the chromosome ends of senescent cells directly contribute to the DNA damage response, and that uncapped telomeres directly associate with many, but not all, DNA damage response proteins. Finally, the authors show that inactivation of DNA damage checkpoint kinases in senescent cells can restore cell-cycle progression into S phase. Thus, the authors conclude that telomere-initiated senescence reflects a DNA damage checkpoint response that is activated with a direct contribution from dysfunctional telomeres.

A DNA damage checkpoint response in telomere-initiated senescence

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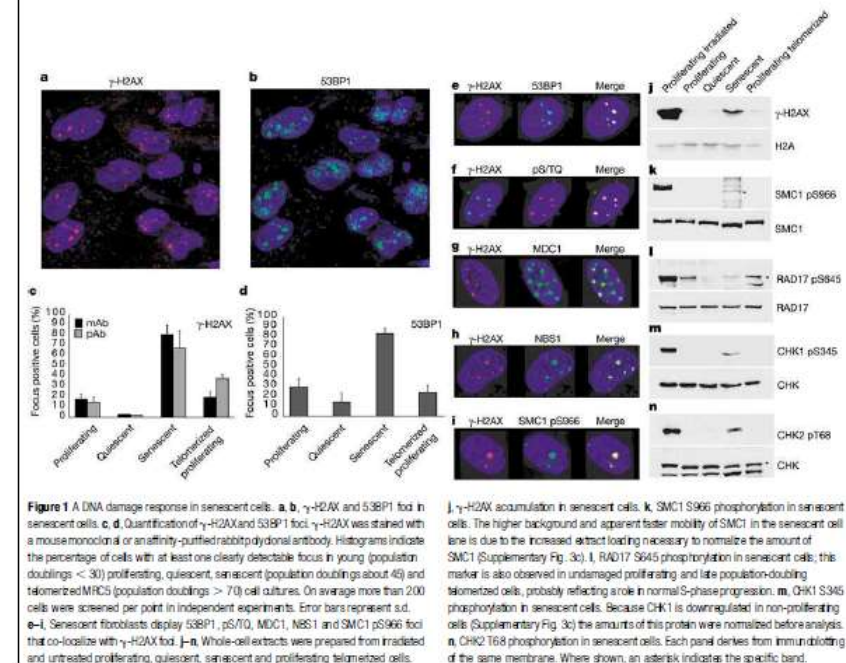
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Most human somatic cells can undergo only a limited number of population doublings *in vitro*¹. This exhaustion of proliferative potential, called senescence, can be triggered when telomeres—the ends of linear chromosomes—cannot fulfil their normal protective functions. Here we show that senescent human fibroblasts display molecular markers characteristic of cells bearing DNA double-strand breaks. These markers include nuclear foci of phosphorylated histone H2AX and their co-localization with DNA repair and DNA damage checkpoint factors such as 53BP1,

MDC1 and NBS1. We also show that senescent cells contain activated forms of the DNA damage checkpoint kinases CHK1 and CHK2. Furthermore, by chromatin immunoprecipitation and whole-genome scanning approaches, we show that the chromosome ends of senescent cells directly contribute to the DNA damage response, and that uncapped telomeres directly associate with many, but not all, DNA damage response proteins. Finally, we show that inactivation of DNA damage checkpoint kinases in senescent cells can restore cell-cycle progression into S phase. Thus, we propose that telomere-initiated senescence reflects a DNA damage checkpoint response that is activated with a direct contribution from dysfunctional telomeres.

Senescence in primary human diploid fibroblasts (HDFs) can be triggered by telomere erosion². We speculated that shortened, dysfunctional telomeres might activate events that, under other circumstances, are elicited by DNA double-strand breaks (DSBs). Cells react to DSBs by triggering the DNA damage checkpoint response, which arrests cell-cycle progression until the DNA damage has been removed. Enforcement of the DNA damage checkpoint relies on the concerted activities of the upstream protein kinases ATM and ATR, the downstream transducer kinases CHK1 and CHK2, and mediators such as 53BP1 and MDC1 (refs 3–7). In addition to targeting proteins such as RAD17, SMC1 and p53, ATM and ATR phosphorylate S139 of histone H2AX molecules flanking the site of DNA damage⁸. Phosphorylated H2AX (γ -H2AX)—a



d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA damage checkpoint response in telomere-initiated senescence. *Nature*. 2003 Nov 13;426(6963):194-8.

2003: Francis Collins

Identified a specific mutation in the LMNA gene (encoding lamin A) as the cause of progeria (a genetic disease of premature aging). The mutation leads to the production of a truncated protein which disrupts nuclear structure and function.

The 2003 paper by Francis Collins and colleagues, entitled "Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome", published in *Nature*, is a landmark study that identified the genetic cause of Hutchinson-Gilford Progeria Syndrome (HGPS), a rare and fatal genetic disorder characterized by accelerated aging in children.

The study revealed that HGPS is caused by recurrent, spontaneous point mutations in the LMNA gene, which encodes the nuclear envelope protein lamin A. Specifically, the researchers found that a single nucleotide substitution (c.1824C>T) in exon 11 of the LMNA gene leads to the activation of a cryptic splice site. This results in the production of a truncated and toxic form of lamin A called progerin, which lacks 50 amino acids near the C-terminus. Progerin disrupts the normal structure and function of the nuclear lamina, leading to nuclear abnormalities, cellular dysfunction, and the characteristic features of accelerated aging seen in progeria patients.

The discovery was groundbreaking because it not only pinpointed the molecular basis of progeria but also provided a framework for understanding how defects in nuclear architecture contribute to disease. The study involved genetic analysis of 20 progeria patients, 18 of whom carried the same c.1824C>T mutation, demonstrating that this mutation is the predominant cause of HGPS. The researchers also showed that progerin accumulates in the nuclei of affected cells, causing misshapen nuclei and impaired cellular function. This work laid the foundation for subsequent research into therapeutic strategies, such as farnesyltransferase inhibitors (FTIs), and highlighted the broader relevance of lamin A and nuclear structure in normal aging.

Recurrent *de novo* point mutations in lamin A cause Hutchinson–Gilford progeria syndrome

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Hutchinson–Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by features reminiscent of marked premature ageing^{1,2}. Here, we present evidence of mutations in lamin A (LMNA) as the cause of this disorder. The HGPS gene was initially localized to chromosome 1q by observing two cases of uniparental isodisomy of 1q—the inheritance of both copies of this material from one parent—and one case with a 6-megabase paternal interstitial deletion. Sequencing of LMNA, located in this interval and previously implicated in several other heritable disorders^{3,4}, revealed that 18 out of 20 classical cases of HGPS harboured an identical *de novo* (that is, newly arisen and not inherited) single-base substitution, G608G (GGC > GGT), within exon 11. One additional case was identified with a different substitution within the same codon. Both of these mutations result in activation of a cryptic splice site within exon 11, resulting in production of a protein product that deletes 50 amino acids near the carboxy terminus. Immunofluorescence of HGPS fibroblasts with antibodies directed against lamin A revealed that many cells show visible abnormalities of the nuclear membrane. The discovery of the molecular basis of this disease may shed light on the general phenomenon of human ageing.

Francis Collins et al., Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature*. 2003 May 15;423(6937):293-8.

2008: Curb and Willcox

One allele of a gene associated with the IGF-1 pathway (FOXO3) more than doubles the risk of long-term survival in a Hawaiian population.

Human longevity is a complex phenotype with a significant familial component, yet little is known about its genetic antecedents. Increasing evidence from animal models suggests that the insulin/IGF-1 signaling (IIS) pathway is an important, evolutionarily conserved biological pathway that influences aging and longevity. However, to date human data have been scarce. Studies have been hampered by small sample sizes, lack of precise phenotyping, and population stratification, among other challenges.

Therefore, to more precisely assess potential genetic contributions to human longevity from genes linked to IIS signaling, we chose a large, homogeneous, long-lived population of men well-characterized for aging phenotypes, and we performed a nested-case control study of 5 candidate longevity genes. Genetic variation within the FOXO3A gene was strongly associated with human longevity. The OR for homozygous minor vs. homozygous major alleles between the cases and controls was 2.75 ($P = 0.00009$; adjusted $P = 0.00135$).

Long-lived men also presented several additional phenotypes linked to healthy aging, including lower prevalence of cancer and cardiovascular disease, better self-reported health, and high physical and cognitive function, despite significantly older ages than controls. Several of these aging phenotypes were associated with FOXO3A genotype. Long-lived men also exhibited several biological markers indicative of greater insulin sensitivity and this was associated with homozygosity for the FOXO3A GG genotype. Further exploration of the FOXO3A gene, human longevity and other aging phenotypes is warranted in other populations.

References: Attia p. 69.

FOXO3A genotype is strongly associated with human longevity

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Edited by Cynthia J. Kenyon, University of California, San Francisco, CA, and approved July 9, 2008 (received for review February 5, 2008)

Human longevity is a complex phenotype with a significant familial component, yet little is known about its genetic antecedents. Increasing evidence from animal models suggests that the insulin/IGF-1 signaling (IIS) pathway is an important, evolutionarily conserved biological pathway that influences aging and longevity. However, to date human data have been scarce. Studies have been hampered by small sample sizes, lack of precise phenotyping, and population stratification, among other challenges. Therefore, to more precisely assess potential genetic contributions to human longevity from genes linked to IIS signaling, we chose a large, homogeneous, long-lived population of men well-characterized for aging phenotypes, and we performed a nested-case control study of 5 candidate longevity genes. Genetic variation within the FOXO3A gene was strongly associated with human longevity. The OR for homozygous minor vs. homozygous major alleles between the cases and controls was 2.75 ($P = 0.00009$; adjusted $P = 0.00135$). Long-lived men also presented several additional phenotypes linked to healthy aging, including lower prevalence of cancer and cardiovascular disease, better self-reported health, and high physical and cognitive function, despite significantly older ages than controls. Several of these aging phenotypes were associated with FOXO3A genotype. Long-lived men also exhibited several biological markers indicative of greater insulin sensitivity and this was associated with homozygosity for the FOXO3A GG genotype. Further exploration of the FOXO3A gene, human longevity and other aging phenotypes is warranted in other populations.

genes | insulin | healthy aging | disease | disability

Human longevity is a complex phenotype with multiple determinants. While non-genetic factors, including diet, physical activity, health habits, and psychosocial factors are important, up to 50% of the variation in human lifespan might be explained by genetic differences (1–5). Several studies suggest that about 25% of the variation in human lifespan in average-lived populations can be explained by genetic factors, but in populations with larger numbers of exceptional survivors, the genetic contribution to lifespan may be much higher. For example, family studies of nonagenarians and centenarians show that sibling relative risk, a common method for assessing potential genetic contribution to a complex phenotype (6), is particularly high and grows with increasing age of the proband (7–10). However, studies of candidate “longevity-associated” genes in humans, hereafter referred to as “longevity genes,” have generally been disappointing. Few replications have been observed across populations, with the exception of the *APOE* gene (3).

In contrast, there have been several robust genetic findings in model organisms of aging (11–13). For example, variation in single genes can result in substantial differences in lifespan in model organisms, particularly with genes that are considered part of the insulin/IGF-1 (IIS) signaling pathway (14–18).

Mutations that increase SIR-2 activity or that decrease insulin/IGF-1 signaling both increase the lifespan of *C. elegans* by activating the DAF-16/FOXO protein (19, 20). In mammalian cells, a Sir2

homolog “SIRT1,” influences several downstream transcription events affecting lifespan, including the cellular response to stress. SIRT1 accomplishes this by regulating the FOXO (Forkhead box transcription) factors, a family of proteins that function as sensors in the IIS pathway and influence mammalian longevity (17).

Genetic knock-out models in mammals (and other species) have also supported the IIS hypothesis. For example, mice with a fat-specific insulin receptor knockout (fIRKO) have reduced fat mass, protected against age-related obesity, and have extended longevity (21). Many other mutations in the IIS pathway appear to impact longevity in mice. These include mutations in the IGF-1 receptor (22), IRS-1 (22), IRS-2 (23), PAPP-A (24), and the Ames Dwarf mouse mutation (22).

The basic molecular pathway of insulin signaling is conserved through evolution, evidence of which can be seen in yeast, flies, worms, rodents, and humans (25). A key regulator of this pathway in worms is the transcription factor DAF-16 (abnormal DAF-16 Formation-16), which is required for the large lifespan extension produced in *C. elegans* by inhibiting insulin/IGF-1 signaling (16). A number of factors appear to extend lifespan in *C. elegans* in a DAF-16 dependent manner, such as AMP kinase (26), 14-3-3 proteins (27), the lin-4 microRNA (28), and heat shock factor (29). Homologues of DAF-16 in several species have been linked to aging phenotypes and longevity (30). For example, the stress responsive Jun-N-k terminal kinase (JNK) pathway appears to require FOXO to prolong lifespan in *Drosophila* (31), and when flies over express dFOXO, the DAF-16 ortholog, it can increase lifespan (32). The convergence of such a diverse array of signals on DAF-16/FOXO suggests that this protein may be an important, evolutionarily conserved “node” in a signaling network that impacts aging and longevity.

The human homologue of DAF-16 includes four FOXOs: FOXO1, FOXO3, FOXO4 and FOXO6. We hypothesize that common, natural variation in the form of single nucleotide polymorphisms (SNPs) in FOXO and related genes might influence human aging and longevity. A connection between insulin, FOXO, oxidative stress, and human longevity would be particularly interesting since oxidative stress has long been a favorite putative mechanism of aging. Since 1956, the free radical theory of aging has hypothesized that aging results partly from damage to DNA, cells, and tissues from cumulative exposure to reactive oxygen molecules

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The authors declare no conflict of interest.

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Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, Masaki KH, Willcox DC, Rodriguez B, Curb JD. FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci U S A*. 2008 Sep 16;105(37):12987–92.

2012: Kathryn Lunetta

Aging is only about 20% heritable.

Genetic factors clearly contribute to exceptional longevity and healthy aging in humans, yet the identification of the underlying genes remains a challenge. Longevity is a complex phenotype with modest heritability. Age-related phenotypes with higher heritability may have greater success in gene discovery. Candidate gene and genome-wide association studies (GWAS) for longevity have had only limited success to date.

The Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium conducted a meta-analysis of GWAS data for longevity, defined as survival to age 90 years or older, that identified several interesting associations but none achieved genome-wide significance. A recent GWAS of longevity conducted in the Leiden Longevity Study identified the ApoE E₄ isoform as deleterious to longevity that was confirmed in an independent GWAS of long-lived individuals of German descent. Notably, no other genetic loci for longevity have been identified in these GWAS.

To examine the conserved genetic mechanisms between the mouse and humans for life span, we mapped the top Cohorts for Heart and Aging Research in Genomic Epidemiology GWAS associations for longevity to the mouse chromosomal map and noted that eight of the ten top human associations were located within a previously reported mouse life-span quantitative trait loci. This work suggests that the mouse and human may share mechanisms leading to aging and that the mouse model may help speed the understanding of how genes identified in humans affect the biology of aging.

Special Issue on Genetics and Aging

Translational

The Search for Longevity and Healthy Aging Genes: Insights From Epidemiological Studies and Samples of Long-Lived Individuals

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Genetic factors clearly contribute to exceptional longevity and healthy aging in humans, yet the identification of the underlying genes remains a challenge. Longevity is a complex phenotype with modest heritability. Age-related phenotypes with higher heritability may have greater success in gene discovery. Candidate gene and genome-wide association studies (GWAS) for longevity have had only limited success to date. The Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium conducted a meta-analysis of GWAS data for longevity, defined as survival to age 90 years or older, that identified several interesting associations but none achieved genome-wide significance. A recent GWAS of longevity conducted in the Leiden Longevity Study identified the ApoE E₄ isoform as deleterious to longevity that was confirmed in an independent GWAS of long-lived individuals of German descent. Notably, no other genetic loci for longevity have been identified in these GWAS. To examine the conserved genetic mechanisms between the mouse and humans for life span, we mapped the top Cohorts for Heart and Aging Research in Genomic Epidemiology GWAS associations for longevity to the mouse chromosomal map and noted that eight of the ten top human associations were located within a previously reported mouse life-span quantitative trait loci. This work suggests that the mouse and human may share mechanisms leading to aging and that the mouse model may help speed the understanding of how genes identified in humans affect the biology of aging. We expect these ongoing collaborations and the translational work with basic scientists to accelerate the identification of genes that delay aging and promote a healthy life span.

Key Words: Longevity—Genetics—Epidemiological studies.

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It is well established that both genetic factors and health-related behaviors influence survival to old age and survival to old age in good health. Longitudinal cohort studies demonstrate that lower levels of cardiovascular risk factors measured in midlife or early older years predict survival and healthy survival to 85 years of age (1,2) and beyond (3,4). Longevity has been observed to cluster within families so that parents and siblings of centenarians have a greater likelihood of attaining advanced age (5–7), and offspring of centenarians appear to have a delay in age-related disease (8,9). Studies of families clustered for longevity both in the United States and Europe (Long Life Family Study and Leiden Longevity Study) have demonstrated that offspring of long-lived participants has more favorable midlife risk

factor profiles and less age-related disease (10,11). Similarly in the community-based Framingham Heart Study, adults with at least one parent surviving to old age have lower risk factor levels compared with individuals whose parents died younger and the risk factor advantage persists over time (12). The genetic contribution to longevity and human aging is likely to result from many genes each with modest effects. Some genes will likely affect longevity by increasing susceptibility to age-related disease and early death, whereas other genes are likely to slow the aging process itself leading to a long life. How genetic factors and their interaction with modifiable behavioral and environmental factors contribute to longevity remains unknown.

Murabito JM, Yuan R, Lunetta KL. The search for longevity and healthy aging genes: insights from epidemiological studies and samples of long-lived individuals. *J Gerontol A Biol Sci Med Sci*. May 2012;67(5): 470–9.

470

2013: Kimber & Chippindale

Antagonistic pleiotropy doesn't hold – at least in fruit flies where pleiotropy is the other way around. Post-reproductive longevity is actively maintained by selection for early-life fitness.

Kimber and Chippindale's 2013 paper investigates the evolutionary mechanisms sustaining extended lifespan in *Drosophila* by exploring the interplay between mutation, environmental conditions, and trade-offs. The study tests the hypothesis that mutations conferring longevity are maintained in populations only under specific environmental contexts, where their fitness costs are minimized.

Using experimental evolution approaches, the authors likely subjected flies with lifespan-extending mutations to varying stressors (e.g., resource scarcity, thermal stress) and tracked lifespan alongside fitness traits like reproduction and survival. Their findings suggest that extended lifespan is condition-dependent: mutations promoting longevity persist in benign environments but are selected against under harsher conditions due to trade-offs with fecundity or stress resilience.

Their results suggest that extended postreproductive longevity is actively maintained by selection for early-life fitness via positive pleiotropy and is not a merely a byproduct of exhaustion of genetic variation or weak drift. Thus mutation-selection balance for early fitness may govern variance in longevity in this system: a balance struck remarkably long after selection for continued survival ceases.

Mutation, Condition, and the Maintenance of Extended Lifespan in *Drosophila*

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Summary

The evolutionary theory of aging predicts that longevity will decline via drift or age-specific tradeoffs when selection favors early life fitness [1–3]. Many *Drosophila melanogaster* populations continually terminated at young adult ages retain surprisingly long postselection lifespans. We compiled three decades of longevity data from the Ives population, demonstrating that postselective longevity was both substantial (30 days) and temporally stable over this period. Recently, alleles with positive pleiotropic effects between adjacent ages, particularly those affecting overall condition, have been integrated into the theory [4–13] and may explain the extended longevity observed. We experimentally tested this hypothesis by isolating 20 hemiclones [14] from Ives and allowing spontaneous mutations to accumulate (MA) for 35 generations. Fitness and longevity were positively genetically correlated in control females, and both traits declined due to MA. Crucially, MA induced a strong positive genetic correlation between the traits in both sexes, implying that mutations with early-life impacts also reduce late-life survival. Our results suggest that extended postreproductive longevity is actively maintained by selection for early-life fitness via positive pleiotropy and is not a merely a byproduct of exhaustion of genetic variation or weak drift. Thus mutation-selection balance for early fitness may govern variance in longevity in this system: a balance struck remarkably long after selection for continued survival ceases.

Results and Discussion

The cornerstone of the evolutionary theory of aging is the decline in the strength of natural selection with increasing age [3, 15], which predicts that two categories of allelic variation will cause senescence. First, deleterious alleles that are only expressed late in life are freer to accumulate, relative to those with early-life impact; this is the mutation-accumulation model attributed to Medawar [1]. Second, alleles that increase early-life reproductive success but reduce longevity as a pleiotropic consequence can be favored; this is the antagonistic pleiotropy model suggested by Williams [2]. An extensive body of experimental work, particularly with demographic selection in the fruit fly *Drosophila melanogaster*, has tested, and supported, these predictions. Using demographic selection, *Drosophila* have been evolved forward and backward for aging-related traits, demonstrating patterns consistent with both antagonistic pleiotropy and mutation accumulation [6,9–, [16–18]. The most straightforward and productive design employs discrete generations of varying duration,

with eggs collected only during a brief window at the end of each generation. In terms of Hamilton's mathematical treatment of the evolutionary theory of aging [15], the force of natural selection in such a population declines abruptly from 100% to zero immediately after a new generation is initiated from eggs. Alleles favoring net fitness at the edge of this Hamiltonian cliff will be favored, whether or not they impact fitness costs at earlier or later ages. While the power and fine-tuning capacity of selection have been showcased in this work (reviewed in [19]), there is one rarely visited anomaly: even after hundreds of generations of selection for early reproduction, outbred fruit flies typically have extraordinarily long lives. For example, although some researchers maintain base fly stocks with overlapping generations, much of the aging research has begun with base populations previously kept on 2-week, discrete generation culture for an extended period of time [20]. In the course of experiments with other objectives, we were struck by the fact that recent longevity assays of the Ives (IV) and IV-derived B populations in our lab, populations that have been maintained on the same 2-week protocol (reproduction at 4–5 days of adult age) since 1981, yielded similar values to those from decades earlier (see data below). Review of published data revealed that multiple outbred laboratory populations of *D. melanogaster* kept on short, discrete generation regimes also survive 4–5 weeks after the age when selection for continued survival ceases (e.g., [21–25]). This surplus longevity presents a paradox for the evolutionary theory that has received only cursory attention. Here we present evidence that (1) confirms our observation that longevity has been stable in the IV system for three decades and (2) suggests that this longevity is sustained by a correlated effect of direct selection.

Does Longevity Decline with Long-Term Domestication?

First, we wanted to confirm the stability of longevity over long time periods of continuous selection. Aware of the caveat that experiment-to-experiment variation in mean longevity can be substantial due to variation in environmental conditions, we compiled 30 years of published longevity data for the IV and B populations. Data employed come from assays using similar experimental designs as much as possible (Supplemental Experimental Procedures and Table S1 available online). Despite the potential for noise, we found that longevity has remained remarkably constant for both males ($r^2 = 0.0067$, slope = -0.0026 , $p = 0.7169$) and females ($r^2 = 0.0053$, slope = -0.0005 , $p = 0.7307$; Figure 1) with no detectable decline. With a mean posteclosion longevity (± 1 SE) of 34.43 days (± 1.50) for males and 34.65 days (± 1.57) for females and a development time of 9–10 days in the stock, the estimated surplus longevity is 30 days.

Early in the experiments of Rose and colleagues on these lines, surplus longevity was attributed to the supposition that all of the variation with antagonistic pleiotropic effects was purged from short-generation populations [36]; finite population size limited the standing genetic variation, creating a fixed floor for longevity reduction. But later longevity evolution experiments employing these and derivative lines showed that antagonistic pleiotropic variation was far from exhausted

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Kimber CM, Chippindale AK. Mutation, condition, and the maintenance of extended lifespan in *Drosophila*. Curr Biol. 2013 Nov 18;23(22):2283–2287.

2015: Stephen Elledge

An autophagy transcription factor called GATA4 links the DNA damage response to cellular senescence. Elledge received the 2015 Lasker Prize work on the DNA damage response.

In their 2015 *Science* paper, Stephen Elledge, Judith Campisi and other colleagues revealed a novel mechanism linking DNA damage response (DDR) to inflammation and cellular senescence via autophagy regulation of the transcription factor GATA4. The study showed that DDR activation, triggered by genotoxic stress, suppresses autophagy—a process that normally degrades GATA4. When autophagy is inhibited, GATA4 accumulates and activates the NF-κB pathway, driving expression of senescence-associated secretory phenotype (SASP) genes, which secrete pro-inflammatory cytokines. This pathway connects DNA damage to chronic inflammation, a hallmark of aging and age-related diseases. The researchers demonstrated that GATA4 stabilization is essential for SASP and senescence, highlighting its role as a critical mediator between genomic instability and inflammatory signaling.

The findings underscored autophagy's dual role in both cell survival and regulating inflammatory responses. By identifying GATA4 as a key substrate of autophagy under DDR, the study provided a mechanistic explanation for how persistent DNA damage promotes senescence-associated inflammation. This work advanced understanding of aging, cancer, and degenerative disorders, suggesting therapeutic strategies targeting GATA4 or autophagy to mitigate inflammation-driven pathologies. The paper bridged DDR, autophagy, and senescence, offering a framework for exploring interventions in age-related chronic inflammation and tissue dysfunction.

The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4

Chunhee Kang, Qikai Xu, Timothy D. Martin, Mamie Z. Li, Marco Demaria, Lavin Aron, Tao Lu, Bruce A. Yankner, Judith Campisi, Stephen J. Elledge¹

INTRODUCTION: Cellular senescence is a program of arrested proliferation and altered gene expression triggered by many stresses. Although it is a potent tumor-suppressive mechanism, senescence has been implicated in several pathological processes including aging, age-associated diseases, and (counter-intuitively) tumorigenesis. One potential mechanism through which senescent cells exert such pleiotropic effects is the secretion of pro-inflammatory cytokines, chemokines, growth factors, and proteases, termed the senescence-associated secretory phenotype (SASP), which affects senescent cells and their microenvironment. The mechanism by which the SASP is initiated and maintained is not well characterized beyond the classical regulators of inflammation, including the transcription factors NF-κB and C/EBPβ.

RATIONALE: In senescence growth arrest, two core senescence-regulating pathways, p53

and p16^{INK4a}/Rb, play a critical role. By contrast, the SASP does not depend on either p53 or p16^{INK4a}, which suggests the existence of an independent senescence regulatory network that controls the SASP. Having observed high levels of induction of microRNA miR-146a during induced senescence in human fibroblasts, we developed a green fluorescent protein-tagged senescence reporter based on a miR-146a promoter fragment. This reporter responded to senescence-inducing stimuli, including replicative exhaustion, DNA damage, and oncogenic RAS activation—all of which activate the SASP. This system allowed us to identify additional regulators of senescence and the SASP.

RESULTS: Through miR-146a promoter analysis, we mapped the critical region for senescence-induced activity and identified the transcriptional regulator responsible for this regulation, GATA4, previously known as a regulator of embryonic

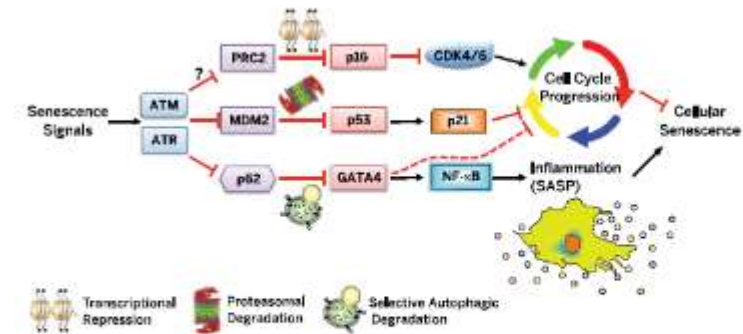
genesis, primarily as a result of increased protein stability.

Under normal conditions, GATA4 binds the p62 autophagy adaptor and is degraded by selective autophagy. Upon senescence induction, however, this selective autophagy was suppressed through decreased interaction between GATA4 and p62, thereby stabilizing GATA4. GATA4 in turn induced

TRAF3IP2 (tumor necrosis factor receptor-associated factor interacting protein 2) and IL1A (interleukin 1A), which activate NF-κB to initiate and maintain the SASP, thus facilitating senescence. GATA4 pathway activation depends on the key DNA damage response (DDR) kinases ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related), as does senescence-associated activation of p53 and p16^{INK4a}. However, the GATA4 pathway is independent of p53 and p16^{INK4a}. Finally, GATA4 protein accumulated in multiple tissues in mice treated with senescence-inducing stimuli and during normal mouse and human aging, including many cell types in the brain; these findings raise the possibility that the GATA4 pathway drives age-dependent inflammation.

CONCLUSION: Our results indicate that GATA4 connects autophagy and the DDR to senescence and inflammation through TRAF3IP2 and IL1A activation of NF-κB. These findings establish GATA4 as a key switch activated by the DDR to regulate senescence, independently of p53 and p16^{INK4a}.

Our in vivo data indicate a potential role of GATA4 during aging and its associated inflammation. Because accumulation of senescent cells is thought to promote aging and aging-associated diseases through the resulting inflammatory response, inhibiting the GATA4 pathway may provide an avenue for therapeutic intervention. ■



GATA4 functions as a key switch in the senescence regulatory network to activate the SASP. The non-senescent state is maintained by inhibitory barriers that prevent cell cycle arrest and inflammation. Upon senescence-inducing signals, ATM and ATR relieve inhibition of the p53 and p16^{INK4a} pathways to induce growth arrest and also block p52-dependent autophagic degradation of GATA4, resulting in NF-κB activation and SASP induction.

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Kang C, Xu Q, Martin TD, Li MZ, Demaria M, Aron L, Lu T, Yankner BA, Campisi J, Elledge SJ. The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. *Science*, Sep 25, 2015;349(6255):aaa5612.

2018: Leslie Gordon

Leslie Gordon and colleagues reported successful results of a clinical trial in children with progeria (a genetic disease of premature aging) for the first time.

The 2021 paper "Association of LonaFarnib Treatment vs No Treatment With Mortality Rate in Patients With Hutchinson-Gilford Progeria Syndrome" by Gordon et al. evaluated the impact of lonaFarnib, a farnesyltransferase inhibitor (FTI), on the survival of patients with Hutchinson-Gilford Progeria Syndrome (HGPS). Lead author Leslie Gordon is the parent of a child with progeria.

This retrospective cohort study compared 27 treated patients from clinical trials with untreated patients from a natural history study. In this cohort study of 27 treated patients with Hutchinson-Gilford progeria syndrome compared with a pool of 103 contemporaneous untreated patients, treatment with lonaFarnib monotherapy compared with no treatment was associated with a significantly lower mortality rate (3.7% vs 33.3%) after a median of 2.2 years of follow-up. The study provided the strongest clinical evidence to date that targeting progerin's farnesylation could mitigate some of the life-threatening effects of HGPS, ultimately contributing to the FDA's 2020 approval of lonaFarnib (Zokinvy) as the first treatment for progeria.

This was the first time, to our knowledge, where a clinical trial showed a direct reduction in mortality in a specific form of aging itself (rather than a disease that is associated with aging).

Research

JAMA | Preliminary Communication

Association of LonaFarnib Treatment vs No Treatment With Mortality Rate in Patients With Hutchinson-Gilford Progeria Syndrome

Leslie B. Gordon, MD, PhD; Heather Shappell, PhD; Joe Massaro, PhD; Ralph B. D'Agostino Sr, PhD; Joan Brazier, MS; Susan E. Campbell, MA; Monica E. Kleinman, MD; Mark W. Kieran, MD, PhD

[Editorial page 1663](#)
[Supplemental content](#)

IMPORTANCE Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare fatal premature aging disease. There is no approved treatment.

OBJECTIVE To evaluate the association of monotherapy using the protein farnesyltransferase inhibitor lonaFarnib with mortality rate in children with HGPS.

DESIGN, SETTING, AND PARTICIPANTS Cohort study comparing contemporaneous (birth date ≥ 1991) untreated patients with HGPS matched with treated patients by age, sex, and continent of residency using conditional Cox proportional hazards regression. Treatment cohorts included patients from 2 single-group, single-site clinical trials (ProLon1 [n = 27; completed] and ProLon2 [n = 36; ongoing]). Untreated patients originated from a separate natural history study (n = 103). The cutoff date for patient follow-up was January 1, 2018.

EXPOSURE Treated patients received oral lonaFarnib (150 mg/m²) twice daily. Untreated patients received no clinical trial medications.

MAIN OUTCOMES AND MEASURES The primary outcome was mortality. The primary analysis compared treated patients from the first lonaFarnib trial with matched untreated patients. A secondary analysis compared the combined cohorts from both lonaFarnib trials with matched untreated patients.

RESULTS Among untreated and treated patients (n = 258) from 6 continents, 123 (47.7%) were female; 141 (54.7%) had a known genotype, of which 125 (88.7%) were classic (c.1824C>T in LMNA). When identified (n = 73), the primary cause of death was heart failure (79.4%). The median treatment duration was 2.2 years. Median age at start of follow-up was 8.4 (interquartile range [IQR], 4.8-9.5) years in the first trial cohort and 6.5 (IQR, 3.7-9.0) years in the combined cohort. There was 1 death (3.7%) among 27 patients in the first trial group and there were 9 deaths (33.3%) among 27 patients in the matched untreated group. Treatment was associated with a lower mortality rate (hazard ratio, 0.12; 95% CI, 0.01-0.93; P = .04). In the combined cohort, there were 4 deaths (6.3%) among 63 patients in the treated group and 17 deaths (27.0%) among 63 patients in the matched untreated group (hazard ratio, 0.23; 95% CI, 0.06-0.90; P = .04).

CONCLUSIONS AND RELEVANCE Among patients with HGPS, lonaFarnib monotherapy, compared with no treatment, was associated with a lower mortality rate after 2.2 years of follow-up. Study interpretation is limited by its observational design.

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JAMA. 2018;319(16):1687-1695. doi:10.1001/jama.2018.3264

Gordon LB et.al. Association of LonaFarnib Treatment vs No Treatment With Mortality Rate in Patients With Hutchinson-Gilford Progeria Syndrome. *JAMA*. 2018 Apr 24;319(16):1687-1695.

2025: Argentieri and Van Duijn

This Harvard/Oxford team uses UK Biobank data to show that genetics are far less important than environmental factors in explaining mortality.

Article Abstract: Both environmental exposures and genetics are known to play important roles in shaping human aging. Here we aimed to quantify the relative contributions of environment (referred to as the exposome) and genetics to aging and premature mortality. To systematically identify environmental exposures associated with aging in the UK Biobank, we first conducted an exposome-wide analysis of all-cause mortality ($n = 492,567$) and then assessed the associations of these exposures with a proteomic age clock ($n = 45,441$), identifying 25 independent exposures associated with mortality and proteomic aging. These exposures were also associated with incident age-related multimorbidity, aging biomarkers and major disease risk factors. Compared with information on age and sex, polygenic risk scores for 22 major diseases explained less than 2 percentage points of additional mortality variation, whereas the exposome explained an additional 17 percentage points. Polygenic risk explained a greater proportion of variation (10.3–26.2%) compared with the exposome for incidence of dementias and breast, prostate and colorectal cancers, whereas the exposome explained a greater proportion of variation (5.5–49.4%) compared with polygenic risk for incidence of diseases of the lung, heart and liver. Our findings provide a comprehensive map of the contributions of environment and genetics to mortality and incidence of common age-related diseases, suggesting that the exposome shapes distinct patterns of disease and mortality risk, irrespective of polygenic disease risk.

Integrating the environmental and genetic architectures of aging and mortality

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Check for updates

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Both environmental exposures and genetics are known to play important roles in shaping human aging. Here we aimed to quantify the relative contributions of environment (referred to as the exposome) and genetics to aging and premature mortality. To systematically identify environmental exposures associated with aging in the UK Biobank, we first conducted an exposome-wide analysis of all-cause mortality ($n = 492,567$) and then assessed the associations of these exposures with a proteomic age clock ($n = 45,441$), identifying 25 independent exposures associated with mortality and proteomic aging. These exposures were also associated with incident age-related multimorbidity, aging biomarkers and major disease risk factors. Compared with information on age and sex, polygenic risk scores for 22 major diseases explained less than 2 percentage points of additional mortality variation, whereas the exposome explained an additional 17 percentage points. Polygenic risk explained a greater proportion of variation (10.3–26.2%) compared with the exposome for incidence of dementias and breast, prostate and colorectal cancers, whereas the exposome explained a greater proportion of variation (5.5–49.4%) compared with polygenic risk for incidence of diseases of the lung, heart and liver. Our findings provide a comprehensive map of the contributions of environment and genetics to mortality and incidence of common age-related diseases, suggesting that the exposome shapes distinct patterns of disease and mortality risk, irrespective of polygenic disease risk.

Human aging is a complex process that initially manifests as subclinical and biological changes that begin to accumulate from mid-life onward^{1–3}. These systemic biological changes are major drivers of common age-related diseases^{4–6} and multimorbidity^{7,8}, which in turn are the major causes of premature mortality worldwide⁹. While there have been major advancements in understanding the complex genetic etiology of age-related diseases, genetic studies show only a modest effect of the genome on lifespan^{10,11}. Instead, a strong argument that nongenetic environmental factors play a key role in aging and premature mortality comes from the observation that global human lifespan

has increased nearly twofold during the past 200 years, while the human genome is expected to have been stable in such a short period^{12,13}. Epidemiological research has made major progress in relating individual environmental and behavioral exposures to age-related diseases and mortality, yet few studies have comprehensively examined the exposome (that is, the total set of interrelated environmental exposures throughout the life course) in relation to these outcomes^{14,15}. In the field of genetic epidemiology, the use of genome-wide approaches has greatly increased the positive predictive value¹⁶ and reproducibility¹⁷ of findings, in particular for genetic variants conveying small effects on

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1016

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Section 8.B: Insight Into Caloric Restriction / Nutrient Pathways

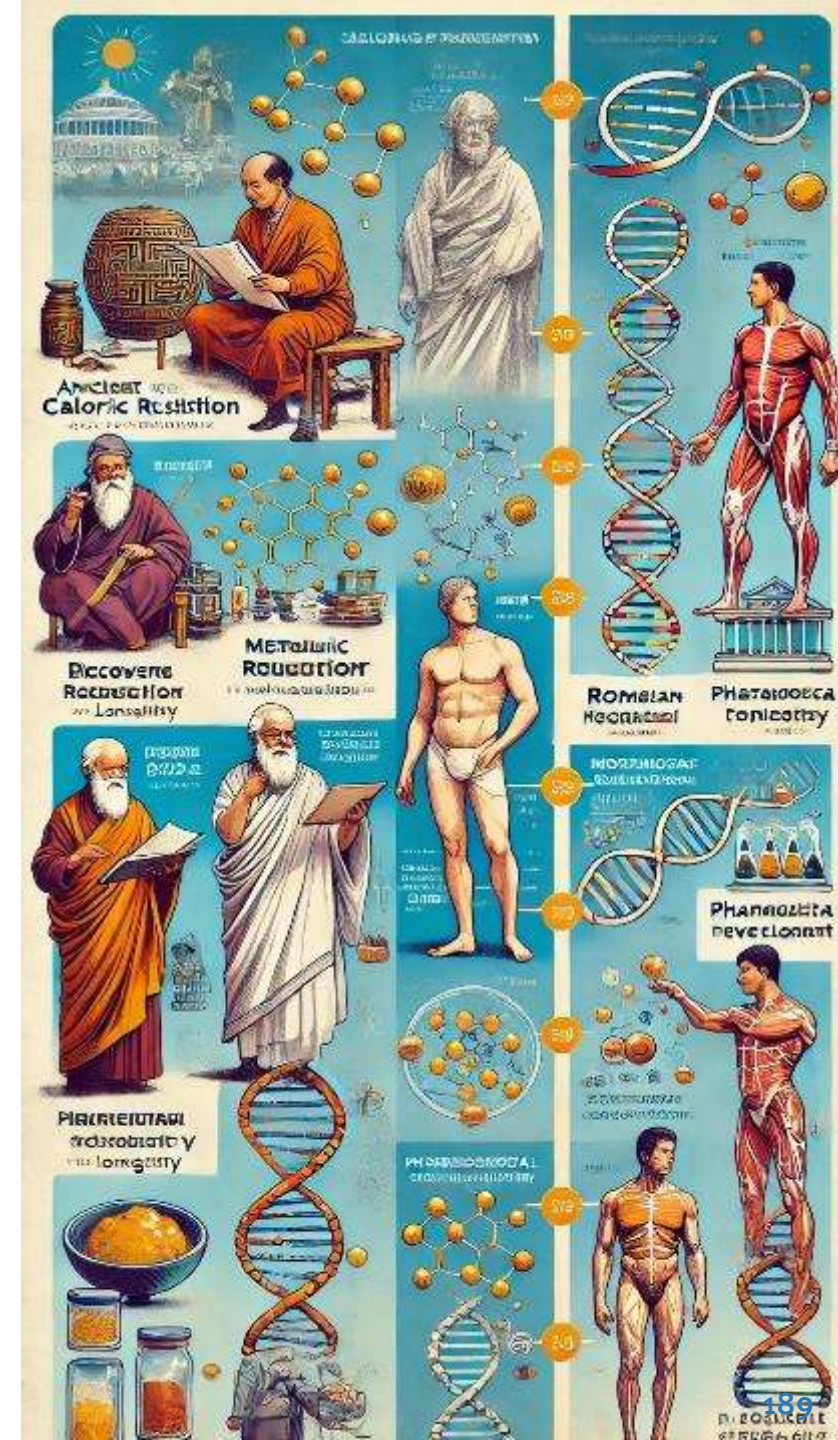
If there was a single takeaway from this report it would be that humanity has understood the relationship between caloric restriction and longevity from the earliest of times.

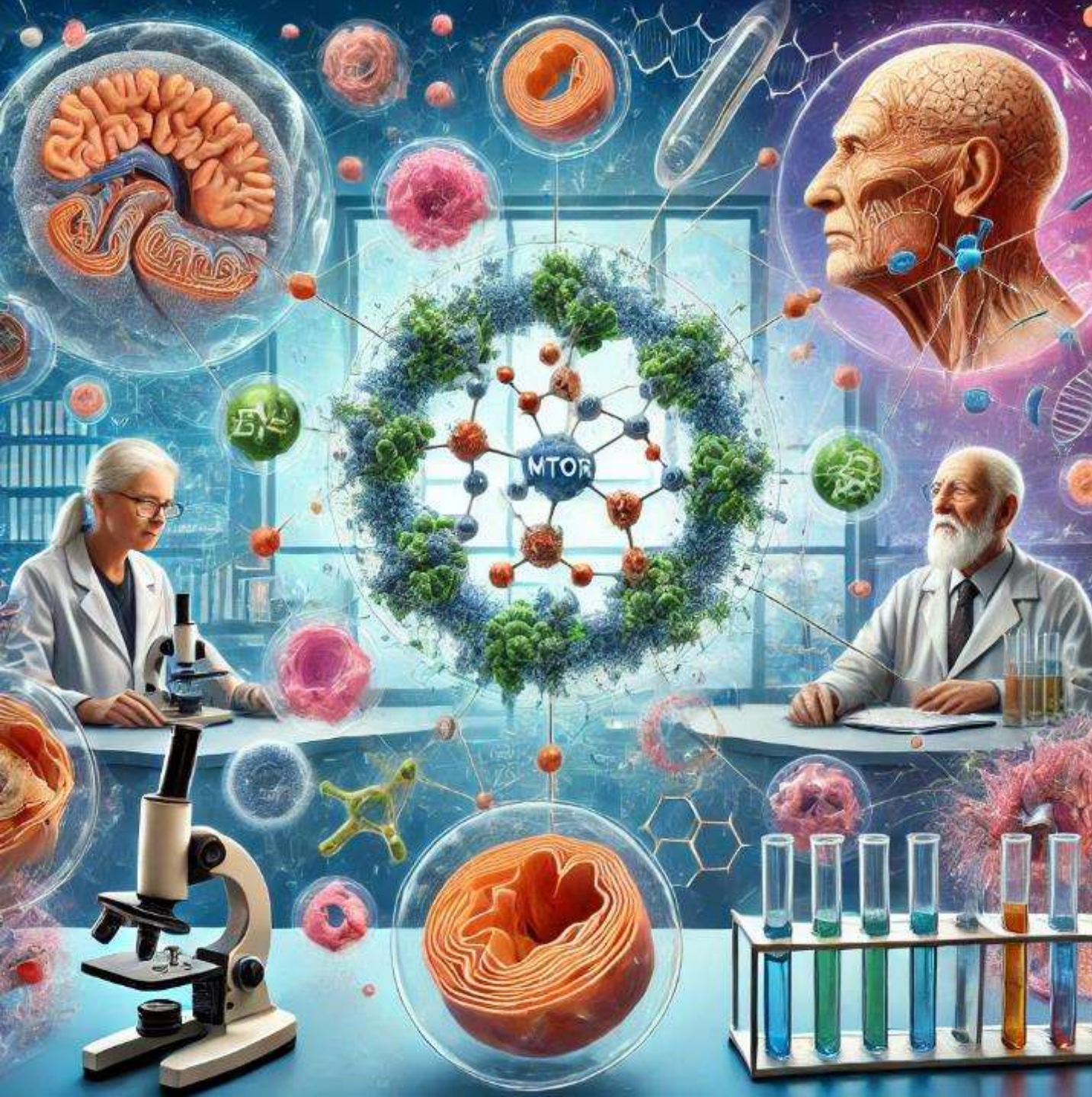
Whether it be the Han-era Chinese, the ancient Greeks, the Romans or the Early Modern Italians the message has always been the same: eat less to live longer.

But the scientific understanding of why diet might impact longevity only started to emerge in the early 20th Century with the emergence of the observation that cross-species differentials in lifespan were correlated with basal metabolic rate.

In the last 50 years insights into the underlying biology of caloric restriction have come at a dizzying pace.

Specifically, caloric restriction has been shown to extend lifespan through a fairly elaborate set of biomolecular interactions. This biology has become central to today's insights into how to best pharmacologically address aging.





Cell Metabolism and Aging

Supporting this theory, David Friedman and Michael Hall discovered the mTOR (mechanistic target of rapamycin) pathway, a key regulator of cellular growth and metabolism. Inhibition of mTOR, particularly through rapamycin, has been shown to extend lifespan in various organisms, including mammals, reinforcing the idea that metabolic regulation is critical to aging.

Building on this, David Sabatini identified the mTOR pathway as a central regulator of nutrient sensing, linking dietary restriction to longevity.

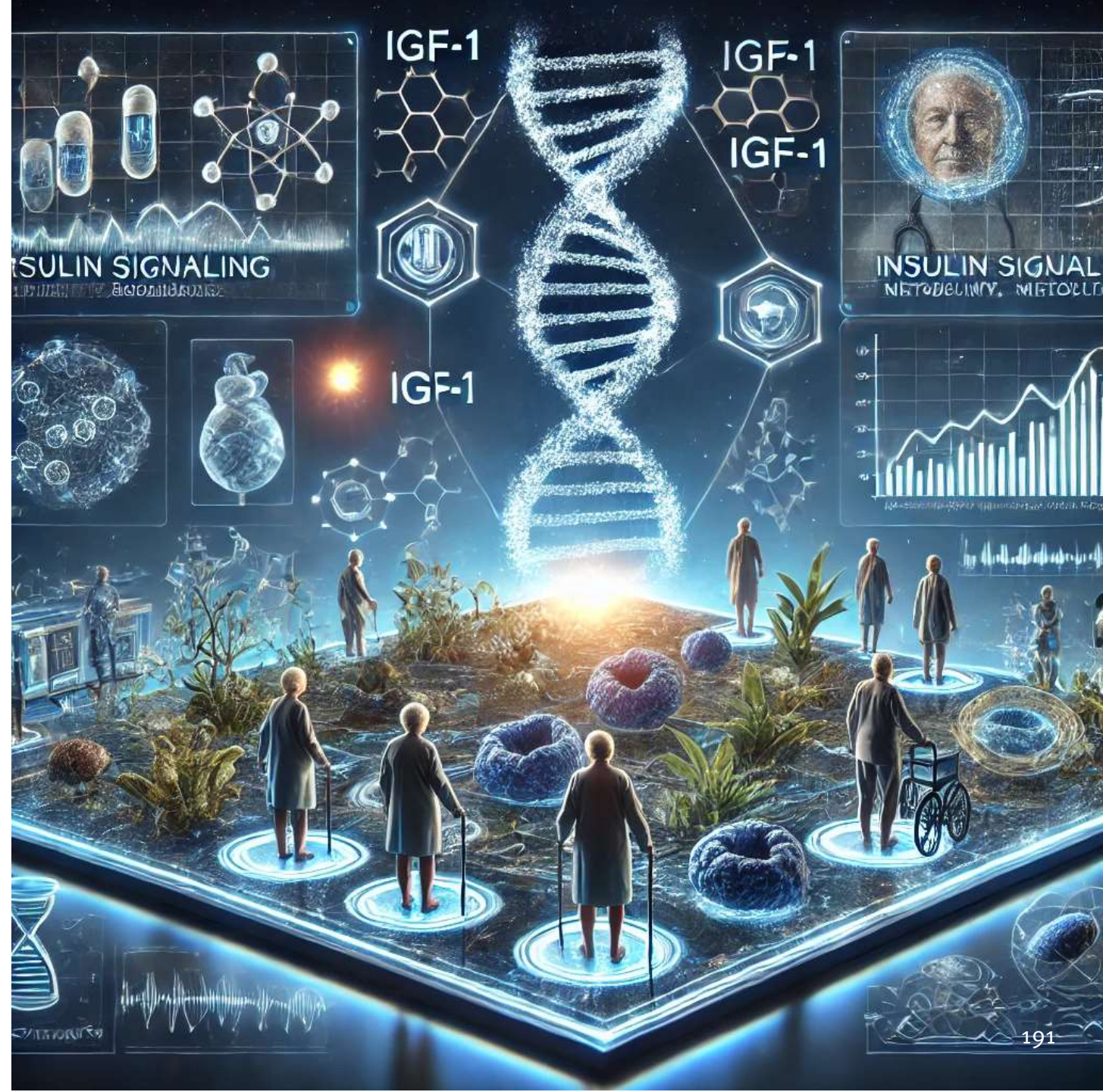
Joseph Heitman expanded on this by exploring how rapamycin, an mTOR inhibitor, could mimic caloric restriction's lifespan-extending effects in yeast, worms, and mammals.

Insulin Pathways Matter in Aging

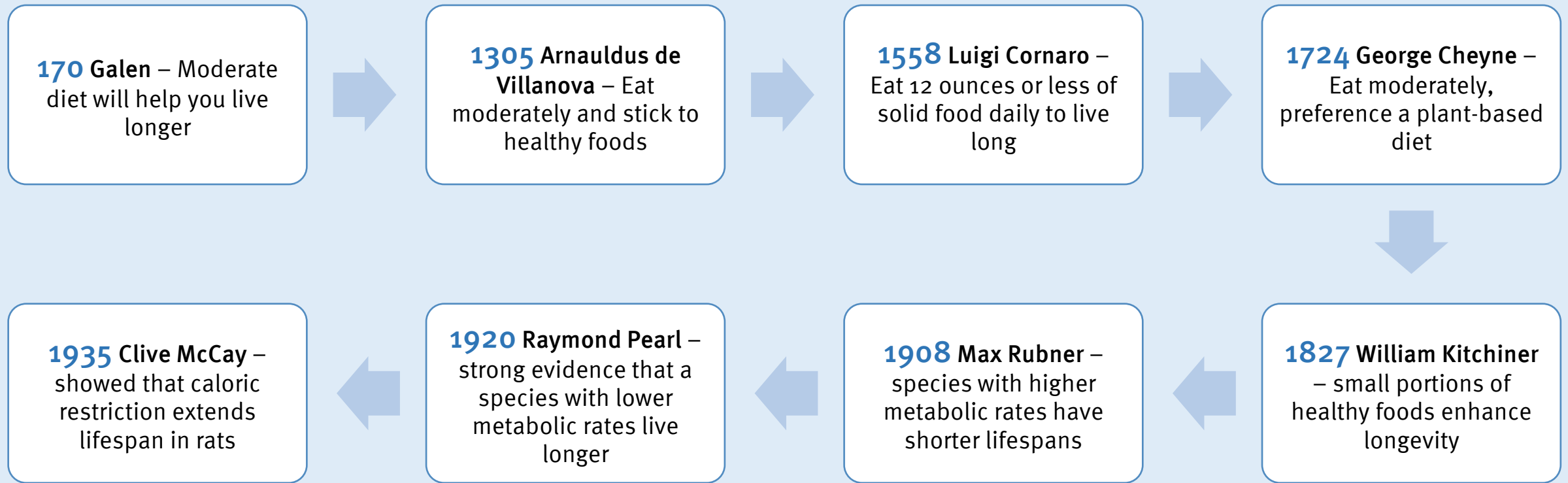
Similarly, genetic and pharmacological inhibition of the insulin/IGF-1 signaling (IIS) pathway has been shown to promote longevity. Research over the past two decades has shown that reduced IIS and mTOR signaling can extend lifespan in a wide range of organisms, from yeast to mammals.

An important finding by Willcox and colleagues (2008) is that FOXO3 variants which are tied to the IGF-1 pathway are linked to substantial differences in human lifespan.

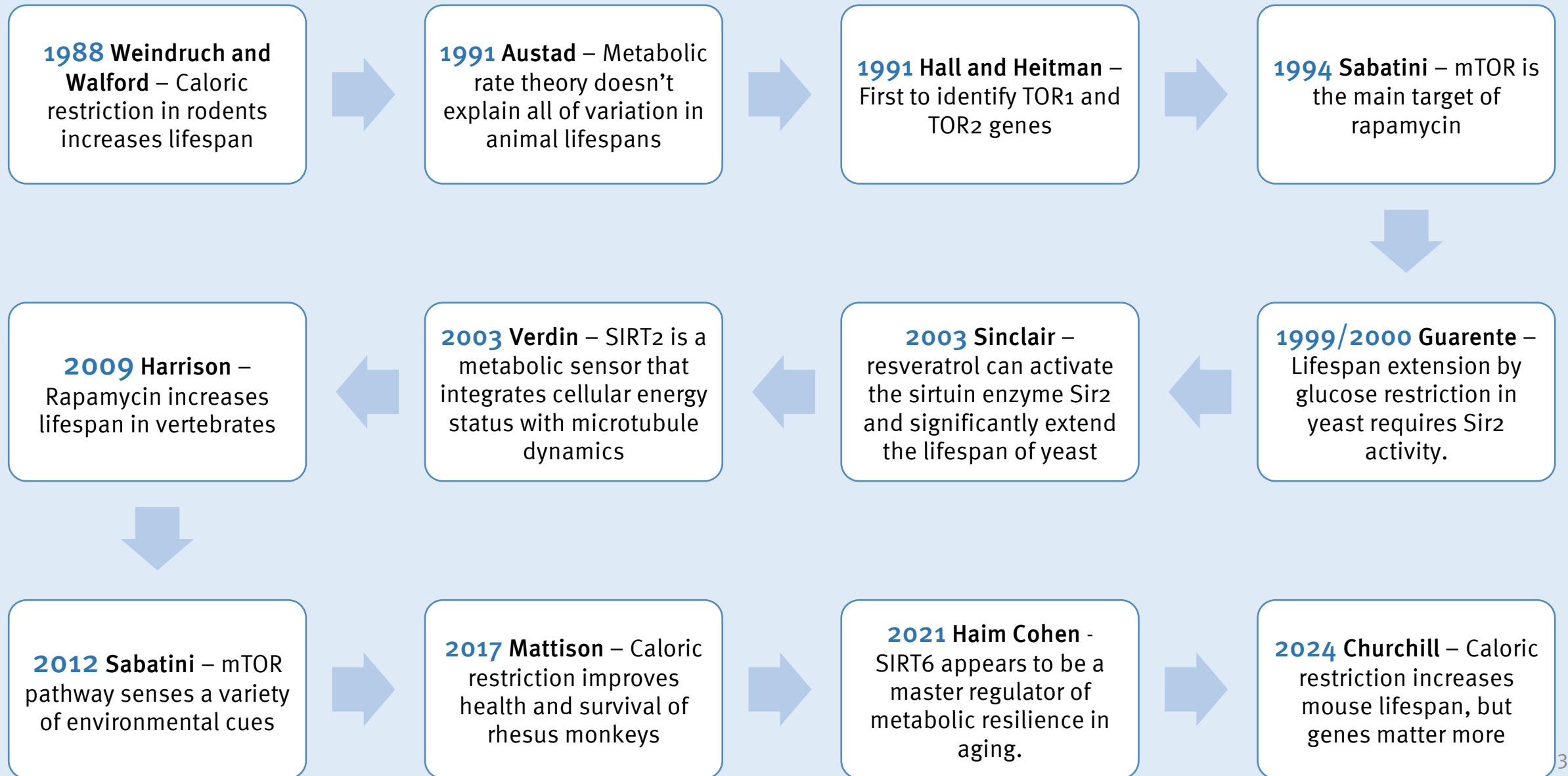
Cumulatively speaking, these findings have led to the development of drugs, such as rapamycin and metformin, that target these pathways and are being tested for their potential to extend healthspan in humans.



Milestones of the Aging and Nutrient Sensing / Caloric Restriction Literature (170AD to 1987)



Aging and Caloric Restriction (1988 to 2024)



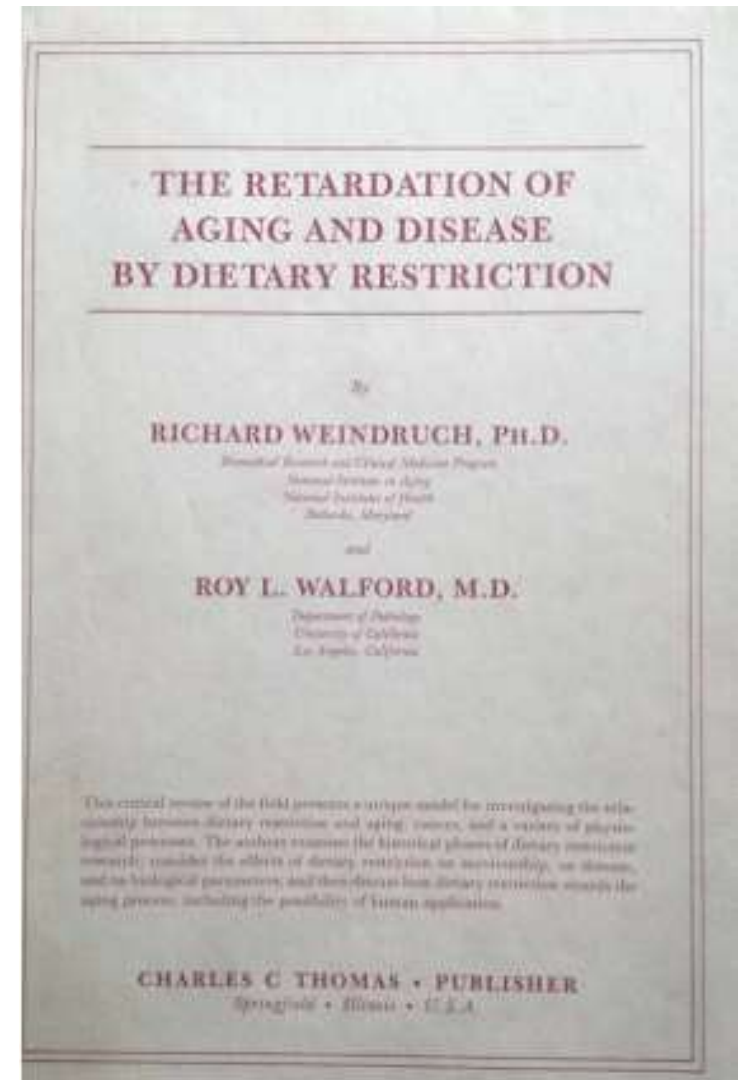
1988: Weindruch and Walford

Conducted rodent studies which showed that caloric restriction could increase lifespan.

In their 1988 book *The Retardation of Aging and Disease by Dietary Restriction*, Richard Weindruch and Roy Walford comprehensively reviewed the effects of caloric restriction (CR) on aging and age-related diseases, primarily based on studies in rodents. The authors presented extensive experimental evidence showing that reducing caloric intake without malnutrition significantly extends lifespan and delays the onset of age-related diseases such as cancer, kidney disease, and autoimmune disorders. They demonstrated that CR not only increases maximum lifespan but also improves overall health and physiological function, including enhanced immune response, reduced oxidative damage, and improved metabolic efficiency.

Weindruch and Walford's work highlighted the potential of dietary restriction as a powerful intervention to modulate the aging process. They proposed that CR slows aging by reducing metabolic rate and oxidative stress, while also enhancing cellular repair mechanisms.

This heavily cited book became a cornerstone in aging research, inspiring numerous studies to explore the molecular and physiological mechanisms underlying the benefits of CR. Their findings have had lasting implications for understanding the relationship between nutrition, metabolism, and aging, and continue to influence research on dietary interventions to promote healthy aging and longevity in humans.



Richard Weindruch and Roy Walford, *The Retardation of Aging and Disease by Dietary Restriction*, 1988. From the medical library of Tim C. Opler.

1991: Steve Austad

Shows some exceptions to metabolic rate theory. This theory is apparently not enough on its own to explain lifespan differentials across species.

This study compared trends in body size, life span, metabolic rate, and ecology of bats and marsupials with those from mammals using a 580 species data base.

The linear logarithmic relationship between mammalian body mass and maximum longevity, deleting bats and marsupials, is used as a standard against which to measure life spans of particular mammal groups.

Bats have maximum life spans a minimum of 3 times those of nonflying eutherians--a trend resulting from neither low basal metabolic rate, the ability to enter torpor, nor large relative brain size. Marsupials live about 80% as long as nonflying eutherians despite averaging lower basal metabolic rates; similarly, there is no effect of heterothermy or relative brain size.

These results directly conflict with predictions of both "rate of living" and brain-size mediated theories of aging.

However, they are consistent with an evolutionary theory that posits exceptionally long-life spans among mammals with reduced environmental vulnerability.

References: Ramakrishnan p. 31.

Mammalian Aging, Metabolism, and Ecology: Evidence From the Bats and Marsupials

Steven N. Austad¹ and Kathleen E. Fischer²

¹Department of Organismic and Evolutionary Biology, The Biological Laboratories, Harvard University.

²Department of Anthropology, Harvard University.

This study compared trends in body size, life span, metabolic rate, and ecology of bats and marsupials with those from mammals generally, using a 580 species data base. The linear logarithmic relationship between mammalian body mass and maximum longevity, deleting bats and marsupials, is used as a standard against which to measure life spans of particular mammal groups. Bats have maximum life spans a minimum of 3 times those of nonflying eutherians—a trend resulting from neither low basal metabolic rate, the ability to enter torpor, nor large relative brain size. Marsupials live about 80% as long as nonflying eutherians despite averaging lower basal metabolic rates; similarly, there is no effect of heterothermy or relative brain size. These results directly conflict with predictions of both "rate of living" and brain-size mediated theories of aging. However, they are consistent with an evolutionary theory that posits exceptionally long life spans among mammals with reduced environmental vulnerability.

THERE are two general theories that provide functional explanations of aging. One of these, the "rate of living" theory (Rubner, 1908; Pearl, 1928), originally proposed that an organism's life span depended upon a genetically determined metabolic potential and the rate at which that potential was expended. In its strong form, this notion held that related species, such as mammals or birds, had similar metabolic potentials and, therefore, the diversity of life spans could be largely explained by differences in metabolic rate (Sacher, 1959; Lindstedt and Calder, 1976).

In its more modern, weaker form, the theory holds that aging is the inevitable consequence of imperfect physiochemical processes, eventually leading to failure of cellular constituents due to the accumulation of biochemical errors or to the gradual buildup of toxic metabolic byproducts (Sohal, 1986). A major prediction of this theory is that animal life span should be inversely correlated with the rate at which physiochemical processes occur. Thus, other things being equal, organisms with low metabolic rates should live longer than organisms with high metabolic rates.

Support for this idea stems from temperature effects on heterothermic and ectothermic vertebrate and insect life spans (e.g., Herreid, 1964; Liu and Walford, 1975; Sohal, 1986) and the inverse relation between maximum longevity and body size or mass-specific basal metabolic rate in birds (Lindstedt and Calder, 1976) and in mammals (e.g., Sacher, 1977). Moreover, a number of writers in recent years have claimed that energy expended per unit body mass per lifetime is relatively constant throughout large subgroups of the eutherian mammals (Stahl, 1962; Cutler, 1978). So intuitively satisfying is the notion of a straightforward relation between metabolic rate and longevity, that widely different phenomena, such as the exceptional life span of bats and the life-extending effects of dietary restriction on laboratory rodents were generally assumed to result from reductions in metabolic rates (Bourliere, 1958; Sacher, 1959, 1975, 1977).

The second general aging theory, the evolutionary theory of aging, attributes senescence to the declining strength of natural selection at successive ages after sexual maturity (Medawar, 1952; Williams, 1957; Hamilton, 1966; Charlesworth, 1980). According to this idea, the power of natural selection to favor advantageous alleles, or eliminate deleterious ones, declines with age after sexual maturity. Aging, therefore, is the genetic consequence of: (a) weak selection against the accumulation of late-acting deleterious alleles, and (b) selection for alleles with pleiotropic early beneficial effects and detrimental late effects, even if the early effects are small and the late effects large. The rate of aging from this perspective will be set chiefly by environmental hazard, i.e., probability of death due to external causes such as predation or environmental catastrophe, and the nature of the genetic trade-offs between reproduction and subsequent survival. Together these factors determine the rate at which natural selection declines with age. Note that the evolutionary theory also suggests that cellular constituents will eventually fail due to accumulated errors or damage, but it differs from the rate of living theory in that the timing of cellular deterioration is set not by physiological limits, but by the action of natural selection operating upon reproductive schedules (Kirkwood, 1981). Evidence for this idea comes from genetic experiments in which artificially increasing the strength of selection at later ages leads to increased life span (Rose and Charlesworth, 1980; Luckinbill et al., 1984), and from the presence or absence of senescence in related invertebrates, depending upon whether or not they exhibit a clear division between soma and germ line, i.e., organisms with and without definable ages (Bell, 1984).

In recent years, general knowledge about the ecology and maximum longevity (assumed to be an index of aging rate) of mammal species both in the wild and in captivity has increased dramatically. Simultaneously, basal metabolic rates have been compiled for an increasing number of species (reviewed in McNab, 1988). This new information

Austad SN, Fischer KE. Mammalian aging, metabolism, and ecology: evidence from the bats and marsupials. *J Gerontol.* 1991 Mar;46(2):B47-53.

1991: Michael Hall and Joseph Heitman

First to identify TOR1 and TOR2, the key genes that drive proteins associated with mTOR. This was a key breakthrough in understanding how Rapamycin impacts aging. Michael Hall received the 2017 Lasker Prize for this work.

The 1991 paper by Heitman, Movva, and Hall, "Targets for Cell Cycle Arrest by the Immunosuppressant Rapamycin in Yeast," published in *Science*, was a landmark study that identified the molecular target of rapamycin and elucidated its mechanism of action. The researchers discovered that rapamycin, a potent immunosuppressant and antifungal agent, exerts its effects by binding to a highly conserved protein called FKBP12 (FK506-binding protein 12).

This complex then inhibits the function of TOR (Target of Rapamycin), a key regulator of cell growth and proliferation. In yeast, TOR (specifically TOR1 and TOR2) was shown to control the cell cycle by regulating nutrient-sensitive signaling pathways, and its inhibition by rapamycin led to cell cycle arrest in the G1 phase.

This study was groundbreaking because it revealed the conserved role of TOR signaling in coordinating cell growth with nutrient availability, a pathway now known as the mTOR pathway in mammals. The identification of TOR as the target of rapamycin provided critical insights into the molecular basis of rapamycin's immunosuppressive and antiproliferative effects. This work laid the foundation for extensive research into the mTOR pathway, which has since been implicated in a wide range of biological processes, including aging, cancer, and metabolism.

References: Campisi p. 184; Ramakrishnan p. 133.

Targets for Cell Cycle Arrest by the Immunosuppressant Rapamycin in Yeast

JOSEPH HEITMAN,* N. RAO MOVVA, MICHAEL N. HALL†

FK506 and rapamycin are related immunosuppressive compounds that block helper T cell activation by interfering with signal transduction. In vitro, both drugs bind and inhibit the FK506-binding protein (FKBP) proline rotamase. *Saccharomyces cerevisiae* cells treated with rapamycin irreversibly arrested in the G1 phase of the cell cycle. An FKBP-rapamycin complex is concluded to be the toxic agent because (i) strains that lack FKBP proline rotamase, encoded by *FPR1*, were viable and fully resistant to rapamycin and (ii) FK506 antagonized rapamycin toxicity in vivo. Mutations that conferred rapamycin resistance altered conserved residues in FKBP that are critical for drug binding. Two genes other than *FPR1*, named *TOR1* and *TOR2*, that participate in rapamycin toxicity were identified. Nonallelic noncomplementation between *FPR1*, *TOR1*, and *TOR2* alleles suggests that the products of these genes may interact as subunits of a protein complex. Such a complex may mediate nuclear entry of signals required for progression through the cell cycle.

IN HIGHER EUKARYOTES, THE IMMUNOSUPPRESSIVE compounds cyclosporin A (CsA), FK506, and rapamycin inhibit the intermediate steps in signal transduction between the cytoplasm and the nucleus. These three compounds are immunosuppressive because they block signal transduction pathways required for the activation of helper T cells (1, 2).

FK506 and rapamycin are related macrocyclic compounds and share no chemical similarity with CsA, a cyclic undecapeptide (2). Although structurally unrelated, CsA and FK506 inhibit the same step in T cell activation, an antigen-stimulated, Ca^{2+} -dependent signal transduction step that acts through the nu-

clear factor of activated T cells (transcription factor NF-AT) (3). In contrast, rapamycin does not interfere with the response to an antigen but rather impairs a later step in T cell activation, the proliferative response to interleukin-2 (4, 5).

The effects of CsA, FK506, and rapamycin are thought to be mediated by the

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†To whom correspondence should be addressed.

Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*. 1991 Aug 23; 253(5022):905-9.

1994: David Sabatini

This paper identified mTOR (then called RAFT1) as a key regulator of cell growth and a target of rapamycin. The signaling pathway anchored by mTOR is a central regulator of growth in eukaryotes and acts by setting the balance between mass accumulation and mass breakdown processes.

This groundbreaking study identified RAFT1 (later renamed mTOR, mechanistic Target of Rapamycin), a key protein in mammalian cells that binds to the FKBP12-rapamycin complex. The researchers discovered that RAFT1 shares significant homology with the yeast TOR proteins (Target of Rapamycin), which were already known to regulate cell growth in response to nutrients.

Using biochemical techniques, they isolated RAFT1 and demonstrated its interaction with FKBP12, a protein that binds rapamycin, an immunosuppressive drug. This interaction was shown to be critical for rapamycin's inhibitory effects on cell growth, linking RAFT1 to the regulation of cellular processes such as proliferation and metabolism.

The study also highlighted the evolutionary conservation of the TOR pathway between yeast and mammals, suggesting that mTOR plays a central role in nutrient sensing and growth regulation across species.

By identifying RAFT1 as a rapamycin-sensitive protein, the authors identified how mTOR integrates signals from nutrients, growth factors, and energy status to control cell growth and metabolism. This discovery opened the door for future research into mTOR's role in aging, cancer, and other diseases, making it a cornerstone in the field of cell signaling and longevity research.

References: Attia p. 77;

Cell, Vol. 78, 35-43, July 15, 1994, Copyright © 1994 by Cell Press

RAFT1: A Mammalian Protein That Binds to FKBP12 in a Rapamycin-Dependent Fashion and Is Homologous to Yeast TORs

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Summary

The immunosuppressants rapamycin and FK506 bind to the same intracellular protein, the immunophilin FKBP12. The FKBP12-FK506 complex interacts with and inhibits the Ca^{2+} -activated protein phosphatase calcineurin. The target of the FKBP12-rapamycin complex has not yet been identified. We report that a protein complex containing 245 kDa and 35 kDa components, designated rapamycin and FKBP12 targets 1 and 2 (RAFT1 and RAFT2), interacts with FKBP12 in a rapamycin-dependent manner. Sequences (330 amino acids total) of tryptic peptides derived from the 245 kDa RAFT1 reveal striking homologies to the yeast TOR gene products, which were originally identified by mutations that confer rapamycin resistance in yeast. A RAFT1 cDNA was obtained and found to encode a 289 kDa protein (2549 amino acids) that is 43% and 39% identical to TOR2 and TOR1, respectively. We propose that RAFT1 is the direct target of FKBP12-rapamycin and a mammalian homolog of the TOR proteins.

Introduction

The natural products cyclosporin A, FK506, and rapamycin are potent immunosuppressants with realized or potential clinical applications in the prevention of graft rejection after organ transplantation and the treatment of autoimmune disorders (Borel, 1986; Kino et al., 1987; Marfell et al., 1977). These drugs act by inhibiting intermediate steps in the signaling pathways that effect the T-cell response to antigen (for reviews, see Fruman et al., 1994; Kunz and Hall, 1993; Liu, 1993; Schreiber, 1991). This makes them useful probes for identifying the components of those pathways and determining their physiological roles.

The immunosuppressants interact with the immunophilins, which are small, soluble, receptor proteins that mediate their actions. Cyclosporin A (a cyclical undecapeptide) binds to cyclophilin A, whereas FK506 and rapamycin (two related macrolide antibiotics) bind to a distinct receptor protein, FKBP12 (Grandschmacher et al., 1984; Harding et al., 1989; Sekierka et al., 1989). Though cyclophilin and FKBP12 differ markedly in amino acid sequence, both immunophilins have peptidyl-prolyl cis-trans isomerization (rolamase) activity, which is inhibited by their respective ligands (reviewed by Heitman et al., 1992). However,

this inhibition does not appear to explain the effects of the immunosuppressants (Bierer et al., 1990a, 1990b; Tropschug et al., 1989). Instead, the action of cyclosporin A and FK506 derives from the binding of the drug-receptor complexes to the calcium-activated protein phosphatase calcineurin (Liu et al., 1991). This association inhibits the catalytic activity of the phosphatase, which is required for the Ca^{2+} -dependent initial step in the activation of the T lymphocyte via the T cell receptor (Farrag et al., 1991; Kronke et al., 1984).

On the other hand, rapamycin appears to block a later, Ca^{2+} -independent stage in the T cell response. This drug selectively inhibits the IL-2-stimulated G1 to S cell cycle transition that initiates T cell proliferation (Dumont et al., 1990b). Although this inhibition has been correlated with the decreased activity of the 70 kDa S6 kinase (pp70^{src}), a known downstream effector of the IL-2 receptor, the FKBP12-rapamycin complex does not appear to interact directly with this kinase (Chung et al., 1992; Kuo et al., 1992). Similarly, in T cells and other cell types, rapamycin blocks progression of the cell cycle by preventing the activation of the cyclin-dependent kinases p34^{cdc2} and p34^{cdc2}, but an association of the drug-immunophilin complex with the kinases or their respective cyclins has not been demonstrated (Albers et al., 1993; Jayaraman and Marks, 1993; Morice et al., 1993).

In the budding yeast *Saccharomyces cerevisiae*, rapamycin also causes an arrest in the G1 phase of the cell cycle through its binding to a highly conserved FKBP12 homolog (Heitman et al., 1991b). The toxicity of the drug for yeast cells has allowed, through genetic selection, the identification of two homologous genes, which, when mutated, render the cells rapamycin-resistant (Heitman et al., 1991a). This led to the proposal that the products of these genes, which show some amino acid homology to the catalytic domain of the p110 subunit of P-3 kinase, are the targets of rapamycin, hence the designation of the genes as TOR1 and TOR2 (Kunz et al., 1993). Direct support for this proposal, however, has not been presented, and how the TOR gene products confer sensitivity to rapamycin remains to be elucidated. Alternatively, it has been suggested that in the signaling pathway blocked by rapamycin, the TOR proteins, like the S6 kinase and the cyclin-dependent kinases, lie downstream of the direct target of the FKBP12-rapamycin complex (Albers et al., 1993; Heitman et al., 1994). This model assumes that the TOR mutations lead to the constitutive activation of the TOR1 and TOR2 proteins.

Besides binding to calcineurin in a FK506-dependent manner, FKBP12 can also interact with calcium-channel proteins, the ryanodine receptor, which mediates calcium-induced calcium release (Jayaraman et al., 1992; Timmerman et al., 1993), and the inositol 1,4,5-trisphosphate (IP_3) receptor (A. Cameron, A. Kaplan, D. M. S., J. Steiner, and S. H. S., unpublished data). These associations do not require FK506 or rapamycin; indeed, these drugs dissociate the FKBP12-channel complex.

Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell*. 1994 Jul 15;78(1):35-43.

1999: Leonard Guarente Lab

Sir2/3/4 complex and SIR2 regulate longevity in yeast.

In the late 1990's a study from the Guarente lab at MIT demonstrated that deletion of Sir2 shortens yeast lifespan and that Sir2 overexpression extends yeast lifespan. This paper followed a 1995 paper showing that SIR2 regulates longevity in yeast.

The study demonstrates that SIR2, a conserved NAD⁺-dependent histone deacetylase, influences longevity through two distinct pathways. First, the SIR2/3/4 protein complex promotes lifespan extension by suppressing recombination at the ribosomal DNA (rDNA) locus, thereby reducing the accumulation of extrachromosomal rDNA circles (ERCs), which are linked to aging in yeast.

The findings highlighted the multifaceted role of SIR2 in aging, showing that it can modulate longevity through both chromatin silencing and genomic stability. This work provided critical insights into the molecular mechanisms of aging and established SIR2 as a key player in the regulation of lifespan, laying the groundwork for further research into conserved aging pathways in higher eukaryotes, including humans.

This work eventually became controversial after gerontologist David Sinclair helped to found a biotech Sirtris. This company was bought by GSK but failed clinically; many have seen the leap to translation as premature. Leonard Guarente continues to argue that the basic biology here is right. (See a March 2025 [article](#) on this in the *Wall Street Journal* and a spirited 2013 [article](#) defending the science and answering the critics by Guarente).

References: Campisi p. 184; Ramakrishnan p. 158.

The *SIR2/3/4* complex and *SIR2* alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms

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The *SIR* genes are determinants of life span in yeast mother cells. Here we show that life span regulation by the Sir proteins is independent of their role in nonhomologous end joining. The short life span of a *sir3* or *sir4* mutant is due to the simultaneous expression of a and α mating-type information, which indirectly causes an increase in rDNA recombination and likely increases the production of extrachromosomal rDNA circles. The short life span of a *sir2* mutant also reveals a direct failure to repress recombination generated by the Fob1p-mediated replication block in the rDNA. Sir2p is a limiting component in promoting yeast longevity, and increasing the gene dosage extends the life span in wild-type cells. A possible role of the conserved SIR2 in mammalian aging is discussed.

[Key Words: Aging; yeast; SIR; rDNA; recombination; nonhomologous end-joining]

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In the budding yeast *Saccharomyces cerevisiae*, the *SIR* (silent information regulator) genes serve several functions. *SIR1*, *SIR2*, *SIR3*, and *SIR4* were first identified as necessary components for the transcriptional repression of the silent mating type loci, *HML* and *HMRI* (Ivy et al. 1986; Rine and Herskowitz 1987). Loss of silencing at these loci results in coexpression of α and α mating type genes and sterility in haploid strains. An additional function of *SIR2*, *SIR3*, and *SIR4* was demonstrated by showing that reporter genes positioned at telomere-proximal sequences exhibit positional effect variegation (PEV) of gene expression (Cottachling et al. 1990). Sir3p and Sir4p can be visualized at telomeric locations microscopically, and sir mutations result in a loss of PEV, telomere shortening, and the constitutive expression of telomeric reporter genes (Aparicio et al. 1991; Palladino et al. 1993).

A growing body of evidence suggests that the Sir proteins are also involved in nonhomologous end joining (NHEJ), which is used to repair breaks in DNA by ligation of the free ends (for review, see Critchlow and Jackson 1998). In mammalian cells, DNA protein kinase and KU70/85 carry out NHEJ, which can occur in response to double strand breaks. In yeast, *HDF1* and *HDF2* encode Ku70p and Ku80p and, together with other genes including *DNL4*, *XR52*, *MRE11*, and *RAD50*, are required for NHEJ (Boulton and Jackson 1996a,b; Moore and Haber 1996; Teo and Jackson 1997). Sir4p interacts with Hdf1p in a two-hybrid assay, and efficient NHEJ

requires *SIR2*, *SIR3*, and *SIR4* (Tsukamoto et al. 1997; Boulton and Jackson 1998). Furthermore, genetic and biochemical experiments imply a direct role for the Sir proteins and Hdf1p in NHEJ (Martin et al. 1999; Mills et al. 1999). Immunofluorescence and chromatin immunoprecipitation have shown that Sir3p, as well as Hdf1p, relocalizes from the telomeres to sites of double-strand breaks created by the EcoRI or HO endonucleases (Martin et al. 1999; Mills et al. 1999).

In addition to its roles in HM silencing, telomeric silencing, and NHEJ, Sir2p also functions at the ribosomal DNA (rDNA) locus. In yeast, the rDNA consists of a 9.1 kb unit that is tandemly repeated 100–200 times on chromosome XII (Petes and Borstein 1977; Philippsen et al. 1978; Rustshenko and Sherman 1994). Each unit contains genes encoding the 35S rRNA and the 5S rRNA, separated by a nontranscribed spacer (NTS). Transcription of these genes and ribosome assembly takes place in a subnuclear compartment called the nucleolus (Scheer and Benavente 1990; Melese and Xue 1995; Shaw and Jordan 1995). *SIR2* was initially shown to play a role in the suppression of mitotic recombination in the rDNA (Gottlieb and Esposito 1989). More recently, *SIR2* was shown to be required for transcriptional silencing of reporter genes integrated at the rDNA (Royk et al. 1997; Smith and Boeke 1997). The majority of cellular Sir2p, as assayed by immunofluorescence, is found in the nucleolus (Gotta et al. 1997), and the accessibility of rDNA chromatin is responsive to Sir2p dosage (Fritze et al. 1997).

Recently, it was demonstrated that a cause of aging in yeast is the accumulation of circular species of rDNA

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Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* 1999 Oct 1;13(19):2570-80.

2000: Leonard Guarente Lab

Lifespan extension by glucose restriction in yeast requires Sir2 activity.

This study by Lin, Defossez, and Guarente (2000) investigates how calorie restriction extends lifespan in *Saccharomyces cerevisiae* (yeast) and identifies the critical roles of NAD (nicotinamide adenine dinucleotide) and the SIR2 gene in this process.

The researchers demonstrated that calorie restriction increases cellular NAD levels, which activates SIR2—a gene encoding an NAD-dependent deacetylase enzyme. SIR2 activity, dependent on elevated NAD, was shown to mediate lifespan extension by modifying chromatin and suppressing genomic instability, a key factor in aging. The findings establish a direct molecular link between calorie restriction and longevity, highlighting NAD as a metabolic sensor and SIR2 as a downstream effector in this pathway.

The study confirms prior hypotheses that calorie restriction extends lifespan through specific biochemical mechanisms rather than generalized metabolic slowdown.

By linking NAD availability to SIR2’s enzymatic function, the authors propose that boosting NAD levels or enhancing SIR2 activity could mimic the benefits of calorie restriction.

This work not only advances understanding of aging in yeast but also suggests conserved pathways relevant to aging in higher organisms, offering potential targets for anti-aging therapies. The paper, published in *Science*, remains foundational in aging research, bridging dietary interventions with molecular longevity mechanisms.

REPORTS

Requirement of NAD and SIR2 for Life-Span Extension by Calorie Restriction in *Saccharomyces cerevisiae*

Su-Ju Lin,* Pierre-Antoine Defossez,* Leonard Guarente†

Calorie restriction extends life-span in a wide variety of organisms. Although it has been suggested that calorie restriction may work by reducing the levels of reactive oxygen species produced during respiration, the mechanism by which this regimen slows aging is uncertain. Here, we mimicked calorie restriction in yeast by physiological or genetic means and showed a substantial extension in life-span. This extension was not observed in strains mutant for SIR2 (which encodes the silencing protein Sir2p) or NPT1 (a gene in a pathway in the synthesis of NAD, the oxidized form of nicotinamide adenine dinucleotide). These findings suggest that the increased longevity induced by calorie restriction requires the activation of Sir2p by NAD.

Aging in budding yeast is measured by the number of mother cell divisions before senescence. Genetic studies have linked aging in this organism to the *SIR* (silent information regulator) genes, which mediate genomic silencing at telomeres, mating type loci, and the repeated ribosomal DNA (rDNA) (1). Sir2p determines life-span in a dose-dependent manner by creating silenced rDNA chromatin, thereby repressing recombination and the generation of toxic rDNA circles (2, 3). Silencing is triggered by the deacetylation of certain lysines in the NH₂-termini of histones H3 and H4 (4). Sir2p has a NAD-dependent histone deacetylase activity (5) that is conserved in Sir2p homologs (5–7).

Glucose enters yeast cells via highly regulated glucose-sensing transporters (Hxt1p) (8) and is then phosphorylated by hexokinases (Hxk1p, Hxk2p, and Glk1p) to generate glucose-6-phosphate (G-6-P) (9). To study the effect of calorie restriction (CR) in yeast (10), we first determined whether life-span could be extended by limiting glucose availability. Yeast cells grown on 0.5% glucose media exhibited a longer life-span than cells grown on 2% glucose media (Fig. 1A). Limiting the glucose availability by mutating *HXK2* also significantly extended the life-span (Fig. 1B).

Glucose activates the yeast cAMP (adenosine 3',5'-monophosphate)-dependent protein kinase (PKA) pathway. Major components of this pathway include guanosine triphosphate/guanosine diphosphate (GTP/GDP)-binding proteins (Ras1p and Ras2p), a GTP-GDP exchange factor (Cdc25p), GTP hydrolysis factors (Tra1p and Tra2p), the adenylyl cyclase (Cdc35p/Cyr1p), phosphodiesterases (Pde1p and Pde2p) that catalyze the hydrolysis of cAMP, a PKA regulatory subunit (Bcy1p), and a PKA catalytic subunit (encoded by three functionally redundant genes, *TPK1*, *TPK2*, and *TPK3*) (11–13). We therefore tested whether limiting flow through this pathway by mutating its components would mimic the life-span extension effect of low glucose concentration. Mutations in *CDC35*, *CDC25*, *TPK1*, *TPK2*, and *TPK3*, all of which reduce the activity of PKA, lengthened life-span, whereas a mutation in *PDE2*, which increases PKA activity, shortened life-span (Table 1). Growth in 0.5% glucose did not further extend the life-span of long-lived

GDP)-binding proteins (Ras1p and Ras2p), a GTP-GDP exchange factor (Cdc25p), GTP hydrolysis factors (Tra1p and Tra2p), the adenylyl cyclase (Cdc35p/Cyr1p), phosphodiesterases (Pde1p and Pde2p) that catalyze the hydrolysis of cAMP, a PKA regulatory subunit (Bcy1p), and a PKA catalytic subunit (encoded by three functionally redundant genes, *TPK1*, *TPK2*, and *TPK3*) (11–13). We therefore tested whether limiting flow through this pathway by mutating its components would mimic the life-span extension effect of low glucose concentration. Mutations in *CDC35*, *CDC25*, *TPK1*, *TPK2*, and *TPK3*, all of which reduce the activity of PKA, lengthened life-span, whereas a mutation in *PDE2*, which increases PKA activity, shortened life-span (Table 1). Growth in 0.5% glucose did not further extend the life-span of long-lived

Table 1. Longevity inversely correlates with PKA activity in multiple strain backgrounds. For each strain, life-span analysis was carried out at least twice independently on a cohort of more than 45 cells, and the values were averaged. SDs were less than 15% of the mean.

Strain background (2)	Genotype	PKA activity	Average life-span
PSY316	Wild type		21.8
	<i>cdc25-10</i>	↓	32.0
	<i>pde2Δ</i>	↑	16.2
W303	Wild type		20.8
	<i>cdc25-10</i>	↓	26.2
A364A	Wild type		21.4
	<i>cdc35-1</i>	↓	37.3
SGV	<i>tpk1Δ tpk2Δ</i>	↓	21.4
	<i>tpk3Δ</i>		
	<i>tpk1Δ tpk2Δ tpk3Δ</i>	↓	26.5

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cdc25-10 mutants, which suggests that low glucose concentration and low PKA activity function in the same pathway to extend life-span (Fig. 1A). Because these effects occurred irrespective of strain background, all further experiments focused on one strain, PSY316.

GPA2 encodes a heterotrimeric GTP-binding protein (G protein) subunit that acts in parallel to Ras1p to activate adenylyl cyclase, and *GPR1* encodes a G protein-coupled receptor that associates with Gpa2p (14, 15). Similar to mutants in the cAMP-PKA pathway, the *gpr1Δ* and *gpa2Δ* mutants exhibited a longer life-span compared to the wild type (Fig. 1C). The above data show that reducing cAMP-PKA activity or reducing

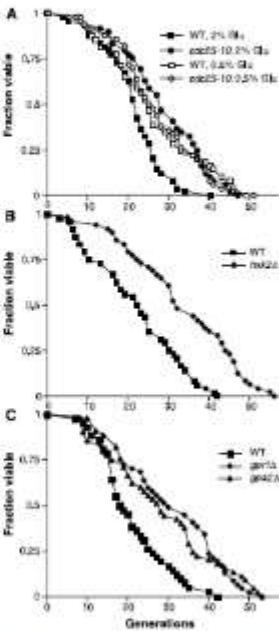


Fig. 1. Limitation of glucose availability or attenuation of glucose signaling extends life-span. (A) Longevity is increased by growth on low-glucose medium or by attenuation of cAMP-PKA signaling. Average life-spans (26) in 2% glucose (number of generations): PSY316 (wild type, WT), 21.2; *cdc25-10* (27), 28.5; in 0.5% glucose, WT, 26.2; *cdc25-10*, 27.4. (B) Deletion of hexokinase gene *HXK2* extends life-span (28). Average life-span: WT, 21.8; *hmk2Δ*, 33.3. (C) Deletion of the glucose-sensing genes *GPR1* or *GPA2* extends life-span. Average life-spans: WT, 21.0; *gpr1Δ*, 29.7; *gpa2Δ*, 29.3.

2003: Eric Verdin

SIRT2 functions as a metabolic sensor that integrates cellular energy status with microtubule dynamics.

Eric Verdin's 2003 paper, "The human Sir2 ortholog, SIRT2, is an NAD⁺-dependent tubulin deacetylase," published in *Molecular Cell*, explores the functional role of SIRT2, a member of the sirtuin family of NAD⁺-dependent deacetylases, which are homologs of the yeast Sir2 protein known for their roles in lifespan regulation and stress responses.

The study identifies SIRT2 as a novel tubulin deacetylase, revealing its ability to deacetylate α -tubulin, a major component of microtubules, in an NAD⁺-dependent manner. This finding establishes a direct link between sirtuin activity and the regulation of microtubule dynamics, which are critical for cellular processes such as cell division, intracellular transport, and cell shape maintenance. The researchers demonstrate that SIRT2 colocalizes with microtubules in mammalian cells and that its deacetylase activity is specific to α -tubulin, as it does not deacetylate histones, another common substrate for sirtuins. This specificity highlights a unique role for SIRT2 in modulating cytoskeletal architecture and function.

The study further investigates the enzymatic mechanism of SIRT2, showing that its deacetylase activity is tightly coupled to cellular NAD⁺ levels, suggesting that SIRT2 functions as a metabolic sensor that integrates cellular energy status with microtubule dynamics. This connection is particularly significant because it implies that SIRT2 could play a role in coordinating cellular responses to metabolic stress or changes in nutrient availability. Additionally, the paper provides evidence that SIRT2-mediated deacetylation of tubulin influences microtubule stability, as hyperacetylation of tubulin, which occurs in the absence of SIRT2 activity, is associated with more stable microtubules.

The Human Sir2 Ortholog, SIRT2, Is an NAD⁺-Dependent Tubulin Deacetylase

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Summary

The silent information regulator 2 protein (Sir2p) of *Saccharomyces cerevisiae* is an NAD-dependent histone deacetylase that plays a critical role in transcriptional silencing. Here, we report that a human ortholog of Sir2p, sirtuin type 2 (SIRT2), is a predominantly cytoplasmic protein that colocalizes with microtubules. SIRT2 deacetylates lysine-40 of α -tubulin both in vitro and in vivo. Knockdown of SIRT2 via siRNA results in tubulin hyperacetylation. SIRT2 colocalizes and interacts in vivo with HDAC6, another tubulin deacetylase. Enzymatic analysis of recombinant SIRT2 in comparison to a yeast homolog of Sir2 protein (Hst2p) shows a striking preference of SIRT2 for acetylated tubulin peptide as a substrate relative to acetylated histone H3 peptide. These observations establish SIRT2 as a bona fide tubulin deacetylase.

Introduction

Reversible histone acetylation is involved in the regulation of transcriptional silencing and activation (Kuo and Allis, 1998). In addition to histones, a rapidly growing number of nonhistone proteins are acetylated on lysine residues (reviewed in Polevoda and Sherman, 2002). The level of acetylation of histones and other acetylated proteins is controlled by the opposing enzymatic activities of histone deacetylases (HDACs) and histone acetyltransferases. HDACs are separated into three distinct classes based on their homology to yeast transcriptional repressors. Class I and class II deacetylases are homologs of the Rpd3p and Hda1p proteins, respectively. Class III HDACs are defined based on their homology to the yeast transcriptional repressor, Sir2p.

The silent information regulator (SIR) proteins mediate transcriptional silencing at a number of loci, including the mating type locus, telomeres, and the rDNA locus (Gasser and Cockell, 2001). Sir2p encodes an NAD-dependent histone deacetylase and is thought to mediate silencing by regulating histone acetylation (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000). The SIR2 gene family is conserved from archaeobacteria to eukaryotes (Frye, 2000). In *S. cerevisiae*, this family consists of Sir2 and four homologs of Sir2 (*Hst1-4*) genes

(Brachmann et al., 1995). Humans have seven proteins with homology to Sir2p, which have been named sirtuins (SIRT1–SIRT7) (Frye, 1999, 2000). Both human SIRT1 and mouse Sir2a deacetylate the transcription factor protein p53 and suppress p53-dependent apoptosis in response to DNA damage (Luo et al., 2001; Vaziri et al., 2001). The human SIRT2 protein shows a cytoplasmic distribution (Afshar and Mumane, 1999; Perrod et al., 2001). Both SIRT2 and Hst2p may regulate rDNA and telomeric silencing indirectly from their cytoplasmic location (Perrod et al., 2001), but could also deacetylate cytoplasmic proteins such as α -tubulin within the microtubule network.

The microtubule network is formed by the polymerization of α - and β -tubulin heterodimers and plays an important role in the regulation of cell shape, intracellular transport, cell motility, and cell division (Nogales, 2000). α - and β -tubulin subunits are subject to numerous post-translational modifications, including tyrosination, phosphorylation, polyglutamylation, polyglycylation, and acetylation (MacRae, 1997). Tubulin acetylation occurs on lysine-40 of the α -tubulin subunit (Nogales et al., 1999; Piperno et al., 1987). A variety of drug treatments and physiological signals have been reported to modulate the level of tubulin acetylation, including the anticancer drug paclitaxel (Taxol) (Piperno et al., 1987), Trichostatin A (TSA) (Grozinger et al., 2001), ras activation (Harrison and Turley, 2001), as well as microtubule interaction of MAP1 and 2C, tau (Takemura et al., 1992), and the herpes simplex virus encoded protein VP22 (Elliott and O'Hare, 1998). Similarly, microtubules associated with stable structures, such as cilia, contain relatively hyperacetylated α -tubulin (Poole et al., 2001). However, a lack of knowledge on the enzymes responsible for the reversible acetylation and deacetylation of tubulin has precluded a thorough analysis of the biological role of tubulin acetylation in microtubule functions.

Results

SIRT2 Is a Cytoplasmic Protein that Colocalizes with the Microtubule Network

Using indirect immunofluorescence and a specific antiserum for endogenous SIRT2, we determined that hSIRT2 was predominantly cytoplasmic (Figure 1A). Merging of the pictures obtained with the SIRT2 and α -tubulin antisera showed strong colocalization between SIRT2 and tubulin (Figure 1A). Fractionation of 293T cells into nuclear and cytoplasmic fractions followed by Western blotting analysis showed that SIRT2 was localized predominantly in the cytoplasm but was also present to a lesser degree in the nucleus (Figure 1B). Probing the same fractions for known cytoplasmic (p65) and nuclear (lamin A) proteins confirmed the purity of our fractions (Figure 1B).

Tubulin Deacetylation by Human SIRT2 In Vivo

The localization of SIRT2 along the microtubule network and the previous observation that α -tubulin is acetyl-

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North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. The human Sir2 ortholog, SIRT2, is an NAD⁺-dependent tubulin deacetylase, *Mol Cell*, Feb 11, 2003; 11(2):437–4, 4.

2003: David Sinclair

The 2003 study by Sinclair and colleagues that resveratrol and other small molecules can activate the sirtuin enzyme Sir2 and significantly extend the lifespan of yeast, suggesting a pharmacological approach to mimicking calorie restriction and promoting longevity. While this paper ended up in some controversy it sparked huge interest in aging research.

This heavily cited 2003 paper by Howitz et al., led by David Sinclair, published in *Nature*, reported the groundbreaking discovery that small molecules can activate sirtuins and extend lifespan in yeast. The authors identified resveratrol, a polyphenol found in red wine, as a potent activator of Sir2, a NAD⁺-dependent histone deacetylase linked to longevity.

Using a novel in vitro fluorescent assay, they showed that resveratrol increased Sir2 activity, and when added to yeast cultures, it significantly extended replicative lifespan—in some cases by as much as 70%.

This study was the first to suggest that aging could be pharmacologically manipulated through small molecules targeting the sirtuin pathway. It also linked dietary compounds like resveratrol to the molecular mechanisms of calorie restriction, which also activates Sir2.

While the work was highly influential and spurred a surge of interest in sirtuin-activating compounds (STACs) as anti-aging therapeutics, it was also met with skepticism regarding the assay's specificity and the mechanism of activation. Nonetheless, the paper was a key milestone in aging research and the commercial pursuit of sirtuin-based longevity drugs.

Reference: Ramakrishnan p. 160.

Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan

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In diverse organisms, calorie restriction slows the pace of ageing and increases maximum lifespan. In the budding yeast *Saccharomyces cerevisiae*, calorie restriction extends lifespan by increasing the activity of Sir2 (ref. 1), a member of the conserved sirtuin family of NAD⁺-dependent protein deacetylases^{2–6}. Included in this family are SIR-2.1, a *Caenorhabditis elegans* enzyme that regulates lifespan⁷, and SIRT1, a human deacetylase that promotes cell survival by negatively regulating the p53 tumour suppressor^{8–10}. Here we report the discovery of three classes of small molecules that activate sirtuins. We show that the potent activator resveratrol, a polyphenol found in red wine, lowers the Michaelis constant of SIRT1 for both the acetylated substrate and NAD⁺, and increases cell survival by stimulating SIRT1-dependent deacetylation of p53. In yeast, resveratrol mimics calorie restriction by stimulating Sir2, increasing DNA stability and extending lifespan by 70%. We discuss possible evolutionary origins of this phenomenon and suggest new lines of research into the therapeutic use of sirtuin activators.

Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*. Sep 11, 2003; 425(6954):191–6.

2009: Harrison and Miller

First study to show that rapamycin extends lifespan in a vertebrate animal. Harrison and colleagues work demonstrated that rapamycin, fed to mice in a dose that substantially inhibits TOR signaling, can extend their lifespan by up to 14%.

This study, published in *Nature* in 2009, represents a landmark discovery in aging research by demonstrating that rapamycin, an inhibitor of the mTOR (mechanistic target of rapamycin) pathway, extends lifespan in mammals.

Prior research had shown that inhibiting the TOR signaling pathway could prolong lifespan in simpler organisms like yeast, nematodes, and fruit flies, but it was unknown whether this effect could be replicated in mammals. The study found that administering rapamycin to genetically heterogeneous mice starting at 600 days of age (equivalent to late middle age in humans) significantly increased both median and maximal lifespan.

Specifically, rapamycin extended the age at which 90% of the mice had died by 14% in females and 9% in males. This effect was consistent across three independent test sites, suggesting robustness and generalizability. Importantly, the treatment did not alter disease patterns in the mice, indicating that rapamycin's lifespan-extending effects were not due to changes in specific disease susceptibilities but rather a broader impact on aging processes.

The study also included a separate cohort of mice that began receiving rapamycin at 270 days of age (early adulthood), which similarly showed increased survival rates in both males and females.

Rapamycin fed late in life extends lifespan in genetically heterogeneous mice

David E. Harrison^{1*}, Randy Strong^{2*}, Zelton Dave Sharp³, James F. Nelson⁴, Clinton M. Astle¹, Kevin Flurkey¹, Nancy L. Nadon⁵, J. Erby Wilkinson⁶, Krystyna Frenkel⁷, Christy S. Carter^{8†}, Marco Pahor^{8†}, Martin A. Javors⁹, Elizabeth Fernandez² & Richard A. Miller^{10*}

Inhibition of the TOR signalling pathway by genetic or pharmacological intervention extends lifespan in invertebrates, including yeast, nematodes and fruitflies^{1–3}; however, whether inhibition of mTOR signalling can extend lifespan in a mammalian species was unknown. Here we report that rapamycin, an inhibitor of the mTOR pathway, extends median and maximal lifespan of both male and female mice when fed beginning at 600 days of age. On the basis of age at 90% mortality, rapamycin led to an increase of 14% for females and 9% for males. The effect was seen at three independent test sites in genetically heterogeneous mice, chosen to avoid genotype-specific effects on disease susceptibility. Disease patterns of rapamycin-treated mice did not differ from those of control mice. In a separate study, rapamycin fed to mice beginning at 270 days of age also increased survival in both males and females, based on an interim analysis conducted near the median survival point. Rapamycin may extend lifespan by postponing death from cancer, by retarding mechanisms of ageing, or both. To our knowledge, these are the first results to demonstrate a role for mTOR signalling in the regulation of mammalian lifespan, as well as pharmacological extension of lifespan in both genders. These findings have implications for further development of interventions targeting mTOR for the treatment and prevention of age-related diseases.

Because incidences of most diseases rise rapidly with age⁴, interventions that delay ageing would greatly benefit health^{5,6}. So far, dietary additives that delay ageing and increase lifespan in rodent models have shown only weak effects^{7–11}. Before clinical studies are considered, anti-ageing interventions must be repeatable and effective in many mouse genotypes, and not merely postpone strain-specific diseases^{12–14}.

The National Institute on Aging Interventions Testing Program (ITP) evaluates agents that may delay ageing and increase lifespan in genetically heterogeneous mice^{15–17}. Agents are chosen as summarized at <http://www.nia.nih.gov/Research/Information/ScientificResources/InterventionsTestingProgram.htm>. Studies are simultaneously replicated at three test sites: The Jackson Laboratory (TJL), the University of Michigan (UM), and the University of Texas Health Science Center (UT). BALB/cByJ × C57BL/6J F1 (C56F1) females and C3H/HeJ × DBA/2J F1 (C3D2F1) males are supplied to each site by The Jackson Laboratory, and mated to produce genetically heterogeneous populations in which each animal is genetically unique, but a full

sibling of all other mice in the population¹⁸. Sufficient mice are used to provide 80% power to detect a 10% increase (or decrease) in mean lifespan with respect to unmanipulated controls of the same sex, even if data from one of the three test sites were to be unavailable. Here we report that dietary encapsulated rapamycin increases mouse survival, including survival to the last decade, a measure of maximal lifespan.

Rapamycin reduces function of the rapamycin target kinase TOR and has anti-neoplastic activities; genetic inhibition of TOR extends lifespan in short-lived model organisms. In male and female mice at each of three collaborating research sites, median and maximum lifespan were extended by feeding encapsulated rapamycin starting at 600 days of age (Fig. 1). We analysed the data set as of 1 February 2009, with 2% (38 of 1,901) of mice still alive. For data pooled across sites, a log-rank test rejected the null hypothesis that treatment and control groups did not differ ($P < 0.0001$); mice fed rapamycin were longer lived than controls ($P < 0.0001$) in both males and females. Expressed as mean lifespan, the effect sizes were 9% for males and 13% for females in the pooled data set. Expressed as life expectancy at 600 days (the age of first exposure to rapamycin), the effect sizes were 28% for males and 38% for females. Mice treated with other agents (enalapril and CAPE (caffeic acid phenethyl ester)) evaluated in parallel did not differ from controls at the doses used (Supplementary Fig. 1).

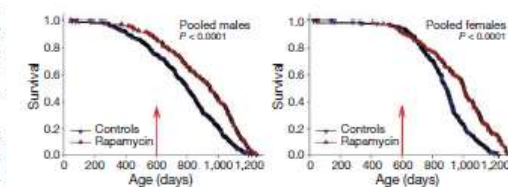


Figure 1 | Survival plots for male and female mice, comparing control mice to those fed rapamycin in the diet starting at 600 days of age, pooling across the three test sites. P values were calculated by the log-rank test. Four per cent of the control mice and three per cent of rapamycin-assigned mice were removed from the experiment for technical reasons. Only five animals (three controls, two rapamycin) were removed after the start of rapamycin treatment at 600 days. Thus, there were no significant differences between groups in censoring.

¹The Jackson Laboratory, Bar Harbor, Maine 04609, USA. ²Geriatric Research, Education and Clinical Center and Research Service, South Texas Veterans Health Care System, Department of Pharmacology, and Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, Texas 78229, USA. ³Institute of Biotechnology/Department of Molecular Medicine, and Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, Texas 78245, USA. ⁴Department of Physiology and Barshop Institute for Longevity and Aging Studies at The University of Texas Health Science Center at San Antonio, Texas 78229, USA. ⁵Division of Aging Biology, National Institute on Aging, Bethesda, Maryland 20892, USA. ⁶Unit for Laboratory Animal Medicine, University of Michigan School of Medicine, Ann Arbor, Michigan 48109-2200, USA. ⁷Environmental Medicine, NY University School of Medicine, New York 10016, USA. ⁸Wake Forest University School of Medicine, Department of Internal Medicine Section on Gerontology and Geriatrics, Winston-Salem, North Carolina 27157, USA. ⁹Department of Psychiatry, The University of Texas Health Science Center at San Antonio, Texas 78229, USA. ¹⁰Department of Pathology and Geriatrics Center, University of Michigan, and Ann Arbor VA Medical Center, Ann Arbor, Michigan 48109-2200, USA. [†]Present address: Department of Aging and Geriatric Research, College of Medicine, Institute on Aging, University of Florida, Gainesville, Florida 32611, USA.

*These authors contributed equally to this work.

2012: David Sabatini

The mechanistic target of rapamycin (mTOR) signaling pathway senses and integrates a variety of environmental cues to regulate organismal growth and homeostasis. This is one of the most heavily cited papers in the history of aging research.

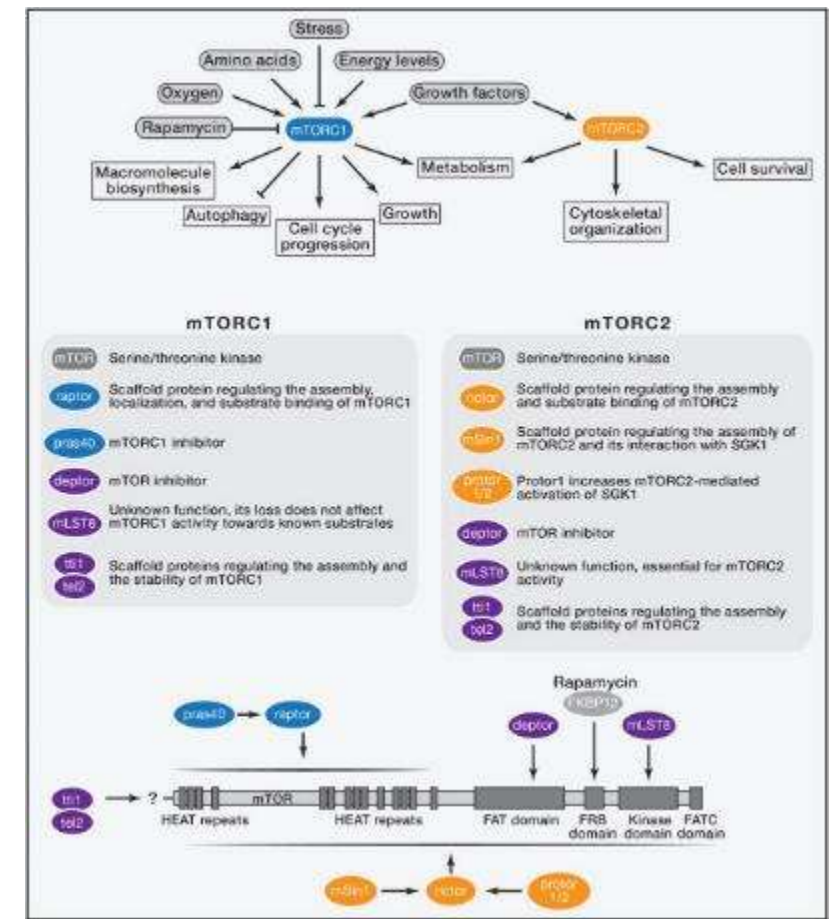
This review explores the central role of the mechanistic Target of Rapamycin (mTOR) pathway in regulating cell growth, metabolism, and survival. mTOR is a critical integrator of nutrients, growth factors, and energy status, which influence cellular processes like protein synthesis, autophagy, and lipid metabolism.

The mTOR pathway is organized into two distinct complexes: mTORC1 and mTORC2. mTORC1 is primarily responsible for sensing nutrient availability and promoting anabolic processes, while mTORC2 regulates cell survival and cytoskeletal organization. Dysregulation of mTOR signaling is implicated in a wide range of diseases, including cancer, metabolic disorders, and neurodegenerative conditions, making it a key target for therapeutic intervention.

Laplane and Sabatini also highlight the dual nature of mTOR signaling: while it is essential for normal growth and development, its overactivation can drive pathological conditions such as tumorigenesis and insulin resistance.

The paper emphasizes the potential of mTOR inhibitors, like rapamycin and its analogs, in treating diseases linked to mTOR hyperactivity.

Additionally, the authors discuss the emerging role of mTOR in aging, noting that inhibition of mTORC1 extends lifespan in model organisms.



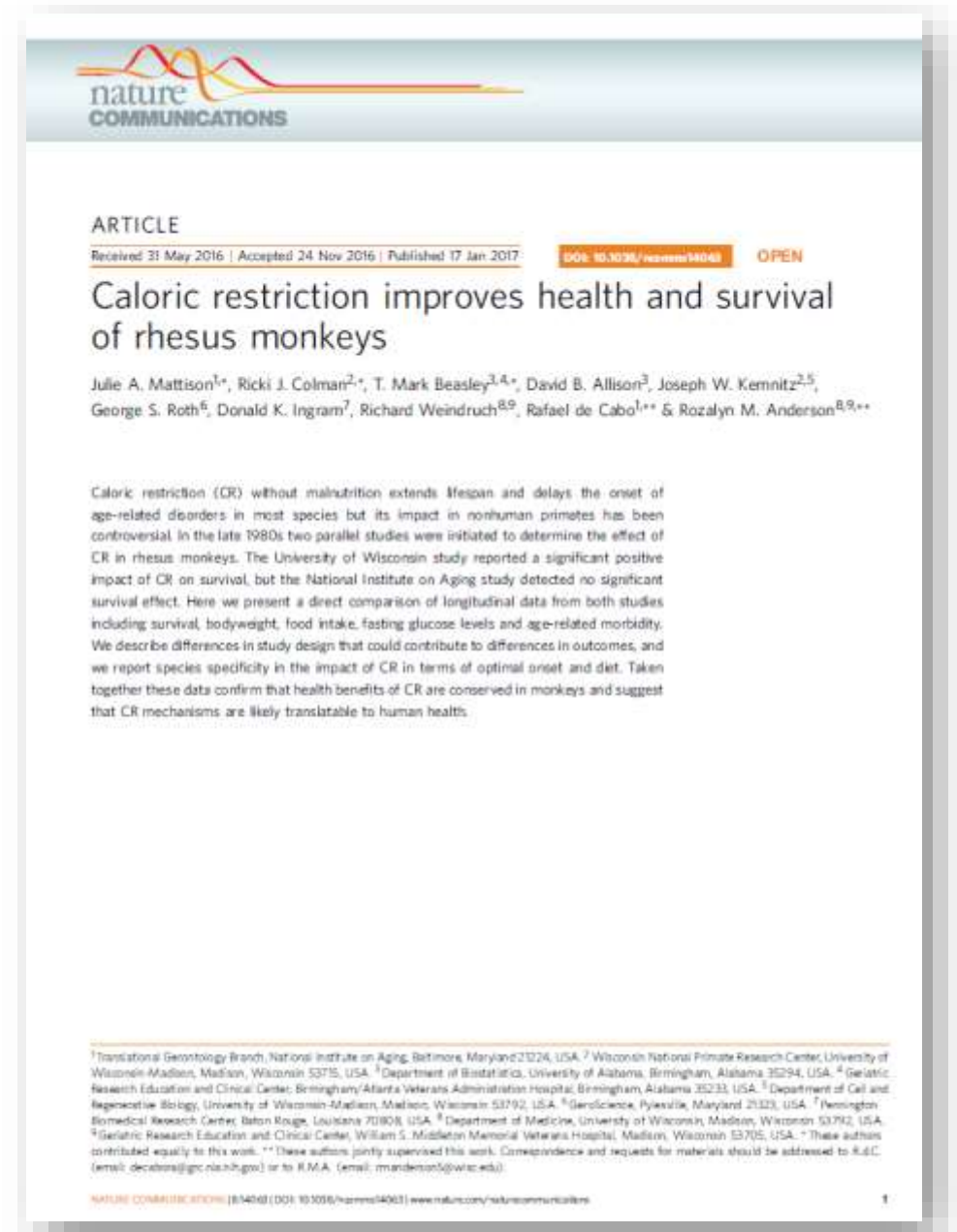
Mathieu Laplane and David Sabatini, mTOR Signaling in Growth Control and Disease *Cell*, 2012, Volume 149, Issue 2, 274 - 293

2017: Mattison & Anderson

Caloric restriction improves health and survival of rhesus monkeys.

The 2017 paper by Mattison et al., titled "Caloric restriction improves health and survival of rhesus monkeys", published in *Nature Communications*, presents findings from a long-term study on the effects of caloric restriction (CR) on health and lifespan in rhesus monkeys. The study, conducted over several decades, compared monkeys on a calorie-restricted diet (30% fewer calories than controls) with those on a standard diet. The results showed that caloric restriction significantly improved health outcomes, including reduced incidence of age-related diseases such as cancer, cardiovascular disease, and diabetes. Additionally, CR monkeys exhibited better metabolic health, including improved insulin sensitivity and lower levels of inflammation, compared to their non-restricted counterparts. These findings suggest that caloric restriction can delay the onset of age-related pathologies and promote healthier aging in primates.

In terms of survival, the study found that caloric restriction extended lifespan in rhesus monkeys, although the effect was more pronounced in reducing morbidity rather than mortality. The CR group had a lower overall mortality rate and a delayed onset of age-related diseases, contributing to an increased healthspan. The study also highlighted that the benefits of caloric restriction were more evident when implemented earlier in life, emphasizing the importance of timing in dietary interventions. These results align with previous findings in smaller organisms, such as yeast, worms, and mice, and provide strong evidence that caloric restriction can improve health and survival in primates, including humans.



Mattison JA, Colman RJ, Beasley TM, Allison DB, Kemnitz JW, Roth GS, Ingram DK, Weindruch R, de Cabo R, Anderson RM. Caloric restriction improves health and survival of rhesus monkeys. *Nat Commun.* Jan 17, 2017; 8:14063. 204

2021: Haim Cohen

This paper positions SIRT6 as one of the most promising genetic targets for aging interventions, with the added advantage that its overexpression not only extends lifespan but also preserves physiological function. This paper's findings suggest that SIRT6 is a master regulator of metabolic resilience in aging.

In this 2021 *Nature Communications* paper, Haim Cohen and colleagues build upon earlier work on the longevity-related protein SIRT6 by exploring its role in restoring energy homeostasis and promoting healthy aging. Using transgenic mice that overexpress SIRT6, the researchers observed not only significant lifespan extension in male mice—up to 27%—but also an improvement in multiple markers of healthspan, including physical activity, metabolic function, and resistance to frailty. Interestingly, these effects were more pronounced than in their earlier 2012 study, likely due to higher levels of SIRT6 expression. Importantly, SIRT6 overexpression improved glucose homeostasis, reduced insulin resistance, and enhanced mitochondrial function, particularly in aged animals.

Mechanistically, the study found that SIRT6 acts as a key regulator of energy metabolism by repressing genes involved in glycolysis and upregulating those involved in mitochondrial respiration and fatty acid oxidation. This shift promotes a metabolic state similar to that seen in calorie restriction—a known intervention that extends lifespan across species. The authors also demonstrated that SIRT6 helps maintain NAD⁺ levels, reduces chronic inflammation, and promotes genomic stability, all of which are linked to delayed aging. Metabolomic and transcriptomic analyses supported the conclusion that SIRT6 orchestrates a broad, systemic reprogramming of energy utilization in favor of a more youthful profile.



Roichman A, Elhanati S, Aon MA, Abramovich I, Di Francesco A, Shahar Y, Avivi MY, Shurgi M, Rubinstein A, Wiesner Y, Shuchami A, Petrover Z, Lebenthal-Loinger I, Yaron O, Lyashkov A, Ubaida-Mohien C, Kanfi Y, Lerrer B, Fernández-Marcos PJ, Serrano M, Gottlieb E, de Cabo R, Cohen HY. Restoration of energy homeostasis by SIRT6 extends healthy lifespan. *Nat Commun*. May 28, 2021; 12(1):3208.

2024: Gary Churchill

Caloric restriction improves lifespan in a range of mice but genes matter more.

In their 2024 **Nature** paper, "Dietary restriction impacts health and lifespan of genetically diverse mice," Gary Churchill and colleagues at Calico Life Sciences explored how various dietary restriction (DR) protocols affect lifespan and health in genetically diverse female Diversity Outbred (DO) mice. Mice were assigned to one of five regimens: ad libitum feeding (AL), 20% caloric restriction (20% CR), 40% caloric restriction (40% CR), one-day intermittent fasting per week (1D IF), or two-day intermittent fasting per week (2D IF). All DR protocols extended lifespan compared to AL, with 40% CR yielding the most significant increase—up to 36.3% longer median lifespan. However, individual responses varied, with some mice experiencing negative effects such as excessive weight loss and impaired immune function, suggesting that DR benefits are not universal.

A key finding was that genetic variation played a major role in determining how mice responded to DR. Some genotypes were better able to maintain body weight, immune health, and energy levels under restriction, leading to extended lifespans, while others suffered adverse effects. This highlights the importance of personalized approaches to dietary interventions, as not all individuals may benefit equally from caloric restriction. The study emphasizes that while DR can promote longevity, its effects are complex and influenced by genetic factors, pointing to the need for tailored nutritional strategies to optimize health and lifespan.

Dietary restriction impacts health and lifespan of genetically diverse mice

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Caloric restriction extends healthy lifespan in multiple species¹. Intermittent fasting, an alternative form of dietary restriction, is potentially more sustainable in humans, but its effectiveness remains largely unexplored^{2–5}. Identifying the most efficacious forms of dietary restriction is key for developing interventions to improve human health and longevity⁶. Here we performed an extensive assessment of graded levels of caloric restriction (20% and 40%) and intermittent fasting (1 and 2 days fasting per week) on the health and survival of 960 genetically diverse female mice. We show that caloric restriction and intermittent fasting both resulted in lifespan extension in proportion to the degree of restriction. Lifespan was heritable and genetics had a larger influence on lifespan than dietary restriction. The strongest trait associations with lifespan included retention of body weight through periods of handling—an indicator of stress resilience, high lymphocyte proportion, low red blood cell distribution width and high adiposity in late life. Health effects differed between interventions and exhibited inconsistent relationships with lifespan extension. 40% caloric restriction had the strongest lifespan extension effect but led to a loss of lean mass and changes in the immune repertoire that could confer susceptibility to infections. Intermittent fasting did not extend the lifespan of mice with high pre-intervention body weight, and two-day intermittent fasting was associated with disruption of erythroid cell populations. Metabolic responses to dietary restriction, including reduced adiposity and lower fasting glucose, were not associated with increased lifespan, suggesting that dietary restriction does more than just counteract the negative effects of obesity. Our findings indicate that improving health and extending lifespan are not synonymous and raise questions about which end points are the most relevant for evaluating aging interventions in preclinical models and clinical trials.

Caloric restriction (CR) delays the onset of age-related diseases and extends lifespan in multiple species¹. In humans, compliance with CR is challenging, and interest has shifted to more permissive forms of dietary restriction (DR), such as time-restricted feeding and intermittent fasting (IF) that have proven to be effective in promoting organismal health^{2–5}. In mice, regular periods of fasting can convey considerable benefits without reduction in overall energy intake⁶. Mice on CR also experience prolonged periods of daily fasting, and the health benefits of CR can be optimized by feeding at a specific time of the day, suggesting that both caloric intake and timing of feeding contribute to physiological response and lifespan extension^{7–9}. Despite the importance of these observations, limited information is available regarding the differences between CR and IF in relation to healthy ageing and longevity⁴.

Responses to DR vary across individuals, and the mechanisms underlying this variability remain largely unclear. Studies in mice and non-human primates have shown that the effects of DR are influenced by individual characteristics including sex, body size and composition, and genetics^{10–12}. Human clinical studies testing the effects of DR on health have largely focused on changes in body weight, adiposity, energy metabolism and cardiometabolic risk factors^{13–15}. There has been less exploration of the long-term effects of DR because studies in humans are limited by their small sample size and short duration. Moreover, the safety and efficacy of DR may depend on factors such

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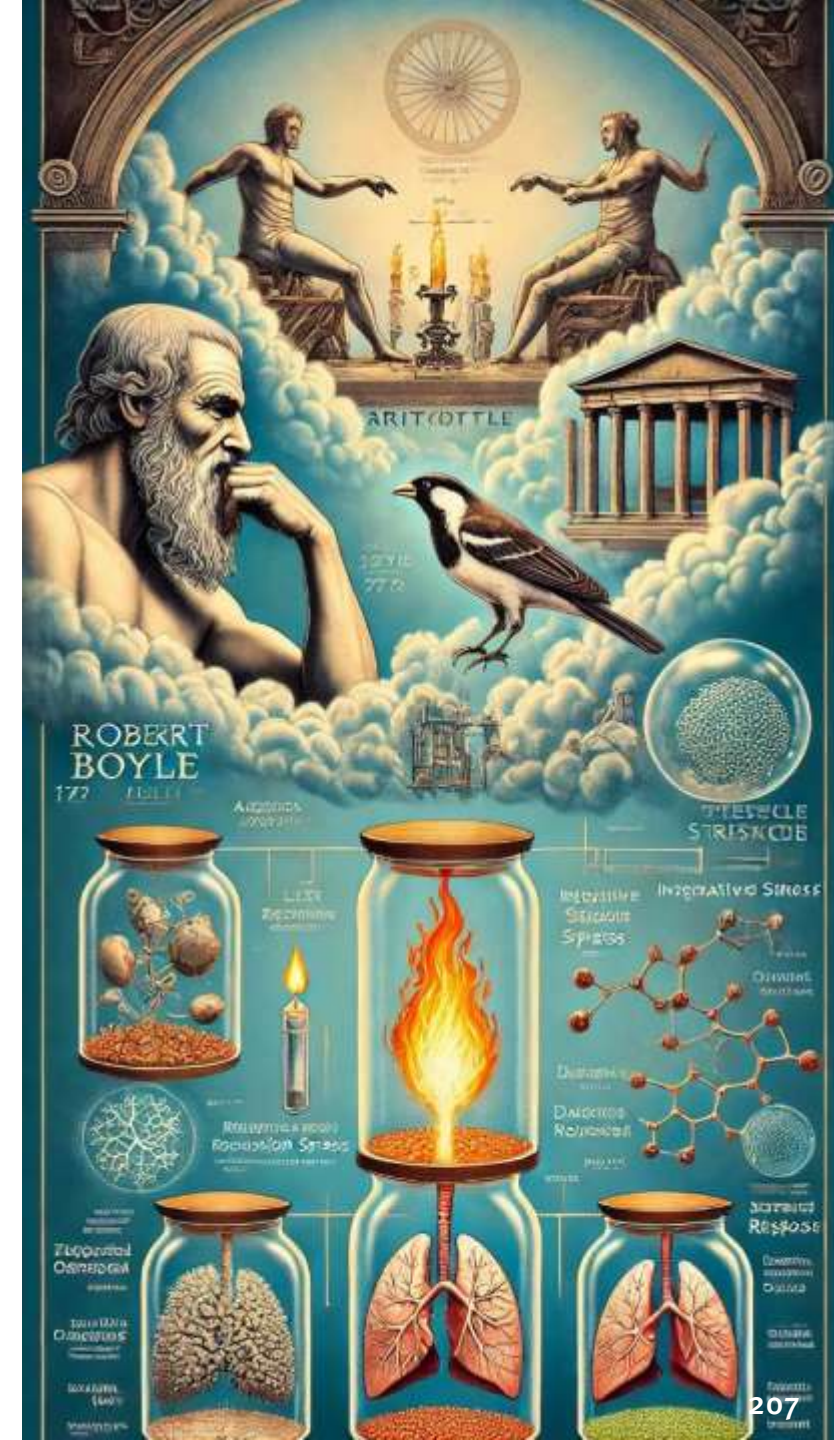
Section 8.C: Oxidative Stress, Mitochondria and Aging

Very early on, humans hypothesized that aging is linked to respiratory processes. More than 2000 years ago, Aristotle argued that respiration brings life but that it ultimately leads to our death.

Robert Boyle made a similar observation in his *Tracts* in 1672 that life was like a flame. It required air to exist. To be clear, he put candles inside clear bell jars and noted that they eventually burned out. He also put finches in the same environment and noted that they died too but took longer to get there. His conclusion was not so far fetched.

Of course, oxygen wasn't discovered until a century later through the works of Priestley, Scheele and Lavoisier. So, it would not have been possible for Boyle to link the consumption of oxygen to physiologic processes.

Thus, oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidant defenses, was later implicated in aging. The free radical theory of aging, proposed by Harman in the 1950s, posits that ROS-induced damage accumulates over time, leading to cellular dysfunction.



The Integrated Stress Response, Unfolded Proteins and Mitochondria

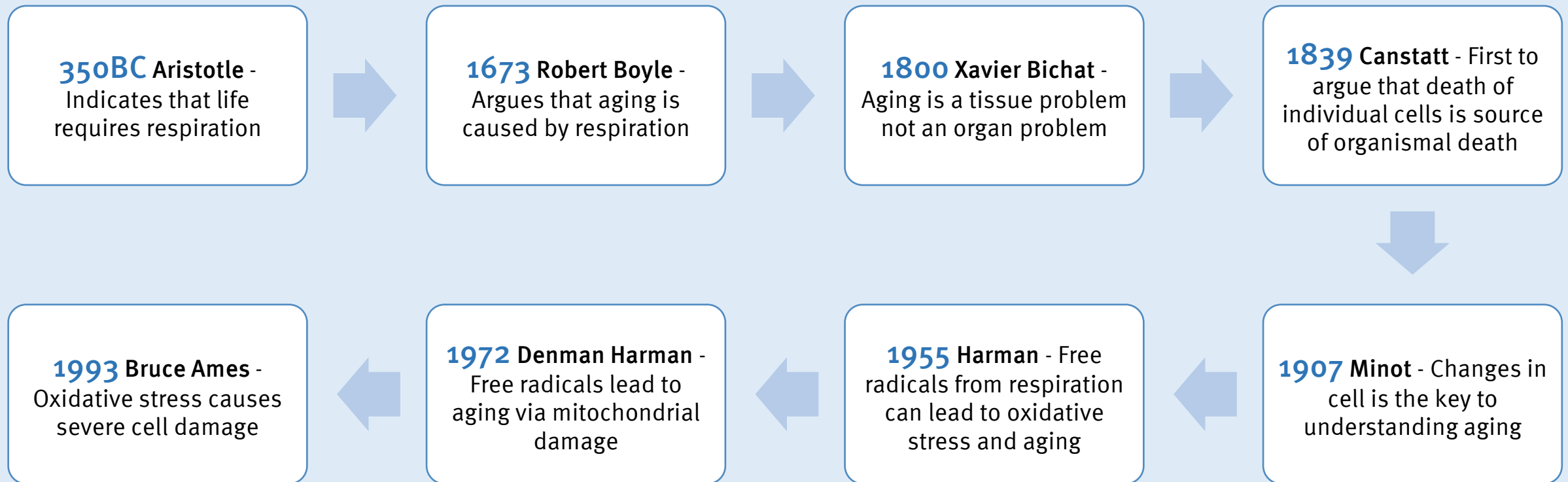
While early studies supported this theory, more recent research has revealed a more nuanced role for ROS in aging. For example, low levels of ROS can act as signaling molecules that promote cellular repair and longevity.

The unfolded protein response (UPR) and the integrated stress response (ISR) are cellular stress response pathways that play critical roles in maintaining protein homeostasis (proteostasis) and ensuring cellular survival under stress conditions. These pathways are closely linked to aging, as the accumulation of misfolded proteins and the decline in stress response efficiency are hallmarks of aging. Activation of the ISR can promote survival under stress, but chronic activation can contribute to age-related decline.

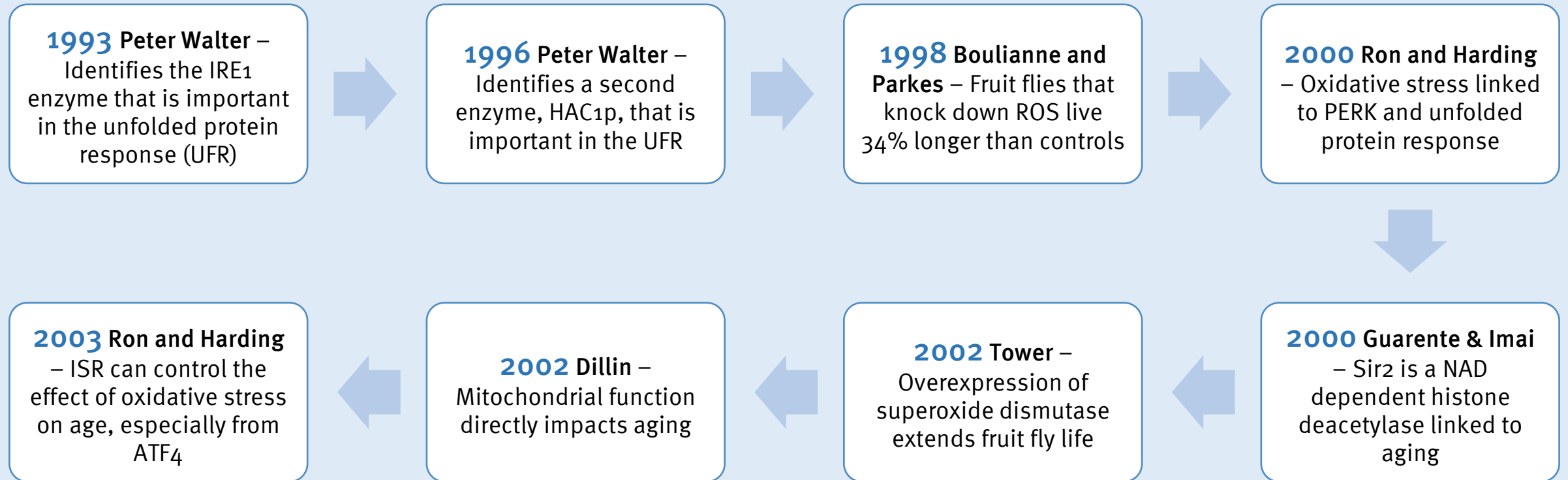
The involved pathways appear to be complex. We note that the effect of the ISR on mitochondrial health is intermediated by a number of other pathways and factors, including NAD dependent proteins, ATF4 and the sirtuin system. It's also important to note that evidence persists to the present that control of oxidative stress and mitochondrial damage can extend lifespan. A paper by Tower and colleagues, for example, showed in 2002 that superoxide dismutase which protects against oxidative damage can extend life in an *in vivo* model.



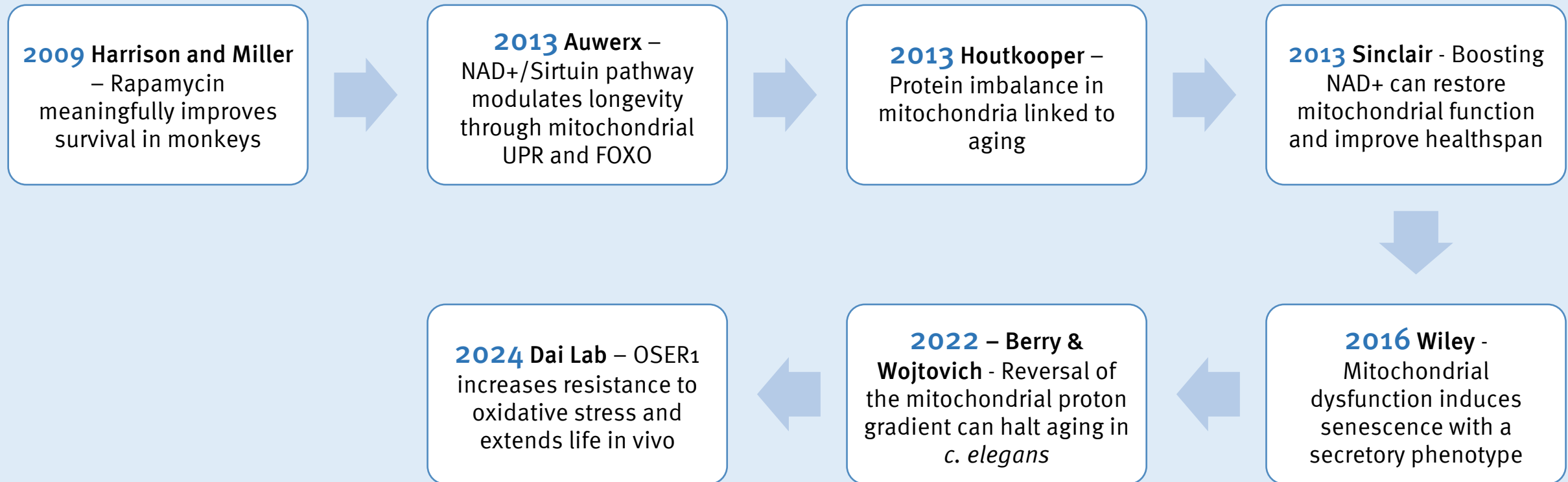
Oxidative Stress and Aging (350BC to 1993)



Oxidative Stress and Aging (1994 to 2007)



Oxidative Stress and Aging (2008 to 2025)



1993: Bruce Ames

This is the second most cited paper on aging research of all time. It highlights the damage that can be caused by reactive oxygen species because of cellular metabolism.

In their 1993 paper published in *PNAS*, Bruce N. Ames, Mark K. Shigenaga, and Tory M. Hagen explore the role of oxidative damage in aging and degenerative diseases. The authors propose that reactive oxygen species (ROS), generated as byproducts of normal metabolism, cause cumulative damage to DNA, proteins, and lipids, contributing to the aging process and the development of diseases such as cancer, cardiovascular disease, and neurodegenerative disorders.

They argue that the body's antioxidant defenses, including enzymes like superoxide dismutase and dietary antioxidants such as vitamins C and E, play a critical role in mitigating this damage. However, with age, the balance between oxidants and antioxidants shifts, leading to increased oxidative stress and cellular dysfunction. The paper highlights the importance of maintaining adequate antioxidant levels through diet and lifestyle to slow the progression of age-related diseases.

The authors also discuss the potential therapeutic implications of their findings, suggesting that boosting antioxidant defenses could help prevent or delay the onset of degenerative diseases. They emphasize the need for further research to understand the mechanisms of oxidative damage and the efficacy of antioxidant interventions. The paper underscores the interconnectedness of diet, metabolism, and aging, proposing that oxidative stress is a key factor in the decline of cellular function over time.

Review

Oxidants, antioxidants, and the degenerative diseases of aging

(cancer/mutation/endogenous DNA adducts/oxygen radicals)

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Contributed by Bruce N. Ames, June 14, 1993

ABSTRACT Metabolism, like other aspects of life, involves tradeoffs. Oxidant by-products of normal metabolism cause extensive damage to DNA, protein, and lipid. We argue that this damage (the same as that produced by radiation) is a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts. Antioxidant defenses against this damage include ascorbate, tocopherol, and carotenoids. Dietary fruits and vegetables are the principal source of ascorbate and carotenoids and are one source of tocopherol. Low dietary intake of fruits and vegetables doubles the risk of most types of cancer as compared to high intake and also markedly increases the risk of heart disease and cataracts. Since only 9% of Americans eat the recommended five servings of fruits and vegetables per day, the opportunity for improving health by improving diet is great.

The degenerative diseases associated with aging include cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts. The functional degeneration of somatic cells during aging appears, in good part, to contribute to these diseases. The relationship between cancer and age in various mammalian species illustrates this point. Cancer increases with about the fifth power of age in both short-lived species, such as rats, and long-lived species, such as humans. Thus a marked decrease in age-specific cancer rates has accompanied the marked increase in lifespan that has occurred in 60 million years of mammalian evolution; i.e., cancer rates are high in a 2-year-old rat, but low in a 2-year-old human. One important factor in longevity appears to be basal metabolic rate, which is about 7 times higher in a rat than in a human and which could markedly affect the level of endogenous oxidants and other mutagens produced as by-products of metabolism. The level of oxidative DNA damage appears to be roughly related to metabolic rate in a number of mammalian species (1–3).

Oxidation and Damage to DNA, Protein, and Lipids

Oxidative damage to DNA, proteins, and other macromolecules accumulates with age and has been postulated to be a major, but not the only, type of endogenous damage leading to aging (4–9). Superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$), which are mutagens produced by radiation, are also by-products of normal metabolism (10–12). Lipid peroxidation gives rise to mutagenic lipid epoxides, lipid hydroperoxides, lipid alkoxy and peroxy radicals, and enals (α,β -unsaturated aldehydes) (13, 14). Singlet oxygen, a high-energy and mutagenic form of oxygen, can be produced by transfer of energy from light, the respiratory burst from neutrophils, or lipid peroxidation (15).

Animals have numerous antioxidant defenses, but since these defenses are not perfect, some DNA is oxidized. Oxidatively damaged DNA is repaired by enzymes that excise the lesions, which are then excreted in the urine. Methods have been developed to assay several of these excised damaged bases in the urine of rodents and humans (1, 16), almost all of which appear as the free base from repair by glycosylases. We estimate that the number of oxidative hits to DNA per cell per day is about 100,000 in the rat and about 10,000 in the human. DNA-repair enzymes efficiently remove most, but not all, of the lesions formed (4). Oxidative lesions in DNA accumulate with age, so that by the time a rat is old (2 years) it has about 2 million DNA lesions per cell, which is about twice that in a young rat. Mutations also accumulate with age (17, 18). For example, the somatic mutation frequency in human lymphocytes, of which the contribution of oxidative DNA lesions is unknown, is about 9 times greater in elderly people than in neonates (17). The importance of oxidative DNA lesions in cancer and aging is underscored by the existence of specific repair glycosylases that excise these lesions from DNA. In the case of 8-oxo-2'-deoxyguanosine, a lesion formed from oxidative damage to guanine residues in

DNA, loss of a specific glycosylase activity leads to an appreciable increase in the spontaneous mutation rate (19), indicating the intrinsic mutagenic potential of this DNA lesion. Many other oxidative DNA lesions are likely to be important as well (10).

Mitochondrial DNA (mtDNA) from rat liver has more than 10 times the level of oxidative DNA damage than does nuclear DNA from the same tissue (20). This increase may be due to a lack of mtDNA repair enzymes, a lack of histones protecting mtDNA, and the proximity of mtDNA to oxidants generated during oxidative phosphorylation. The cell defends itself against this high rate of damage by a constant turnover of mitochondria, thus presumably removing those damaged mitochondria that produce increased oxidants. Despite this turnover, oxidative lesions appear to accumulate with age in mtDNA at a higher rate than in nuclear DNA (Fig. 1). Oxidative damage could also account for the mutations in mtDNA that accumulate with age (21, 22).

Endogenous oxidants also damage proteins and lipids. Stadtman and his colleagues (7, 23, 24) have shown that the activity of proteolytic enzymes that hydrolyze oxidized proteins is not sufficient to prevent an age-associated increase of oxidized proteins. In two human diseases associated with premature aging, Werner syndrome and progeria, oxidized proteins increase at a much higher rate than is normal (7). Fluorescent pigments, which are thought to be due in part to crosslinks between protein and lipid peroxidation products, also increase with age (14, 25).

Sources and Effects of Oxidants

Four endogenous sources appear to account for most of the oxidants produced by cells. (i) As a consequence of normal aerobic respiration, mitochondria consume O_2 , reducing it by sequential steps to produce H_2O (Fig. 2). Inevitable by-products of this process, as stated above,

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1993: Peter Walter

Peter Walter and his two students Jeffrey Cox and Caroline Shamu found a key transcription factor, IRE1, required for the function of the unfolded protein response (UPR) within the endoplasmic reticulum. The same factor was simultaneously discovered by the Japanese researcher Kazutoshi Mori.

Aging is associated with changes in the expression of ER chaperones and folding enzymes, leading to the impairment of proteostasis, and accumulation of misfolded proteins. Specifically, as organisms age, the efficiency of the UPR declines, leading to the accumulation of misfolded proteins and ER stress. This contributes to cellular dysfunction and is associated with age-related diseases such as neurodegenerative disorders (e.g., Alzheimer's and Parkinson's diseases), diabetes, and cardiovascular diseases. Further, the initiation of UPR prevents the elimination of unfolded proteins, leading to endoplasmic reticulum (ER) stress.

The 1993 study by Cox, Shamu, and Walter identified a critical mechanism underlying the unfolded protein response (UPR) in the endoplasmic reticulum (ER). The authors discovered that transcriptional activation of genes encoding ER-resident chaperones, such as BiP, during ER stress requires a transmembrane protein kinase, later known as IRE1.

Through experiments conducted in yeast, they demonstrated that this kinase senses the accumulation of unfolded proteins in the ER lumen and initiates a signaling cascade to upregulate stress-responsive genes.

Their work established IRE1 as a central mediator linking ER stress to adaptive gene expression, revealing a foundational pathway for maintaining protein-folding homeostasis. This discovery significantly advanced understanding of cellular stress responses, providing a framework for exploring related mechanisms in diseases involving protein misfolding.

Transcriptional Induction of Genes Encoding Endoplasmic Reticulum Resident Proteins Requires a Transmembrane Protein Kinase

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Summary

The transcription of genes encoding soluble proteins that reside in the endoplasmic reticulum (ER) is induced when unfolded proteins accumulate in the ER. Thus, an intracellular signal transduction pathway must exist that mediates communication between the ER lumen and the nucleus. We have identified a gene in *S. cerevisiae*, *IRE1*, that is required for this pathway: *ire1*[−] mutants cannot activate transcription of *KAR2* and *PDI1*, which encode the ER resident proteins BiP and protein disulfide isomerase. Moreover, *IRE1* is essential for cell viability under stress conditions that cause unfolded proteins to accumulate in the ER. *IRE1* encodes a transmembrane serine/threonine kinase that we propose transmits the unfolded protein signal across the ER or inner nuclear membrane. *IRE1* is also required for inositol prototrophy, suggesting that the induction of ER resident proteins is coupled to the biogenesis of new ER membrane.

Introduction

In all eukaryotic cells, secreted and transmembrane proteins must be folded and assembled correctly in the endoplasmic reticulum (ER) before they can exit the ER. Folding in the ER is assisted by a set of enzymes including BiP, a member of the HSP70 family of molecular chaperones (reviewed in Gething and Sambrook, 1992). BiP is thought to bind transiently to nascent proteins as they are translocated into the ER lumen to assist proper folding and to prevent aggregation of folding intermediates (Pelham, 1986; Haas and Wabl, 1983; Ng et al., 1989; Machamer et al., 1990). BiP also binds to partially assembled and misfolded proteins and thereby may help target dead-end products to degradative pathways in the ER (Ng et al., 1990; Gething et al., 1988; Knittler and Haas, 1992). Another well-characterized ER luminal protein that is required for proper protein folding is protein disulfide isomerase (PDI), which catalyzes the formation of disulfide bonds (Freedman, 1989).

Interestingly, the synthesis of these ER resident proteins is regulated according to demand for them inside the organelle. In mammalian cells, for example, synthesis of BiP, PDI, and glucose-related protein GRP94, a member of the HSP90 family, is induced when unfolded proteins accumulate in the ER (Lee, 1987; Kozutsumi et al., 1988; Dorner et al., 1992). Experimentally, the accumulation of unfolded proteins in the ER is induced by various treatments: the inhibition of glycosylation either by glucose starvation or by the addition of drugs such as tunicamycin

or 2-deoxyglucose; the addition of reducing agents, such as β -mercaptoethanol, which are thought to affect protein folding by preventing proper disulfide bond formation; the expression of folding-defective mutant secretory proteins; or the addition of calcium ionophores that deplete calcium stores in the ER. Induction of the ER resident proteins occurs at the transcriptional level (Lee, 1987). Thus, an unfolded protein response pathway must exist that allows the transduction of a signal from the ER lumen, where unfolded proteins accumulate, to the cell nucleus, where transcription is activated.

Lee and colleagues have identified a promoter element in the BiP gene of mammalian cells that is required for BiP induction in response to unfolded proteins. The sequence of this element is conserved among different mammalian species (Resendez et al., 1988). Genes encoding other ER luminal proteins are coordinately regulated with the BiP gene. For example, GRP94 contains similar upstream promoter elements, and it has been proposed that the two genes are regulated by a common transcription factor(s) (Chang et al., 1989; Li and Lee, 1991; Li et al., 1992).

In the yeast *Saccharomyces cerevisiae*, BiP is encoded by the *KAR2* gene, and, as in higher cells, its transcription is induced when unfolded proteins accumulate in the ER (Normington et al., 1989; Rose et al., 1989). However, unlike the mammalian BiP gene, *KAR2* transcription is also induced by heat shock. An analysis of the *KAR2* promoter has revealed that the unfolded protein response is regulated by a 22 bp unfolded protein response element (UPRE) that is distinct from the heat shock element in the *KAR2* promoter (Kohno et al., 1993; Mori et al., 1992). The UPRE is sufficient to activate transcription from a heterologous promoter in response to the accumulation of unfolded proteins in the ER lumen. Here, we describe the results of a genetic screen in which we used a UPRE-controlled reporter gene to isolate yeast mutants defective in the unfolded protein response pathway.

Results

Construction of a *lacZ* Reporter Gene That Is Activated by the Accumulation of Unfolded Proteins in the ER Lumen

To isolate *S. cerevisiae* mutants defective in *KAR2* induction, we first made a *lacZ* reporter gene that is induced when unfolded proteins accumulate in the ER. Because transcription from the intact *KAR2* promoter occurs at a relatively high basal level even in the absence of *KAR2* induction, we chose to construct a hybrid promoter with a lower basal level. To this end, we took advantage of the observation that the UPRE in the *KAR2* promoter can function as an upstream activating sequence when fused to a heterologous promoter (Mori et al., 1992). Thus, we synthesized a 38 bp DNA fragment bearing the UPRE from *KAR2* and inserted it upstream of a crippled *CYC1* promoter that, in the absence of an upstream activating

1996: Peter Walter

A second key paper by the Peter Walter lab finds that the unfolded protein response requires the transcription factor HAC1P.

Cells respond to an accumulation of unfolded proteins in the endoplasmic reticulum (ER) by increasing transcription of genes encoding ER-resident proteins.

The information is transmitted from the ER lumen to the nucleus by an intracellular signaling pathway, the unfolded protein response (UPR). We have identified a basic-leucine zipper transcription factor, Hac1p, that is required for the UPR and binds to the UPR element in the promoter of UPR-regulated genes.

Surprisingly, Hac1p is found in UPR-activated cells only, and its level is controlled by regulated splicing of its mRNA. Splicing replaces the C-terminal tail of Hac1p with a different peptide that renders Hac1p more resistant to an otherwise extremely rapid ubiquitin-dependent degradation. We propose that the complex regulation of Hac1p expression serves to provide multiple levels at which the UPR can be controlled.

This work and that of Cox, Shamu and Walter (1993) were important insofar as they provided most of the know how into the mechanism required for the UPR.

Peter Walter received the Lasker Prize in 2015 acknowledging the important contribution of these two papers.

A Novel Mechanism for Regulating Activity of a Transcription Factor That Controls the Unfolded Protein Response

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Summary

Cells respond to an accumulation of unfolded proteins in the endoplasmic reticulum (ER) by increasing transcription of genes encoding ER-resident proteins. The information is transmitted from the ER lumen to the nucleus by an intracellular signaling pathway, the unfolded protein response (UPR). We have identified a basic-leucine zipper transcription factor, Hac1p, that is required for the UPR and binds to the UPR element in the promoter of UPR-regulated genes. Surprisingly, Hac1p is found in UPR-activated cells only, and its level is controlled by regulated splicing of its mRNA. Splicing replaces the C-terminal tail of Hac1p with a different peptide that renders Hac1p more resistant to an otherwise extremely rapid ubiquitin-dependent degradation. We propose that the complex regulation of Hac1p expression serves to provide multiple levels at which the UPR can be controlled.

Introduction

The unfolded protein response (UPR) pathway allows eukaryotic cells to respond to changing conditions in the endoplasmic reticulum (ER) by regulating the synthesis of ER-resident proteins. The accumulation of unfolded proteins in the ER leads to increased transcription of genes encoding ER-localized chaperones that assist in protein folding (Kozutsumi et al., 1988; Lee, 1987). The ER and nucleus are distinct membrane-bounded compartments of the cell. The unfolded protein signal, therefore, must be sensed in the lumen of the ER, be transferred across a membrane (either the ER or the inner nuclear membrane with which it is contiguous), and be received by the transcriptional apparatus in the nucleus, where it modulates gene expression (reviewed in Shamu et al., 1994; McMillan et al., 1994).

Although present in all eukaryotic cells examined, the UPR is best understood in the yeast *Saccharomyces cerevisiae*. Experimentally, the UPR can be activated in yeast by preventing glycosylation, e.g., by treating cells with tunicamycin (Tm) or by preventing disulfide-bond formation with reducing agents such as 2-mercaptoethanol. Genes known to be up-regulated by such treatments include *KAR2* (encoding Kar2p or BiP), *PDI1* (encoding protein disulfide isomerase), *EUG1* (encoding a PDI-like protein), and *FKB2* (encoding a peptidyl-prolyl *cis*-trans isomerase). These genes encode proteins that help catalyze the correct folding of proteins that transit through the ER (reviewed by Gething and Sambrook, 1992; Shamu et al., 1994; Sweet, 1993).

To date, only two components of the UPR have been identified. One of these is the transmembrane kinase

Ire1p, encoded by the nonessential gene *IRE1*. Cells in which *IRE1* has been deleted do not increase transcription of ER-resident proteins in response to induction of the UPR (Cox et al., 1993; Mori et al., 1993). Ire1p is located in the ER or inner nuclear membrane or both and is likely to be the component that transmits the unfolded protein signal across the membrane. Its N-terminal domain lies in the lumen of the ER, where it presumably detects the accumulation of unfolded proteins, and its kinase domain lies in the cytoplasm (or nucleus), where it is presumably responsible for transmitting the unfolded protein signal to the appropriate downstream component or components. Ire1p, like most receptor transmembrane kinases, is thought to be activated by oligomerization and phosphorylation by neighboring Ire1p molecules (Shamu and Walter, 1996).

Interestingly, *ire1* mutants do not grow in the absence of inositol in the growth medium (Nikawa and Yamashita, 1992). Models to explain the link between inositol metabolism and the UPR suggest the intriguing possibility that the *de novo* synthesis of phospholipids and the up-regulation of ER content proteins are linked (Cox et al., 1993; Mori et al., 1993; J. S. C., R. Chapman, and P. W., unpublished data). Thus, the ER membrane may coordinately expand to accommodate an increase in ER content proteins. The unfolded protein response may therefore be more than a stress response pathway; it may execute, or be intimately linked to, a cellular program or programs that allow cells to maintain the appropriate abundance of ER during homeostasis (Nunnari and Walter, 1996).

A second known component of the pathway is the unfolded protein response element (UPRE), a 22 bp sequence present in the promoters of genes that are activated by the UPR. When situated within a heterologous promoter, the UPRE is sufficient to activate transcription in response to the accumulation of unfolded proteins (Kohn et al., 1993; Mori et al., 1992). Mutational analysis has defined the nucleotides within the UPRE that are essential for its ability to activate transcription (Mori et al., 1992).

The putative UPRE binding factor that is responsible for altering gene expression has proven elusive. Gel-shift experiments have identified an activity present in cell extracts that binds specifically to a UPRE probe, regardless of the activity of the UPR (Mori et al., 1992). However, the gene or genes encoding this factor have not been identified, and its participation in the UPR remains unclear. Furthermore, genetic screens to isolate mutants incapable of activating the UPR have isolated different alleles of *IRE1* yet have not identified a gene encoding a DNA-binding protein.

We describe here the identification of a transcription factor, Hac1p, that regulates the UPR by binding to the UPRE only when the signaling pathway is activated. Our studies reveal a complex and unusual regulation of Hac1p activity that distinguishes the UPR from other known signaling pathways.

1998: Boulianne and Parkes

Fruit flies that knock down ROS live 34% longer than controls.

The 1998 paper "Extension of *Drosophila* lifespan by overexpression of human SOD1 in motorneurons" by Boulianne and investigates the role of oxidative stress in aging by overexpressing the human gene for superoxide dismutase 1 (SOD1), an enzyme that neutralizes superoxide radicals, in the motorneurons of fruit flies (*Drosophila melanogaster*).

The researchers hypothesized that reducing oxidative damage in neurons, which are particularly vulnerable to oxidative stress, could extend lifespan. They found that flies overexpressing SOD1 in motor neurons exhibited a significant extension of lifespan, providing strong evidence for the role of oxidative stress in aging and highlighting the importance of neuronal health in determining overall longevity.

The study demonstrated that targeted overexpression of SOD1 in motor neurons led to a 33% increase in median lifespan and a 40% increase in maximum lifespan compared to control flies. This finding suggested that reducing oxidative damage specifically in neurons could have a profound impact on aging, likely because neurons are long-lived cells that are critical for maintaining organismal function. The results supported the free radical theory of aging, which posits that the accumulation of oxidative damage over time is a major driver of aging.

Importantly, the study also underscored the tissue-specific nature of aging processes, as the lifespan extension was achieved without widespread overexpression of SOD1 throughout the body. This work paved the way for further research into the role of oxidative stress in aging and the potential for targeted antioxidant interventions to promote healthy aging.

Extension of *Drosophila* lifespan by overexpression of human SOD1 in motorneurons

Tony L. Parkes¹, Andrew J. Elia², Dale Dickinson¹, Arthur J. Hilliker¹, John P. Phillips¹ & Gabrielle L. Boulianne²

Reactive oxygen (RO) has been identified as an important effector in ageing and lifespan determination¹⁻³. The specific cell types, however, in which oxidative damage acts to limit lifespan of the whole organism have not been explicitly identified. The association between mutations in the gene encoding the oxygen radical metabolizing enzyme CuZn superoxide dismutase (SOD1) and loss of motorneurons in the brain and spinal cord that occurs in the life-shortening paralytic disease, Familial Amyotrophic Lateral Sclerosis (FALS; ref. 4), suggests that chronic and unrepaired oxidative damage occurring specifically in motor neurons could be a critical causative factor in ageing.

To test this hypothesis, we generated transgenic *Drosophila* which express human SOD1 specifically in adult motorneurons. We show that overexpression of a single gene, SOD1, in a single cell type, the motorneuron, extends normal lifespan by up to 40% and rescues the lifespan of a short-lived *Sod* null mutant. Elevated resistance to oxidative stress suggests that the lifespan extension observed in these flies is due to enhanced RO metabolism. These results show that SOD activity in motorneurons is an important factor in ageing and lifespan determination in *Drosophila*.

Expression of a human SOD1 transgene (HS) in *Drosophila* motorneurons was achieved using the yeast GAL4/UAS system⁵⁻⁷. The D42-GAL4 activator used in these experiments is expressed broadly during embryogenesis but becomes restricted to motorneurons and interneurons within the larval nervous system, and with the exception of a few unidentified neurons within the central brain, is restricted to motorneurons in the adult nervous system. The HS transgene consists of a human SOD1 cDNA coupled to a yeast UAS element within a *Drosophila* P-transformation vector. Two independent UAS-HS transgenic lines, designated HS1 and HS2, were used. Because lifespan is strongly affected by variation in genetic background, a series of genetic

schemes was employed to introduce the D42-GAL4 and UAS-HS transgenes into uniform *Sod*^{+/+} or *Sod*^{-/-} genetic backgrounds, and to construct expressing and non-expressing strains that were essentially co-isogenic.

In situ hybridization shows that expression of the HS transgene is limited to adult motorneurons, including a set of five bilaterally symmetrical motorneurons that control flight muscles in *Drosophila* (Fig. 1). Similar results were obtained for whole-mount preparations of *Drosophila* larvae (data not shown). To confirm that functional human SOD protein was expressed in these cells, whole fly extracts were analysed by immunoblot and SOD activity assays (Fig. 2). Using an antibody to the human protein that does not cross-react with *Drosophila* Sod, substantial levels of human SOD were detected in flies arising from a cross between the D42-GAL4 and the UAS-HS lines, while no human SOD was detected in the D42-GAL4 line or in either of the two UAS-HS lines. Assay of SOD activity in GAL4-UASHS transgenic flies that are also homozygous for a *Sod* null mutation demonstrates that the transgenic HS is enzymatically active.

To determine the consequences of SOD1 overexpression in motorneurons on normal longevity, we genetically introduced

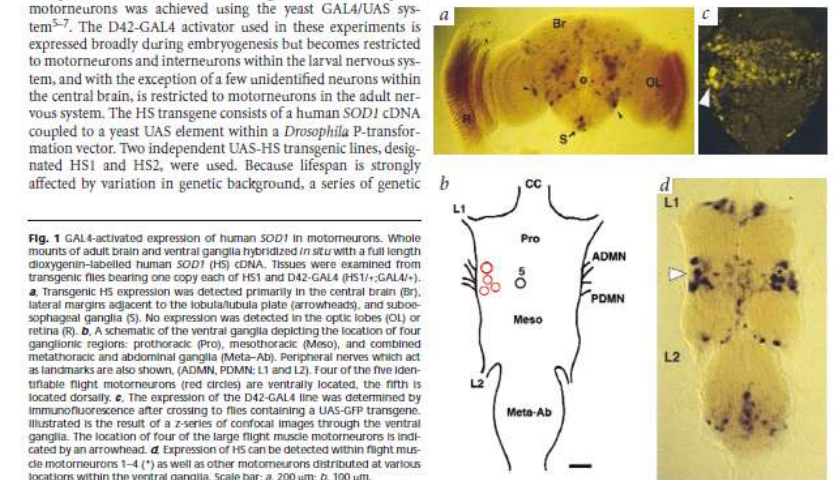


Fig. 1 GAL4-activated expression of human SOD1 in motorneurons. Whole mounts of adult brain and ventral ganglia hybridized *in situ* with a full length dioxygenin-labelled human SOD1 (HS) cDNA. Tissues were examined from transgenic flies bearing one copy each of HS1 and D42-GAL4 (HS1⁺;GAL4⁺). **a**, Transgenic HS expression was detected primarily in the central brain (Br), lateral margins adjacent to the lobula/tubula plate (arrowheads), and suboesophageal ganglia (S). No expression was detected in the optic lobes (OL) or retina (R). **b**, A schematic of the ventral ganglia depicting the location of four ganglionic regions: prothoracic (Pro), mesothoracic (Meso), and combined metathoracic and abdominal ganglia (Meta-Ab). Peripheral nerves which act as landmarks are also shown. (ADMN, PMDN; L1 and L2). Four of the five identifiable flight motorneurons (red circles) are ventrally located, the fifth is located dorsally. **c**, The expression of the D42-GAL4 line was determined by immunofluorescence after crossing to flies containing a UAS-GFP transgene. Illustrated is the result of a z-series of confocal images through the ventral ganglia. The location of four of the large flight muscle motorneurons is indicated by an arrowhead. **d**, Expression of HS can be detected within flight muscle motorneurons 1-4 (*) as well as other motorneurons distributed at various locations within the ventral ganglia. Scale bar: a, 200 μ m; b, 100 μ m.

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2000: Ron and Harding

This paper identified PERK as a key sensor of endoplasmic reticulum stress, which is closely linked to oxidative stress and the unfolded protein response. This work laid the foundation for understanding how the integrated stress response (ISR) integrates various stress signals to regulate cellular homeostasis.

In their landmark 2000 paper, "Perk is essential for translational regulation and cell survival during the unfolded protein response," published in *Molecular Cell*, Heather Harding and colleagues identified PERK (PKR-like ER kinase) as a critical sensor and mediator of the unfolded protein response (UPR), a cellular stress response related to endoplasmic reticulum (ER) dysfunction.

The study demonstrated that PERK is activated in response to the accumulation of misfolded proteins in the ER, a condition that can arise from oxidative stress, nutrient deprivation, or other cellular stressors. Upon activation, PERK phosphorylates the eukaryotic initiation factor 2 α (eIF2 α), which reduces global protein synthesis to alleviate the burden on the ER. However, this phosphorylation also selectively promotes the translation of specific stress-responsive genes, such as the transcription factor ATF4, which activates pathways to restore ER homeostasis. This work established PERK as a central player in the UPR, linking ER stress to translational control and cell survival. The study further revealed that PERK-deficient cells were unable to attenuate protein synthesis under ER stress, leading to an overload of misfolded proteins and increased cell death. This highlighted the essential role of PERK in maintaining cellular viability during stress conditions. Harding et al. provided a foundational understanding of how cells adapt to ER stress and integrate stress responses to promote survival. This paper was pivotal in defining the Integrated Stress Response (ISR), as it connected PERK's function to broader cellular stress pathways, including those involving oxidative stress.

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Perk Is Essential for Translational Regulation and Cell Survival during the Unfolded Protein Response

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Summary

Misfolded proteins in the endoplasmic reticulum (ER) inhibit translation initiation. This response is believed to be mediated by increased phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) and is hypothesized to reduce the work load imposed on the folding machinery during stress. Here we report that mutating the gene encoding the ER stress-activated eIF2 α kinase PERK abolishes the phosphorylation of eIF2 α in response to accumulation of misfolded proteins in the ER resulting in abnormally elevated protein synthesis and higher levels of ER stress. Mutant cells are markedly impaired in their ability to survive ER stress and inhibition of protein synthesis by cycloheximide treatment during ER stress ameliorates this impairment. PERK thus plays a major role in the ability of cells to adapt to ER stress.

Introduction

To attain their proper three-dimensional structure, secreted, membrane-bound, and resident proteins of the exocytic compartment must fold in the lumen of the endoplasmic reticulum (ER). This process is often perturbed when cells are deprived of essential nutrients or exposed to toxins or as a consequence of mutations in the synthesized proteins themselves (Lee, 1992). Conditions that impair protein folding in the ER have a significant adverse effect on cellular survival. However, little is known about the mechanisms involved. At least two functionally distinct signaling pathways have evolved to help maintain a proper folding environment in the ER. The first well-characterized component of the unfolded protein response (UPR) mediates the transcriptional induction of genes encoding ER chaperones, disulfide exchange proteins, and prolyl-peptidyl isomerases (Kozutsumi et al., 1988; Gething and Sambrook, 1992). In yeast, signaling in this pathway is initiated by the product of the *IRE1* gene, an ER resident transmembrane stress receptor that responds to a luminal signal correlating with the accumulation of misfolded proteins (Cox et al., 1993; Mori et al., 1993).

A second arm of the UPR consists of a profound attenuation of translation initiation (reviewed in Brostrom and Brostrom, 1998). This correlates with enhanced phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2 α) (Prostko et al.,

1993). Phosphorylated eIF2 α (eIF2 α -P) binds the guanine nucleotide exchange factor eIF2B with high affinity interfering with the assembly of a 43S translation initiation complex (Clemens, 1996). It is believed that reduced protein synthesis rates during ER stress serve to reduce the load of substrates presented to the folding machinery in the ER lumen. However, the homeostatic role of this response has not been tested experimentally.

Recently, an ER resident transmembrane protein kinase known as PERK (or PEK; Shi et al., 1998) has been shown to be activated by ER stress (Harding et al., 1999). PERK phosphorylates eIF2 α in vitro and, when overexpressed in cells, attenuates protein synthesis (Shi et al., 1998; Harding et al., 1999). PERK is highly likely to participate in coupling ER stress to translation inhibition because its luminal domain is related in sequence to that of IRE1, suggesting that these two arms of the UPR are activated by the same signal(s).

Here we report on the phenotypic consequences of an induced recessive mutation in mouse *Perk*. Our data prove that *Perk* is required for both the phosphorylation of eIF2 α and the attenuation of translation that occurs in response to ER stress. Furthermore, reduced survival of *Perk*^{-/-} cells, when exposed to agents that cause ER stress, suggests that in this physiological context reduced translation is an adaptive response.

Results

The *Perk* gene was targeted in mouse embryonic stem cells. The mutation replaces the transmembrane domain by insertion of a *Neo*-expressing gene (Figure 1A). Homozygosity for the mutant allele was attained with high frequency by culturing *Perk*^{+/-} ES cells in the presence of high concentrations of the selection agent, G418 (Figure 1B). Cells carrying the homozygous mutation expressed 10-fold less *Perk* mRNA than the wild-type cells (Figure 1C). The predicted mutant gene product is either a secreted or truncated protein that could not be properly targeted to the ER membrane. Western blot analysis of detergent lysates from *Perk*^{-/-} cells reveals the absence of PERK protein, whereas the expression of IRE1 α is unaffected in these cells (Figure 1D).

When exposed to agents that induce ER stress, wild-type cells exhibit a profound attenuation in global translation rates. This is reflected in the reduced incorporation of [³⁵S]methionine/cysteine into cellular proteins following a short labeling pulse in the presence of ER stress-inducing toxins (Figure 2A). By contrast, *Perk*^{-/-} cells do not reduce translation rates when exposed to either thapsigargin, tunicamycin, or dithiothreitol, agents that have been shown to activate PERK in wild-type cells (Harding et al., 1999). Rather, when exposed to agents that cause ER stress, the mutant cells reproducibly exhibited an increase in content of ³⁵S-labeled cellular proteins. This small increase may be due to a decrease in secretion that occurs during ER stress. Arsenite, a stress-causing agent that induces phosphorylation of eIF2 α and attenuates translation (Brostrom

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Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D. Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol Cell*. 2000 May;5(5):897-904.

2000: Guarente and Imai

The researchers demonstrated that Sir2, previously known for its role in transcriptional silencing and longevity, functions as an NAD⁺-dependent histone deacetylase.

The heavily cited 2000 study by Imai and Guarente, titled "Transcriptional Silencing and Longevity Protein Sir2 is an NAD-Dependent Histone Deacetylase", published in *Nature*, provides a groundbreaking discovery about the molecular mechanism of the yeast Sir2 protein, linking it to both gene silencing and lifespan regulation.

The researchers demonstrated that Sir2, previously known for its role in transcriptional silencing and longevity, functions as an NAD⁺-dependent histone deacetylase. This enzymatic activity requires NAD⁺ as a cofactor, connecting Sir2's function to cellular energy status. By deacetylating histones, Sir2 promotes chromatin condensation and gene silencing, particularly at regions such as telomeres, ribosomal DNA (rDNA), and silent mating-type loci. The study also showed that Sir2's deacetylase activity is essential for its role in extending lifespan, as mutations that disrupt this activity abolish its ability to promote longevity. This finding established a direct link between metabolism (via NAD⁺ levels) and epigenetic regulation of aging.

The study's findings positioned Sir2 as a central player in the regulation of lifespan, providing a mechanistic explanation for how calorie restriction, which increases NAD⁺ levels, extends lifespan in yeast. By linking Sir2's activity to NAD⁺, the authors proposed that Sir2 acts as a metabolic sensor, translating changes in cellular energy status into changes in gene expression and chromatin structure. This work laid the foundation for understanding the conserved role of sirtuins (the mammalian homologs of Sir2) in aging and metabolism across species.

Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase

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Yeast Sir2 is a heterochromatin component that silences transcription at silent mating loci¹, telomeres² and the ribosomal DNA^{3,4}, and that also suppresses recombination in the rDNA⁵ and extends replicative life span⁶. Mutational studies indicate that lysine 16 in the amino-terminal tail of histone H4 and lysines 9, 14 and 18 in H3 are critically important in silencing, whereas lysines 5, 8 and 12 of H4 have more redundant functions⁷⁻⁹. Lysines 9 and 14 of histone H3 and lysines 5, 8 and 16 of H4 are acetylated in active chromatin and hypoacetylated in silenced chromatin, and overexpression of Sir2 promotes global deacetylation of histones^{8,10}, indicating that Sir2 may be a histone deacetylase. Deacetylation of lysine 16 of H4 is necessary for binding the silencing protein, Sir3 (ref. 8). Here we show that yeast and mouse Sir2 proteins are nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases, which deacetylate lysines 9 and 14 of H3 and specifically lysine 16 of H4. Our analysis of two *SIR2* mutations supports the idea that this deacetylase activity accounts for silencing, recombination suppression and extension of life span *in vivo*. These findings provide a molecular framework of NAD-dependent histone deacetylation that connects metabolism, genomic silencing and ageing in yeast and, perhaps, in higher eukaryotes.

Sir2 is a limiting component that promotes longevity in yeast mother cells. Cells lacking Sir2 have a reduced replicative life span and cells with an extra copy of *SIR2* display a much longer life span than wild type⁶. This extension probably results from a hypersilencing in the rDNA which reduces recombination and the production of extrachromosomal rDNA circles, a known cause of senescence in ageing mother cells¹¹. *SIR2* homologues have been identified in many organisms ranging from bacteria to humans¹². The *Salmonella* homologue, *cobB*, has been implicated in a pyrimidine transfer reaction¹³ and both CobB and eukaryotic Sir2 proteins possess ADP-ribosyltransferase activity^{14,15}.

Because Sir2 proteins use NAD as a substrate in a ADP-ribosylation reaction, we examined whether NAD could be a co-factor necessary for deacetylase activity. We used purified recombinant Sir2 in a reaction with NAD and a peptide of the histone H3 N-terminal tail (residues 1–20) di-acetylated at lysines 9 and 14. We reacted 5 µg of recombinant yeast Sir2 (79 pmoles) (Fig. 1a), 10 µg of the H3 peptide (4.2 nmoles) and increasing concentrations of NAD and analysed the products by high-pressure liquid chromatography (HPLC). The peptide that reacted in the absence of NAD gave

Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature*. 2000 Feb 17;403(6771):795-800.

2002: Tower and Sun

Overexpression of superoxide dismutase extends fruity fly life.

The 2002 study by Sun et al., published in *Genetics*, investigates the effects of overexpressing mitochondrial Mn-superoxide dismutase (MnSOD) on the lifespan of *Drosophila melanogaster* (fruit flies). Using a transgenic "FLP-out" system, the researchers induced MnSOD overexpression in adult flies through a brief heat pulse, ensuring that control and experimental flies had identical genetic backgrounds. The study found that MnSOD overexpression increased enzyme levels by up to 75%, leading to proportional increases in lifespan. Mean lifespan increased by an average of 16%, with some lines showing 30-33% increases, while maximum lifespan increased by an average of 15%, with one line showing a 37% increase.

Interestingly, simultaneous overexpression of catalase with MnSOD did not further extend lifespan, suggesting that catalase is already present in sufficient quantities in flies. Overexpression of Cu/ZnSOD also extended lifespan, indicating that reducing oxidative stress through SOD enzymes is a key factor in longevity. Importantly, the lifespan extension was not associated with reduced metabolic activity, as measured by oxygen consumption.

This study provides strong evidence supporting the free radical theory of aging, which posits that oxidative damage contributes to aging. By demonstrating that increased MnSOD activity reduces oxidative stress and extends lifespan without compromising metabolic activity, the authors highlight the importance of mitochondrial antioxidant defenses in aging. The findings also suggest that MnSOD and Cu/ZnSOD play distinct but complementary roles in mitigating oxidative damage. The study's use of the FLP-out system allowed precise temporal control of gene expression, offering a valuable tool for aging research. Overall, this work underscores the potential of targeting oxidative stress pathways to promote healthy aging and longevity.

Induced Overexpression of Mitochondrial Mn-Superoxide Dismutase Extends the Life Span of Adult *Drosophila melanogaster*

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ABSTRACT

A transgenic system ("FLP-out") based on yeast FLP recombinase allowed induced overexpression of MnSOD enzyme in adult *Drosophila melanogaster*. With FLP-out a brief heat pulse (HP) of young, adult flies triggered the rearrangement and subsequent expression of a MnSOD transgene throughout the adult life span. Control (no HP) and overexpressing (HP) flies had identical genetic backgrounds. The amount of MnSOD enzyme overexpression achieved varied among six independent transgenic lines, with increases up to 75%. Life span was increased in proportion to the increase in enzyme. Mean life span was increased by an average of 16%, with some lines showing 30–33% increases. Maximum life span was increased by an average of 15%, with one line showing as much as 37% increase. Simultaneous overexpression of catalase with MnSOD had no added benefit, consistent with previous observations that catalase is present in excess in the adult fly with regard to life span. Cu/ZnSOD overexpression also increases mean and maximum life span. For both MnSOD and Cu/ZnSOD lines, increased life span was not associated with decreased metabolic activity, as measured by O₂ consumption.

CURRENT theory suggests that aging results from the decreased force of natural selection acting on older individuals (ROSE 1991; PARTRIDGE and BARTON 1993; CHARLESWORTH 1994; KIRKWOOD 1995; KIRKWOOD and AUSTAD 2000). Reduction in the force of natural selection with age allows each generation to inherit mutations that have deleterious effects at late ages. The accumulation of such mutations in the germ line results in reduced fitness of old organisms, including an imbalance between damage and repair and a failure to maintain somatic tissue structure and function.

Reactive oxygen species (ROS) are produced as a toxic byproduct of normal cellular metabolism, and oxidative damage is hypothesized to be one cause of aging in metazoans (HARMAN 1956; STADTMAN 1992; MARTIN *et al.* 1996; SOHAL and WEINDRUCH 1996; WALLACE 1999; FINKEL and HOLBROOK 2000). The enzymes catalase and superoxide dismutase (SOD) are major defenses against ROS in all cells. SOD exists in two forms inside the eukaryotic cell, Cu/ZnSOD primarily in the cytoplasm and MnSOD in the mitochondria. The major ROS produced by the cell is mitochondrial superoxide, which is converted by SOD to H₂O₂. Catalase converts H₂O₂ to molecular oxygen and water. These and other cellular defenses against ROS are not completely effi-

cient. Oxidatively damaged macromolecules accumulate in every aging organism examined, and oxidative damage is implicated in the etiology of most human aging-related diseases.

Numerous studies demonstrate the correlation between aging and oxidative damage in *Drosophila*. In *Drosophila*, aging has been found to correlate with increased levels of protein carbonyls and 8-oxo-Guanine in the DNA (ORR and SOHAL 1994; SOHAL *et al.* 1995). *Drosophila* lacking either catalase or Cu/ZnSOD have reduced life span (MACKAY and BEWLEY 1989; PHILLIPS *et al.* 1989; ORR *et al.* 1992; GRISWOLD *et al.* 1993). Moreover, the stress response protein hsp70 is induced in muscle tissue during *Drosophila* aging and this expression is increased or accelerated in *catalase* and *Cu/ZnSOD* null mutant flies (WHEELER *et al.* 1995, 1999). Finally, genetic selection of *Drosophila* populations for increased life span in the laboratory correlates with increased expression of oxidative stress resistance enzymes and increased oxidative stress resistance (ARKING *et al.* 1991, 2000).

One way to directly test the hypothesis that oxidative damage causes aging is to engineer transgenic organisms with increased oxidative stress defenses and determine the effects on life span. In pioneering experiments *Drosophila* were created that contained *catalase* and *Cu/ZnSOD* transgenes under the control of their native promoters or a constitutive heterologous promoter (SETO *et al.* 1990; REVEILLAUD *et al.* 1991; ORR and SOHAL 1992, 1993, 1994; GRISWOLD *et al.* 1993; SOHAL *et al.* 1995). While in certain cases increased stress resistance and life span were reported, current interpretation of

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2002: Dillin and Kenyon

Mitochondrial activity during development in *C. elegans* establishes lifelong rates of respiration, behavior and aging, which persist independently of mitochondrial function in adulthood, highlighting the critical role of early-life mitochondrial function.

The 2002 paper by Dillin, Kenyon and colleagues, published in *Science*, investigates the role of mitochondrial function in development and its long-term effects on behavior and aging in *Caenorhabditis elegans* (nematodes). The researchers used RNA interference (RNAi) to reduce the activity of the electron transport chain and ATP synthase during development, which resulted in smaller body size, slower behavioral rates, and extended adult lifespan.

Importantly, restoring normal levels of mitochondrial messenger RNA (mRNA) during adulthood did not reverse these effects, as ATP levels remained low, and the phenotypes persisted. Conversely, inhibiting mitochondrial function only during adulthood did not alter behavioral rates or lifespan.

These findings suggest that mitochondrial activity during development establishes a regulatory system that sets lifelong rates of respiration, behavior, and aging, which are maintained independently of mitochondrial function in adulthood.

The study highlights the critical role of early-life mitochondrial function in shaping adult physiology and aging. It proposes that the developing organism uses mitochondrial activity as a "gauge" to determine metabolic and behavioral rates, which are then fixed for the remainder of the organism's life.

Rates of Behavior and Aging Specified by Mitochondrial Function During Development

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To explore the role of mitochondrial activity in the aging process, we have lowered the activity of the electron transport chain and adenosine 5'-triphosphate (ATP) synthase with RNA interference (RNAi) in *Caenorhabditis elegans*. These perturbations reduced body size and behavioral rates and extended adult life-span. Restoring messenger RNA to near-normal levels during adulthood did not elevate ATP levels and did not correct any of these phenotypes. Conversely, inhibiting respiratory-chain components during adulthood only did not reset behavioral rates and did not affect life-span. Thus, the developing animal appears to contain a regulatory system that monitors mitochondrial activity early in life and, in response, establishes rates of respiration, behavior, and aging that persist during adulthood.

During a systematic screen of a *C. elegans* chromosome I RNAi library (1, 2), we found that animals grown on bacteria expressing double-stranded RNA (dsRNA) encoding a component of the mitochondrial ATP synthase (*atp-3*) lived much longer than normal (3) (Fig. 1A, table S1). RNAi of three genes encoding respiratory-chain components also extended life-span: *nuc-2*, which encodes a component of complex I (NADH/ubiquinone oxidoreductase); *cyc-1*, which encodes a component of complex III (cytochrome c reductase); and *cco-1*, which encodes a component of complex IV (cytochrome c oxidase) (Fig. 1A, table S1) (3). Treatment of wild-type animals with antimycin A, which inhibits complex III (4), increased life-span as well (3) (Fig. 1D, table S1).

RNAi of respiratory-chain components also

affected growth and behavior. The animals were smaller than normal. They were well proportioned (fig. S1, table S2) and did not have an obvious decrease in cell number. We counted cells in two postembryonic lineages, the vulval and epidermal seam cell lineages (5), and found that cell number was normal (3). Thus, in these tissues (and likely in others as well), a metabolite whose level is regulated by mitochondrial respiration acts as a signal to control cell size. Small body size itself is unlikely to extend life-span, because mutants defective in *daf-4*, which encodes a transforming growth factor- β type II receptor (6), are small but not long-lived (7). Respiratory-chain RNAi also decreased the rate of growth to adulthood, as well as the rates of pharyngeal pumping (eating) and defecation (table S2). In addition, the animals moved more slowly than normal (table S2).

Inhibition of a component of respiratory chain complex III, *isp-1*, in *C. elegans* has been shown to reduce oxygen consumption (8). Inhibiting respiratory-chain components and ATP synthase would also be predicted to reduce ATP levels. We found that ATP levels were reduced 60 to 80% in animals subjected to *cyc-1* (complex III) or *atp-3* (ATP synthase) RNAi and 40 to 60% in animals treated with *nuc-2* (complex I) or *cco-1* (complex IV) RNAi (3) (Fig. 2).⁶ These findings, and the fact that all these proteins are known to function together in the processes of respiration and ATP production, suggest that reducing the rates of respira-

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Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C. Rates of behavior and aging specified by mitochondrial function during development. *Science*. Dec 20, 2002;298(5602):2398-401

2003: Ron and Harding

This paper further elucidated the role of the integrated stress response (ISR) in coordinating cellular responses to oxidative stress. This work suggested that the ISR is a key mechanism for combating age-related oxidative damage.

In their 2003 paper, "An integrated stress response regulates amino acid metabolism and resistance to oxidative stress," published in *Molecular Cell*, Heather Harding and David Ron expanded on the concept of the Integrated Stress Response (ISR) by demonstrating its role in coordinating cellular adaptation to stress, particularly oxidative stress, through the regulation of amino acid metabolism.

The study showed that the phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) by stress-activated kinases, such as PERK, leads to the selective translation of the transcription factor ATF4. ATF4, in turn, activates genes involved in amino acid synthesis, transport, and metabolism, which are critical for maintaining cellular homeostasis under stress conditions. This work revealed that the ISR not only reduces global protein synthesis to conserve resources but also reprograms cellular metabolism to enhance survival during oxidative stress and other challenges.

The authors further demonstrated that the ISR's regulation of amino acid metabolism is essential for protecting cells against oxidative damage. By inducing ATF4 and its downstream targets, the ISR helps replenish critical amino acids like glutathione precursors, which are vital for antioxidant defense. Harding et al. showed that cells lacking functional ISR pathways were more susceptible to oxidative stress, underscoring the importance of this response in maintaining redox balance and cellular viability. This paper provided a deeper understanding of how the ISR integrates multiple stress signals to regulate metabolic pathways, highlighting its role in promoting resistance to oxidative stress and supporting overall cellular resilience.

An Integrated Stress Response Regulates Amino Acid Metabolism and Resistance to Oxidative Stress

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Summary

Eukaryotic cells respond to unfolded proteins in their endoplasmic reticulum (ER stress), amino acid starvation, or oxidants by phosphorylating the α subunit of translation initiation factor 2 (eIF2 α). This adaptation inhibits general protein synthesis while promoting translation and expression of the transcription factor ATF4. ATF4^{-/-} cells are impaired in expressing genes involved in amino acid import, glutathione biosynthesis, and resistance to oxidative stress. Perk^{-/-} cells, lacking an upstream ER stress-activated eIF2 α kinase that activates ATF4, accumulate endogenous peroxides during ER stress, whereas interference with the ER oxidase ERO1 abrogates such accumulation. A signaling pathway initiated by eIF2 α phosphorylation protects cells against metabolic consequences of ER oxidation by promoting the linked processes of amino acid sufficiency and resistance to oxidative stress.

Introduction

The reversible phosphorylation of serine 51 on the α subunit of eukaryotic translation initiation factor 2 (eIF2 α) is a highly conserved adaptation to stress. Phosphorylation results in reduced activity of the eIF2 complex, reduced rates of translation initiation, and reduced protein synthesis (Hinnebusch, 2000; Dever, 2002). Four mammalian protein kinases are known to couple distinct upstream stress signals to eIF2 α phosphorylation. Among these, GCN2 is activated by uncharged tRNAs (Hinnebusch, 2000) and PERK is activated by client protein load in the endoplasmic reticulum (ER stress) (Ron and Harding, 2000). Furthermore, eIF2 α phosphorylation increases in cells exposed to oxidants or reactive metals,

though the responsible kinase, if one exists, has not been identified (Brostrom et al., 1996). Phosphorylation of eIF2 α thus integrates signaling downstream of several otherwise unrelated stresses.

Stress conditions leading to eIF2 α phosphorylation share the potential for accumulation of misfolded or modified proteins, for which the eIF2 α phosphorylation-mediated inhibition of protein synthesis appears an appropriate response. Consequently, Perk^{-/-} cells are profoundly sensitive to pharmacological agents that perturb protein folding in the ER (Harding et al., 2000b) and mice and humans lacking PERK activity experience progressive destruction of secretory cells with physiologically high levels of ER stress (Delepine et al., 2000; Harding et al., 2001; Zhang et al., 2002). Furthermore, PERK signaling and signaling by the parallel, IRE1-mediated component of the ER unfolded protein response (UPR) exist in a state of partial redundancy (Shen et al., 2001), consistent with PERK's role in reducing ER client protein load (Harding et al., 2000b). However, protein synthesis inhibitors only partially restore ER stress resistance to Perk^{-/-} cells (Harding et al., 2000b), suggesting that PERK controls more than ER client protein load.

In yeast, eIF2 α phosphorylation controls a gene expression program that adapts the organism to amino acid starvation. Gcn4p, a transcription factor whose translation is upregulated under condition of eIF2 α phosphorylation, mediates signaling in this general control response (Hinnebusch, 1993). The mammalian transcription factor ATF4 is similarly regulated. Expression of ATF4 during ER stress is dependent both on PERK activity and on eIF2 α phosphorylation, as Perk^{-/-} cells, eIF2 α ^{SH-ATF4} knock-in cells (that cannot be phosphorylated by eIF2 α kinases), and cells overexpressing an eIF2 α phosphatase are all impaired in activating ATF4 during ER stress (Harding et al., 2000a; Novoa et al., 2001; Scheuner et al., 2001). Furthermore, other stresses that result in eIF2 α phosphorylation, such as amino acid deprivation and exposure to oxidants or reactive metals, also activate ATF4 (Fawcett et al., 1999; Harding et al., 2000a). Together, these findings suggest that ATF4 may be a common downstream component that integrates signaling by stress-induced eIF2 α kinases.

Here, we report on an ATF4-mediated integrated stress response initiated by eIF2 α phosphorylation that insures supply of amino acids for protein and glutathione biosynthesis and protects cells against oxidative stress. Activation of this integrated stress response is shown to have a special role adapting cells to the metabolic consequences of ER activity.

Results

ATF4 and PERK Upregulate Genes Involved in Amino Acid Import and Metabolism and Resistance to Oxidative Stress

In wild-type mouse fibroblasts, ER stress led to rapid and sustained accumulation of ATF4 protein, which was

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Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calton M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell*. 2003 Mar;11(3):619-33. 220

2009: Harrison and Miller

Rapamycin can extend their median and maximal lifespan of mice by up to 14%. This life extension was observed in mice fed rapamycin from 270 days of age and also at a late stage in their life, from age 600 days. These findings point to the TOR pathway as a critical point in the control of ageing in mammals and in the pathogenesis of late-life illnesses.

The 2009 paper by Harrison et al., published in *Nature*, was a landmark study demonstrating that the drug rapamycin can significantly extend lifespan even when administered late in life. Using genetically heterogeneous UM-HET3 mice to ensure broad applicability, the researchers began feeding rapamycin to mice at 600 days of age (roughly 20 months), equivalent to about 60 years in humans. The treatment resulted in a significant increase in both median and maximal lifespan in both male and female mice. Importantly, this intervention occurred after most of the mice had already reached old age, showing that aging can be modulated even late in life, which had not been robustly demonstrated in mammals before.

The study was conducted across three independent testing sites as part of the NIH Interventions Testing Program (ITP), lending strong credibility and reproducibility to the findings. The mice were given encapsulated rapamycin in their chow at 14 parts per million (ppm), a form designed to improve bioavailability and stability. Lifespan extension was observed consistently across the sites, and the statistical analyses showed highly significant results. The findings positioned rapamycin as the first pharmacological agent to robustly extend lifespan in mammals when treatment is started in later life, opening up a new avenue for anti-aging therapeutics and sparking a wave of research into the mTOR signaling pathway as a target for longevity interventions.

LETTERS

Rapamycin fed late in life extends lifespan in genetically heterogeneous mice

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Inhibition of the TOR signalling pathway by genetic or pharmacological intervention extends lifespan in invertebrates, including yeast, nematodes and fruitflies^{1–3}; however, whether inhibition of mTOR signalling can extend lifespan in a mammalian species was unknown. Here we report that rapamycin, an inhibitor of the mTOR pathway, extends median and maximal lifespan of both male and female mice when fed beginning at 600 days of age. On the basis of age at 90% mortality, rapamycin led to an increase of 14% for females and 9% for males. The effect was seen at three independent test sites in genetically heterogeneous mice, chosen to avoid genotype-specific effects on disease susceptibility. Disease patterns of rapamycin-treated mice did not differ from those of control mice. In a separate study, rapamycin fed to mice beginning at 270 days of age also increased survival in both males and females, based on an interim analysis conducted near the median survival point. Rapamycin may extend lifespan by postponing death from cancer, by retarding mechanisms of ageing, or both. To our knowledge, these are the first results to demonstrate a role for mTOR signalling in the regulation of mammalian lifespan, as well as pharmacological extension of lifespan in both genders. These findings have implications for further development of interventions targeting mTOR for the treatment and prevention of age-related diseases.

Because incidences of most diseases rise rapidly with age⁴, interventions that delay ageing would greatly benefit health^{5,6}. So far, dietary additives that delay ageing and increase lifespan in rodent models have shown only weak effects^{7–11}. Before clinical studies are considered, anti-ageing interventions must be repeatable and effective in many mouse genotypes, and not merely postpone strain-specific diseases^{12–14}.

The National Institute on Aging Interventions Testing Program (ITP) evaluates agents that may delay ageing and increase lifespan in genetically heterogeneous mice^{15–17}. Agents are chosen as summarized at <http://www.nia.nih.gov/ResearchInformation/ScientificResources/InterventionsTestingProgram.htm>. Studies are simultaneously replicated at three test sites: The Jackson Laboratory (JL), the University of Michigan (UM), and the University of Texas Health Science Center (UTH). BALB/cByJ × C57BL/6J F1 (CB6F1) females and C3H/HeJ × DBA/2J F1 (CD1F1) males are supplied to each site by The Jackson Laboratory, and mated to produce genetically heterogeneous populations in which each animal is genetically unique, but a full

sibbling of all other mice in the population¹⁸. Sufficient mice are used to provide 80% power to detect a 10% increase (or decrease) in mean lifespan with respect to unmanipulated controls of the same sex, even if data from one of the three test sites were to be unavailable. Here we report that dietary encapsulated rapamycin increases mouse survival, including survival to the last decade, a measure of maximal lifespan.

Rapamycin reduces function of the rapamycin target kinase TOR1 and has anti-neoplastic activities; genetic inhibition of TOR1 extends lifespan in short-lived model organisms. In male and female mice at each of three collaborating research sites, median and maximum lifespan were extended by feeding encapsulated rapamycin starting at 600 days of age (Fig. 1). We analysed the data set as of 1 February 2009, with 2% (34 of 1,901) of mice still alive. For data pooled across sites, a log-rank test rejected the null hypothesis that treatment and control groups did not differ ($P < 0.0001$) in both males and females. Expressed as mean lifespan, the effect sizes were 9% for males and 13% for females in the pooled data set. Expressed as life expectancy at 600 days (the age of first exposure to rapamycin), the effect sizes were 26% for males and 38% for females. Mice treated with other agents (enalapril and CAFE (caffeic acid phenethyl ester)) evaluated in parallel did not differ from controls at the doses used (Supplementary Fig. 1).

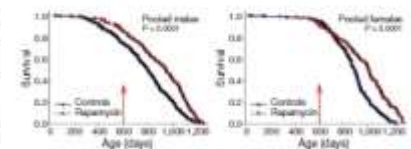


Figure 1 | Survival plots for male and female mice, comparing control mice to those fed rapamycin in the diet starting at 600 days of age, pooling across the three test sites. P values were calculated by the log-rank test. Four per cent of the control mice and three per cent of rapamycin-treated mice were removed from the experiment for technical reasons. Only few animals (three controls, two rapamycin) were removed after the start of rapamycin treatment at 600 days. Thus, there were no significant differences between groups in censoring.

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2013: Johan Auwerx

NAD(+) is an important cofactor regulating metabolic homeostasis and a rate-limiting substrate for sirtuin deacylases. Auwerx et.al. show that NAD(+) levels are reduced in aged mice and *Caenorhabditis elegans* and that decreasing NAD(+) levels results in a further reduction in worm lifespan.

The 2013 study by Mouchiroud et al., titled "The NAD⁺/Sirtuin Pathway Modulates Longevity Through Activation of Mitochondrial UPR and FOXO Signaling", published in *Cell*, investigates how the NAD⁺/sirtuin pathway influences lifespan by regulating mitochondrial function and stress responses. The researchers found that increasing NAD⁺ levels in *Caenorhabditis elegans* (nematodes) activates the sirtuin SIR-2.1, which in turn promotes longevity by enhancing mitochondrial homeostasis and activating the mitochondrial unfolded protein response (UPR^{mt}).

The study demonstrates that the NAD⁺/sirtuin pathway acts upstream of the UPR^{mt}, a protective stress response that maintains mitochondrial function by repairing damaged proteins and improving mitochondrial quality. Activation of this pathway also leads to the upregulation of FOXO transcription factors, which are known to play a critical role in stress resistance and lifespan extension. Together, these mechanisms improve mitochondrial efficiency and reduce the accumulation of damaged mitochondria, thereby promoting longevity. By linking NAD⁺ levels to the activation of the UPR^{mt} and FOXO signaling, the research provides a mechanistic explanation for how boosting NAD⁺ can extend lifespan. The findings also suggest that interventions aimed at increasing NAD⁺ levels, such as calorie restriction or NAD⁺ precursors, could promote healthy aging by enhancing mitochondrial function and stress resistance.

The NAD⁺/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling

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SUMMARY

NAD⁺ is an important cofactor regulating metabolic homeostasis and a rate-limiting substrate for sirtuin deacylases. We show that NAD⁺ levels are reduced in aged mice and *Caenorhabditis elegans* and that decreasing NAD⁺ levels results in a further reduction in worm lifespan. Conversely, genetic or pharmacological restoration of NAD⁺ prevents age-associated metabolic decline and promotes longevity in worms. These effects are dependent upon the protein deacetylase *sir-2.1* and involve the induction of mitochondrial protein imbalance as well as activation of stress signaling via the mitochondrial unfolded protein response (UPR^{mt}) and the nuclear translocation and activation of FOXO transcription factor DAF-16. Our data suggest that augmenting mitochondrial stress signaling through the modulation of NAD⁺ levels may be a target to improve mitochondrial function and prevent or treat age-associated decline.

INTRODUCTION

Alterations in NAD⁺ levels have a powerful metabolic impact because it serves as an obligatory substrate for the deacetylase activity of the sirtuin proteins (Guarente, 2008; Haigis and Sinclair, 2010; Houtkooper et al., 2010a). The best-characterized mammalian sirtuin is SIRT1, which controls mitochondrial function through the deacetylation of targets that include PGC-1 α and FOXO (Chalkiadaki and Guarente, 2012; Houtkooper et al., 2012). The administration of NAD⁺ precursors, such as nicotinamide mononucleotide (Yoshino et al., 2011) or nicotinamide riboside (NR) (Cantó et al., 2012), has proven to be an efficient way to increase NAD⁺ levels and SIRT1 activity, improving metabolic homeostasis in mice.

Furthermore, the NAD⁺-consuming poly(ADP-ribose) polymerase proteins—with PARP1 and PARP2 representing the main PARP activities in mammals—were classically described as DNA repair proteins (Gibson and Kraus, 2012; Schreiber et al., 2006), but recent studies have linked these proteins to metabolism (Asher et al., 2010; Bai et al., 2011a, 2011b; Erener et al., 2012). Indeed, genetic or pharmacological inactivation of PARP1 increased tissue NAD⁺ levels and activated mitochondrial metabolism (Bai et al., 2011b). An association between PARPs and lifespan has been postulated (Grube and Bürkle, 1992; Mangerich et al., 2010), but a causal role remained unclear. A final line of evidence in support of a role for NAD⁺ in metabolic control came from the deletion of an alternative NAD⁺-consuming protein, CD38, which also led to NAD⁺ accumulation and subsequent SIRT1 activation in mice and proved protective against high-fat diet-induced obesity (Barbosa et al., 2007).

Considering the intimate link between metabolism and longevity (Guarente, 2008; Houtkooper et al., 2010b), we hypothesized that increasing NAD⁺ levels may be sufficient to increase mitochondrial activity and extend lifespan (Houtkooper and Auwerx, 2012). Here, we show how supplementation of PARP inhibitors or NAD⁺ precursors led to improved mitochondrial homeostasis through the activation of the worm sirtuin homolog, *sir-2.1*. This improvement involved the disturbed balance between OXPHOS subunits encoded by mitochondrial DNA (mtDNA) and nuclear DNA (nDNA), a state we termed mitochondrial protein imbalance. This associates with the activation of the mitochondrial unfolded protein response (UPR^{mt})—a mitochondrial proteostasis pathway promoting longevity (Durlieux et al., 2011; Yoneda et al., 2004; Zhao et al., 2002)—and subsequent translocation and activation of the FOXO transcription factor *daf-16*—triggering an antioxidant protection program (Honda and Honda, 1999). Together, our results expose a temporal regulation network for sirtuins on eukaryotic lifespan and pharmacological approaches to control this pathway and prevent age-related physiological decline.

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Mouchiroud L, Houtkooper RH, Moullan N, Katsyuba E, Ryu D, Cantó C, Mottis A, Jo YS, Viswanathan M, Schoonjans K, Guarente L, Auwerx J. The NAD⁺/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling. *Cell*, Jul 18, 2013; 154(2):430–41.

2013: David Sinclair

This study highlighted the role of declining NAD⁺ levels in aging and demonstrated that boosting NAD⁺ could restore mitochondrial function and improve healthspan.

The 2013 paper "Declining NAD⁺ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging" by David Sinclair and colleagues., published in *Cell*, explores the role of NAD⁺ (nicotinamide adenine dinucleotide) in aging and mitochondrial dysfunction.

The researchers discovered that NAD⁺ levels decline with age, leading to a breakdown in communication between the nucleus and mitochondria, which is critical for maintaining cellular energy homeostasis. This disruption results in a pseudohypoxic state (a condition mimicking low oxygen levels) even under normal oxygen conditions, impairing mitochondrial function and contributing to age-related metabolic decline. The study identified that the activation of the NAD⁺-dependent deacetylase SIRT1 is compromised in aging cells, leading to reduced mitochondrial biogenesis and function.

Importantly, the authors demonstrated that boosting NAD⁺ levels (e.g., with precursors like nicotinamide riboside, NR) could restore nuclear-mitochondrial communication, reverse mitochondrial dysfunction, and improve metabolic health in aged mice.

However, while the results are promising, questions remain about the long-term safety and efficacy of NAD⁺ boosting strategies in humans, as well as the optimal dosing and delivery methods.

Declining NAD⁺ Induces a Pseudohypoxic State Disrupting Nuclear-Mitochondrial Communication during Aging

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SUMMARY

Ever since eukaryotes subsumed the bacterial ancestor of mitochondria, the nuclear and mitochondrial genomes have had to closely coordinate their activities, as each encode different subunits of the oxidative phosphorylation (OXPHOS) system. Mitochondrial dysfunction is a hallmark of aging, but its causes are debated. We show that, during aging, there is a specific loss of mitochondrial, but not nuclear, encoded OXPHOS subunits. We trace the cause to an alternate PGC-1 α / β -independent pathway of nuclear-mitochondrial communication that is induced by a decline in nuclear NAD⁺ and the accumulation of HIF-1 α under normoxic conditions, with parallels to Warburg reprogramming. Deleting SIRT1 accelerates this process, whereas raising NAD⁺ levels in old mice restores mitochondrial function to that of a young mouse in a SIRT1-dependent manner. Thus, a pseudohypoxic state that disrupts PGC-1 α / β -independent nuclear-mitochondrial communication contributes to the decline in mitochondrial function with age, a process that is apparently reversible.

INTRODUCTION

One of the most conserved and robust phenomena in biology is a progressive decline in mitochondrial function with age, leading to a loss of cellular homeostasis and organismal health (Lanza

and Nair, 2010; Wallace et al., 2010). There is considerable debate, however, about why mitochondrial homeostasis is disrupted in the first place. The original idea of Harman, that reactive oxygen species (ROS) from mitochondria are a primary cause of aging (Harman, 1972), has been challenged by recent studies of long-lived species and genetically altered animals (Lapointe and Hekimi, 2010).

Though most mitochondrial genes have been transferred to the nuclear genome, 13 subunits of the oxidative phosphorylation (OXPHOS) system remain, demanding functional communication between the nucleus and mitochondria to form stoichiometric OXPHOS complexes. This is mediated in large part by the peroxisome proliferator-activated receptor- γ coactivators α and β (PGC-1 α and PGC-1 β), which along with NRF-1 and -2, induce nuclear-encoded proteins, such as TFAM (mitochondrial transcription factor A), that carry out the replication, transcription, and translation of mitochondrial DNA (mtDNA) (Larsson, 2010).

Mammalian sirtuins (SIRT1-7) are a conserved family of NAD⁺-dependent lysine-modifying acylases that control physiological responses to diet and exercise (Haigis and Sinclair, 2010). The expression of SIRT1, an NAD⁺-dependent deacetylase, is elevated in a number of tissues following calorie restriction (CR) (Cohen et al., 2004), an intervention that extends lifespan in diverse species. Overexpression or pharmacological activation of SIRT1 reproduces many of the health benefits of CR, including protection from metabolic decline, cardiovascular disease, cancer, and neurodegeneration (Haigis and Sinclair, 2010; Libert and Guarente, 2013). Some of the health benefits of SIRT1 have also been linked to improved mitochondrial function (Baur et al., 2006; Gerhart-Hines et al., 2007; Price et al., 2012; Rodgers et al., 2005). Indeed, increased expression of neuronal SIRT1 extends

2016: Judith Campisi

Disrupting mitochondrial function, either through genetic manipulation or chemical inhibition, triggers a stress response that leads to permanent cell cycle arrest, a hallmark of senescence.

The 2016 study by Wiley et al., titled "Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype", published in *Cell Metabolism*, investigates how mitochondrial dysfunction drives cellular senescence and the development of a unique pro-inflammatory secretory phenotype, known as the senescence-associated secretory phenotype (SASP). The researchers found that disrupting mitochondrial function, either through genetic manipulation or chemical inhibition, triggers a stress response that leads to permanent cell cycle arrest, a hallmark of senescence.

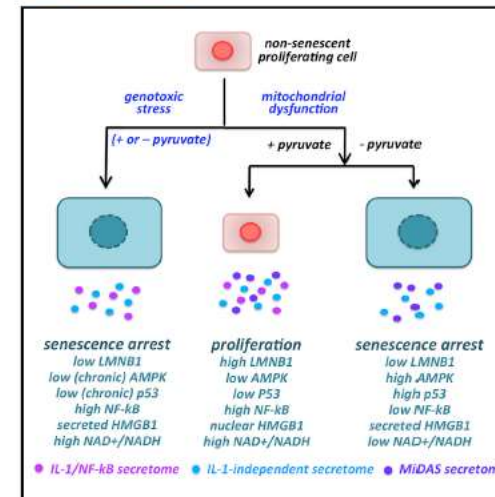
This mitochondrial dysfunction-induced senescence is distinct from other forms of senescence, as it is characterized by a specific SASP profile that includes elevated levels of cytokines, chemokines, and growth factors. These secreted factors can alter the tissue microenvironment, promoting inflammation and potentially contributing to age-related diseases and tissue dysfunction. The study highlights the role of mitochondrial reactive oxygen species (mtROS) and the activation of stress response pathways, such as ATF4 and NF-κB, in driving SASP secretion and amplifying the senescence response.

The findings underscore the importance of mitochondrial health in preventing cellular senescence and its detrimental effects on tissue function.

Cell Metabolism

Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype

Graphical Abstract



Authors

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In Brief

Wiley et al. show that mitochondrial dysfunction induces a senescence state termed MIDAS, causing a distinct secretion profile and mitotic arrest, which can be rescued with pyruvate. MIDAS is controlled by an NAD-AMPK-p53 pathway and occurs in progeroid mice, providing a basis for how dysfunctional mitochondria drive aging phenotypes.

Highlights

- Dysfunctional mitochondria cause cell senescence and a distinct secretory phenotype
- This secretory phenotype can influence the differentiation of certain cell types
- An NAD-AMPK-p53 pathway controls the secretory and mitotic arrest phenotypes
- Mice with dysfunctional mitochondria and premature aging accumulate senescent cells



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CellPress

Wiley CD, Velarde MC, Lecot P, Liu S, Sarnoski EA, Freund A, Shirakawa K, Lim HW, Davis SS, Ramanathan A, Gerencser AA, Verdin E, Campisi J. Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype. *Cell Metabolism*, Feb 9, 2016; 23(2):303-14.

2022: Berry & Wojtovich

Shows that reversal of the mitochondrial proton gradient can substantially halt aging in the nematode *C. elegans*.

In this 2022 paper, Brandon Berry of the University of Washington and Andrew Wojtovich of the University of Rochester explore the biological causes of mitochondrial dysfunction and aging at a granular level that had not been previously attempted.

A core energetic function of mitochondria is to produce ATP, a feat that is achieved by establishing a proton electrochemical gradient across their inner membrane via the oxidation of food. Recent attention has focused on the mitochondrial proton gradient (the mitochondrial ‘charge’), which declines with age. Experimentally controlling mitochondria in isolation from other metabolic processes within intact animals is a first step to disentangling the complex networks that underlie biological aging.

The researchers found that the mitochondrial proton gradient declined with age in *C. elegans*, which could be reversed by optogenetic activation of mitochondria. Importantly, mitochondrial activation throughout the lifespan, beginning in adulthood, resulted in a significant expansion of lifespan compared to control worms that lacked the optogenetic proton pump. This study provides a strong rationale to focus on the mitochondrial proton gradient for future investigations to promote healthy aging in mammals.

Optogenetic rejuvenation of mitochondrial membrane potential extends *C. elegans* lifespan

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 Check for updates

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Mitochondrial dysfunction plays a central role in aging but the exact biological causes are still being determined. Here, we show that optogenetically increasing mitochondrial membrane potential during adulthood using a light-activated proton pump improves age-associated phenotypes and extends lifespan in *Caenorhabditis elegans*. Our findings provide direct causal evidence that rescuing the age-related decline in mitochondrial membrane potential is sufficient to slow the rate of aging and extend healthspan and lifespan.

The causal role of mitochondrial dysfunction and metabolic decline are central questions of aging research^{1–3}. The voltage potential across the inner membrane of mitochondria (membrane potential $\Delta\psi_m$) decreases with age in many model systems^{4–6}. $\Delta\psi_m$ is a fundamental driver of diverse mitochondrial functions, including adenosine triphosphate (ATP) production, immune signaling and genetic and epigenetic regulation⁷. Decreased $\Delta\psi_m$ is an attractive explanation for the complex dysfunctions of aging; however, it is unclear whether decreased $\Delta\psi_m$ is a cause or a consequence of cellular aging.

To test these questions in a metazoan, we used optogenetics to harness light energy using a mitochondria-targeted light-activated proton pump to increase $\Delta\psi_m$. Using a mitochondrial targeting sequence, we previously expressed a rhodopsin-related proton-specific pump⁸ in the inner membrane of mitochondria⁹. We called this tool ‘mitochondria-ON’ (mtON) (Fig. 1a) and previously characterized its optogenetic function¹⁰. mtON isolates $\Delta\psi_m$ as a single experimental variable in vivo and requires both light activation and a cofactor, all-trans-retinal (ATR), for proton pumping activity¹¹. *Caenorhabditis elegans* does not produce ATR, allowing for control conditions of light exposure alone (which can be damaging¹²), mtON protein expression alone and ATR supplementation alone (which does not affect lifespan or physiology in this context; Supplementary Table 1 and previously¹⁰). Only animals supplemented

with ATR and illuminated will have mtON activity and increased $\Delta\psi_m$ (Supplementary Fig. 1)¹⁰.

We found that $\Delta\psi_m$ naturally declines with age in *C. elegans* (Fig. 1b,c), as expected^{13,14}. mtON activation reversed that decline in two different genetic backgrounds (Fig. 1d and Supplementary Figs 2 and 3). mtON activation did not impact mitochondrial mass observed by mitochondrial staining and quantitative proteomics (Supplementary Fig. 1d and Supplementary Fig. 4a). Respiration rates were similar across conditions (Fig. 1e,f) as expected, given mtON’s specificity for $\Delta\psi_m$ alone¹⁰.

We activated mtON throughout lifespan beginning in adulthood and found an increase in lifespan compared to controls (Fig. 2a,b and Supplementary Fig. 1). Lifespan extension was replicated independently across three different strains, different light intensities and in different laboratories (Supplementary Table 1) and was sensitive to the mitochondrial uncoupler, carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) (Fig. 2c), which dissipates $\Delta\psi_m$. FCCP had no effect on lifespan on its own (Fig. 2c) and ATR and light did not affect lifespan in wild-type animals (Supplementary Table 1). When exposed to light below the threshold to maximally activate mtON¹⁰ lifespan was not extended (Supplementary Fig. 6a). These data together indicate that increasing $\Delta\psi_m$ causes increased lifespan.

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2024: Dai Lab

OSER1 is shown to be a downstream target of FOXO genes. OSER1 enhances resistance to oxidative stress and extends lifespan.

The 2024 paper by Song et al., titled "FOXO-regulated OSER1 reduces oxidative stress and extends lifespan in multiple species", published in *Nature Communications*, identifies OSER1 (Oxidative stress-responsive serine-rich protein 1) as a key evolutionarily conserved target of FOXO transcription factors that plays a critical role in aging and stress resistance. The study demonstrates that OSER1 is regulated by FOXO, a well-known longevity-associated transcription factor, and its overexpression extends lifespan in silkworms, nematodes, and fruit flies, while its depletion shortens lifespan. OSER1 enhances resistance to oxidative stress, starvation, and heat shock in flies, and its knockdown in *Caenorhabditis elegans* leads to increased reactive oxygen species (ROS) production, mitochondrial dysfunction, and reduced ATP levels. These findings highlight OSER1 as a central mediator of FOXO's effects on stress resistance and longevity.

The study also reveals that OSER1 is induced by hydrogen peroxide and actively scavenges ROS, both in vitro and in vivo, suggesting a direct role in mitigating oxidative damage. In humans, OSER1 is implicated in oxidative stress response, cellular senescence, and reproduction, with polymorphic variants in the gene associated with longevity. The conservation of OSER1's function across species underscores its importance in maintaining mitochondrial integrity and promoting healthy aging.

FOXO-regulated OSER1 reduces oxidative stress and extends lifespan in multiple species

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 Check for updates

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FOXO transcription factors modulate aging-related pathways and influence longevity in multiple species, but the transcriptional targets that mediate these effects remain largely unknown. Here, we identify an evolutionarily conserved FOXO target gene, Oxidative stress-responsive serine-rich protein 1 (*OSER1*), whose overexpression extends lifespan in silkworms, nematodes, and flies, while its depletion correspondingly shortens lifespan. In flies, overexpression of *OSER1* increases resistance to oxidative stress, starvation, and heat shock, while *OSER1*-depleted flies are more vulnerable to these stressors. In silkworms, hydrogen peroxide both induces and is scavenged by *OSER1* in vitro and in vivo. Knockdown of *OSER1* in *Caenorhabditis elegans* leads to increased ROS production and shorter lifespan, mitochondrial fragmentation, decreased ATP production, and altered transcription of mitochondrial genes. Human proteomic analysis suggests that *OSER1* plays roles in oxidative stress response, cellular senescence, and reproduction, which is consistent with the data and suggests that *OSER1* could play a role in fertility in silkworms and nematodes. Human studies demonstrate that polymorphic variants in *OSER1* are associated with human longevity. In summary, *OSER1* is an evolutionarily conserved FOXO-regulated protein that improves resistance to oxidative stress, maintains mitochondrial functional integrity, and increases lifespan in multiple species. Additional studies will clarify the role of *OSER1* as a critical effector of healthy aging.

Recent investigations into the molecular basis of aging suggest that the FOXO transcription factor family is a critical regulator of genes that modulate aging, lifespan, and the response to oxidative stress^{1,2}. FOXO proteins modulate signaling pathways downstream of insulin/insulin-like growth factor-1 (IGF-1)³, AMPK⁴, TOR^{5,6}, JNK, and dietary restriction (DR)⁷, and integrate signals from these pathways⁸. FOXO-regulated genes and pathways are widely implicated in age-related

diseases such as cancer⁹, Alzheimer's disease^{10,11}, and type 2 diabetes mellitus¹².

Elevated oxidative stress is associated with cancer¹³, chronic kidney disease¹⁴, and neurodegeneration¹⁵. FOXO proteins regulate and are regulated by redox level and oxidative stress¹⁶. For example, FOXOs upregulate catalase (CAT)¹⁷, manganese superoxide dismutase (MnSOD)¹⁸, and sestrins^{19–21} while suppressing the expression of

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Nature Communications | (2024)15:7144

1

Song J, Li Z, Zhou L, Chen X, Sew WQG, Herranz H, Ye Z, Olsen JV, Li Y, Nygaard M, Christensen K, Tong X, Bohr VA, Rasmussen LJ, Dai F. FOXO-regulated OSER1 reduces oxidative stress and extends lifespan in multiple species. *Nature Communications* Aug 21, 2024;15(1):7144.

Section 8.D: Telomere Biology

The discovery of telomeres, their role in cellular aging, and the enzyme telomerase is one of the most significant developments in genetics and aging research.

The story begins with Hermann J. Muller in 1938 when he first described the protective ends of chromosomes and coined the term telomere. Muller, working with *Drosophila*, observed that chromosome ends had a unique function: they prevented fusion and degradation, distinguishing them from random chromosome breaks. Around the same time, Barbara McClintock, through her work on maize (corn), independently observed similar structures and suggested that chromosome ends were protected by specialized mechanisms. However, the molecular nature of telomeres remained unknown for several decades.

A major breakthrough came in the 1960s, when Leonard Hayflick discovered the Hayflick Limit, the observation that human somatic cells divide a limited number of times before entering a state of permanent growth arrest, now known as cellular senescence. This finding challenged the earlier belief that cells could divide indefinitely under the right conditions. Hayflick and his collaborator Paul Moorhead hypothesized that aging at the cellular level was linked to an intrinsic counting mechanism. Later research confirmed that this mechanism was tied to telomere shortening: with each cell division, telomeres became progressively shorter until they reached a critical length, triggering senescence.

In the 1970s and 1980s, researchers uncovered the molecular structure of telomeres. Elizabeth Blackburn and Joseph Gall identified that telomeres in *Tetrahymena* (a single-celled organism) were composed of repetitive DNA sequences.



Telomerase and Senescence

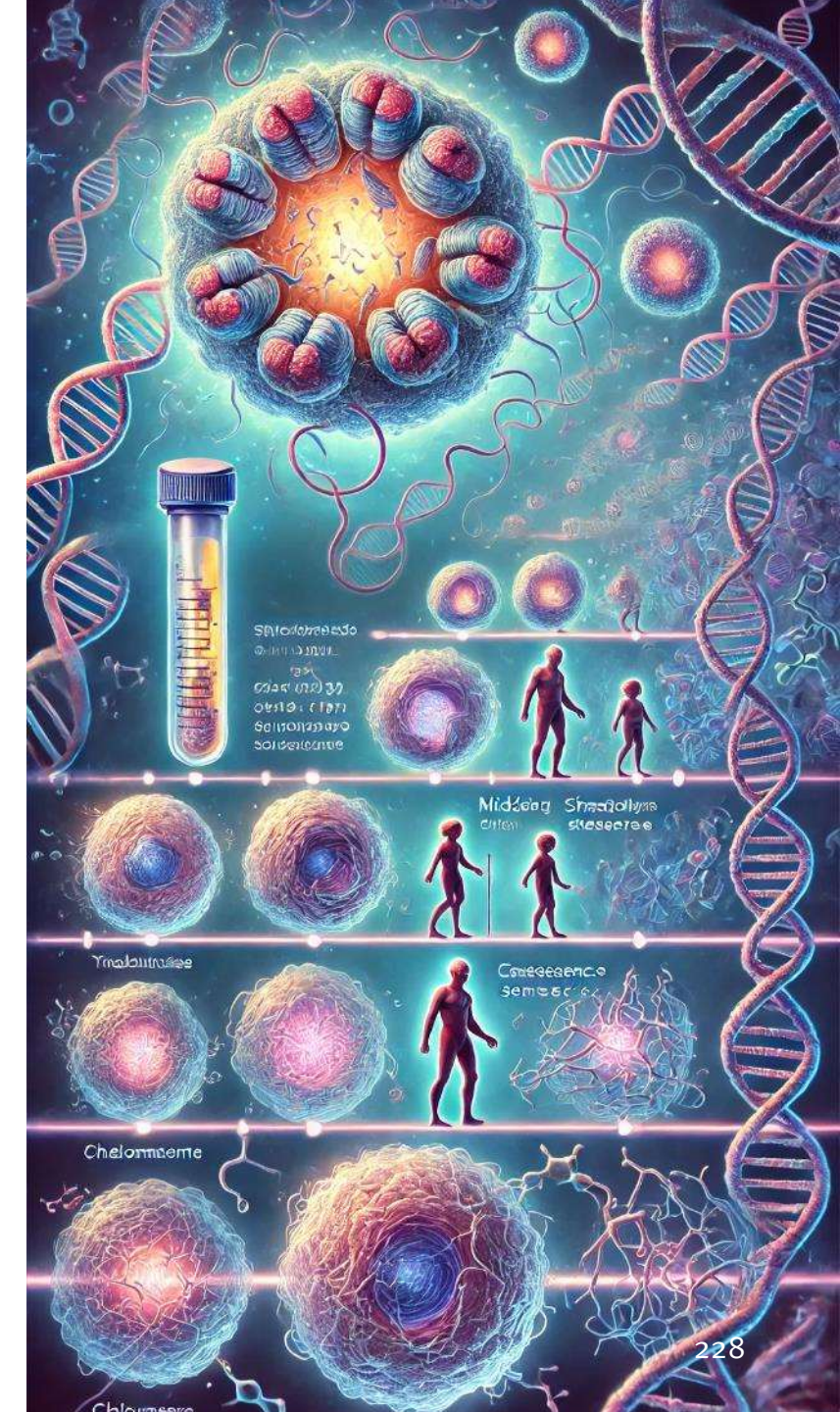
In 1985, Blackburn, along with her graduate student Carol Greider and colleague Jack Szostak, discovered telomerase, an enzyme capable of extending telomeres. Telomerase was found to counteract telomere shortening by adding repetitive nucleotide sequences to the chromosome ends, allowing certain cells (like stem cells, germ cells and cancer cells) to maintain long telomeres and divide indefinitely.

The connection between telomeres, cellular senescence and human aging became clearer in the 1990's and 2000s. Studies revealed that telomerase is active in embryonic and stem cells but largely absent in most adult somatic cells, leading to progressive telomere shortening over time.

This shortening has been linked to age-related decline in tissue function, weakened immune response and increased susceptibility to diseases like cardiovascular disease, fibrosis and neurodegeneration.

Cells that reach critically short telomeres either enter senescence or undergo apoptosis (programmed cell death). However, some dysfunctional senescent cells persist in tissues, secreting inflammatory molecules, which contribute to chronic diseases and aging — a phenomenon known as the senescence-associated secretory phenotype (SASP).

The concept of SASP was formally introduced by Judith Campisi and colleagues (2008) who established it as a hallmark of cellular senescence. This work detailed how senescent cells secrete pro-inflammatory cytokines, growth factors and proteases, linking SASP to both tumor promotion and age-related diseases.



Senolytics and Lifespan

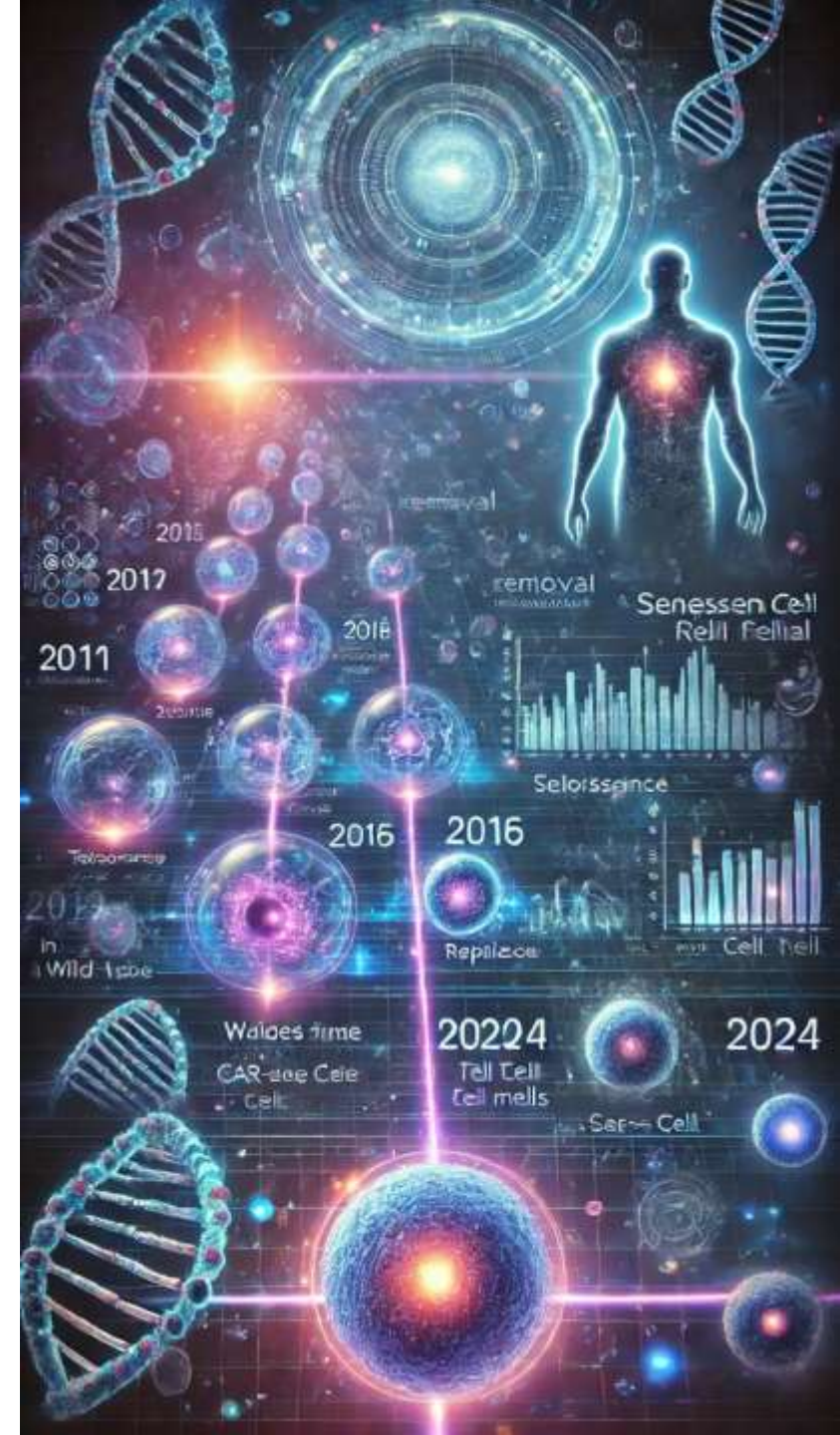
Building on this, Jesus Gil (2013) expanded the understanding of how senescent cells actively communicate with their surroundings. This research highlighted that senescence is not merely a cell-autonomous process but involves extensive crosstalk with neighboring cells, further emphasizing the systemic impact of senescent cells in aging and pathology.

The potential for senescence-targeting interventions was first demonstrated by Baker and van Deursen (2011), who showed that the removal of senescent cells could extend the lifespan of mice. Their findings supported the idea that senescent cells contribute directly to aging and that their selective elimination could offer therapeutic benefits. Their 2016 follow-up paper replicated the 2011 findings from progeroid mice to wild-type mice.

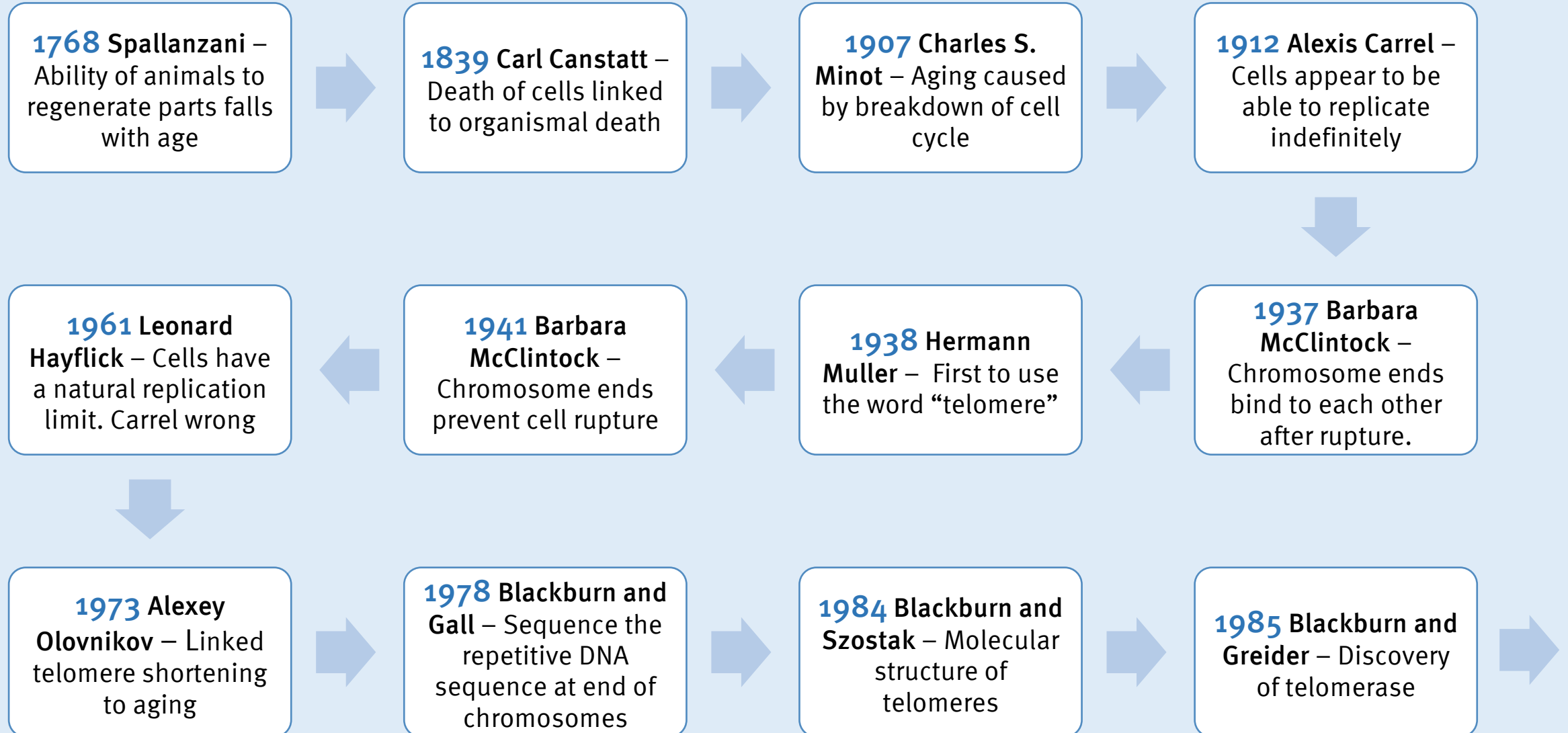
More recently, Sadelain and Lowe in 2020 and 2024 explored the use of Chimeric Antigen Receptor T-cell (CAR-T) therapy to target and eliminate senescent cells. Their studies demonstrated that CAR-T cell therapy could reverse age-related pathologies and was both safe and effective in mice. This approach represents a promising avenue for translating senescence-targeting therapies into clinical applications.

This approach builds on earlier pharmacological strategies using drugs like dasatinib and quercetin, which were shown to selectively clear senescent cells and improve physical function in aged mice (Zhu et al., 2015). The building body of work increasingly suggests that senolytics can extend lifespan by mitigating the pro-inflammatory and degenerative effects of senescent cells, thus maintaining tissue homeostasis and reducing the risk of age-related diseases.

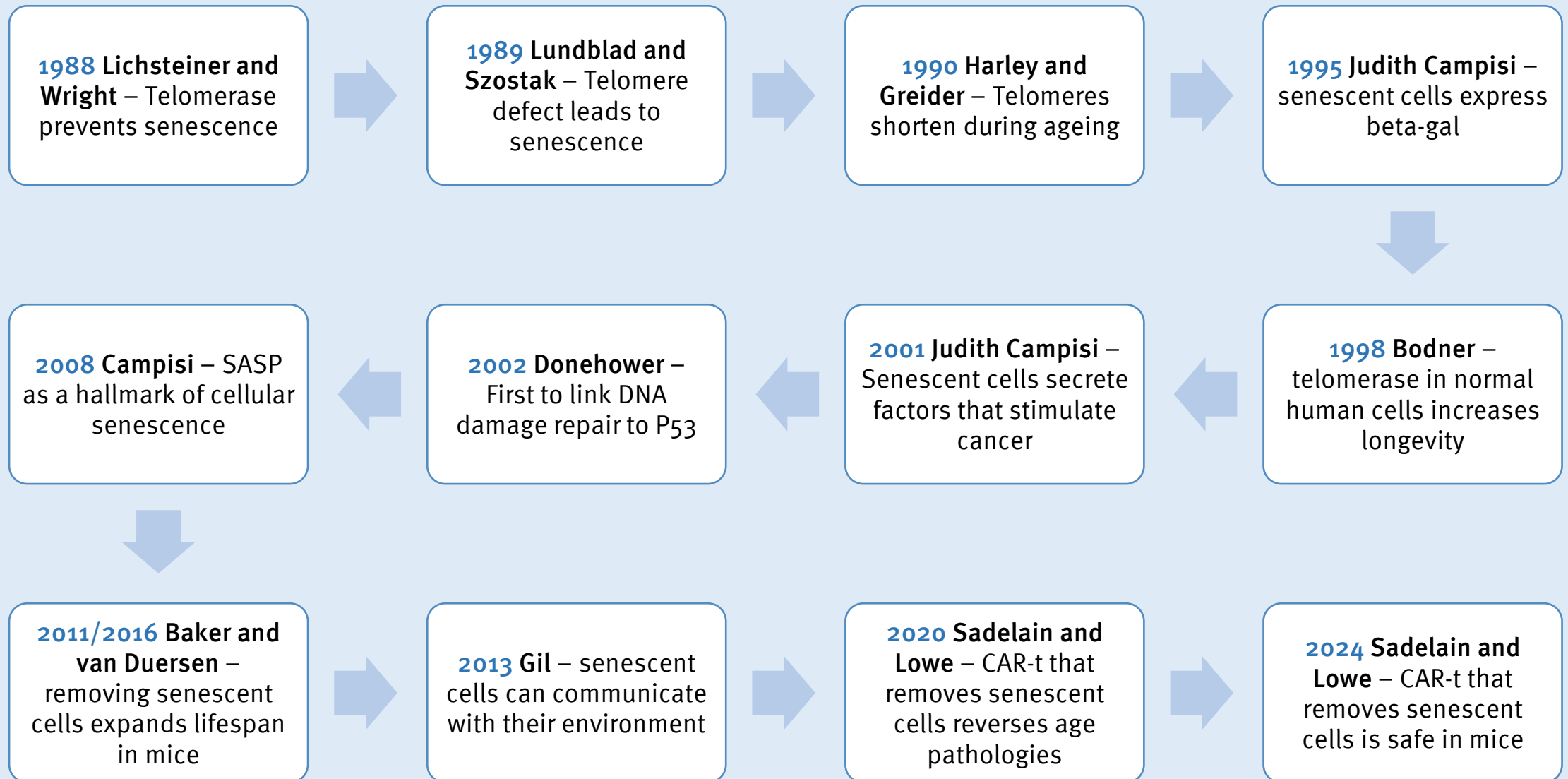
The next page provides a timeline of key milestones in telomere and senescent cell research.



Telomeres and Senescent Cells (1768 to 1987)



Telomeres and Senescent Cells (1988 to 2025)



1978: Blackburn and Gall

Elizabeth Blackburn, working with Joseph Gall, identified the repetitive DNA sequence (TTGGGG) at the ends of chromosomes in the ciliate *Tetrahymena*. This was the first identification of telomeric DNA, revealing that chromosome ends have a unique, conserved structure.

In their 1978 paper published in the *Journal of Molecular Biology*, Elizabeth Blackburn and Joseph Gall made the groundbreaking discovery of repetitive DNA sequences at the ends of chromosomes in the ciliate *Tetrahymena*.

Using biochemical techniques, they identified a simple, repeating sequence (TTGGGG) at the telomeres, the protective caps of chromosomes. This was the first time the structure of telomeric DNA had been characterized, revealing that chromosome ends are not random or unstructured but instead consist of a conserved, repetitive pattern. The authors hypothesized that these repeats play a critical role in protecting chromosome ends from degradation and fusion, providing a foundation for understanding how chromosomes maintain their integrity during cell division.

The discovery of telomeric repeats was a major milestone in molecular biology, as it provided the first clue to the molecular nature of telomeres and their function. Blackburn and Gall's work laid the groundwork for subsequent research into the mechanisms of chromosome protection and replication. Their findings suggested that telomeres serve as a buffer, preventing the loss of essential genetic information during DNA replication and shielding chromosome ends from being recognized as broken DNA. This paper marked the beginning of a new era in the study of telomeres, ultimately leading to the discovery of telomerase and its role in aging, cancer, and cellular biology.

J. Mol. Biol. (1978) **120**, 33–53

A Tandemly Repeated Sequence at the Termini of the Extrachromosomal Ribosomal RNA Genes in *Tetrahymena*

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(Received 19 August 1977)

The extrachromosomal genes coding for ribosomal RNA (rDNA) in the ciliated protozoan *Tetrahymena thermophila* were studied with respect to sequences occurring at their termini. The linear rDNA molecules had previously been shown to be palindromic (Karrer & Gall, 1976; Engberg *et al.*, 1976). Within the terminal rDNA fragment produced by restriction endonuclease digestion, a tandemly repeated hexanucleotide sequence 5' (C-C-C-C-A-A)_n 3' was found, where *n* is between 20 and 70. This fragment was heterogeneous in length as judged by gel electrophoresis. The repeating sequence was preferentially synthesized when rDNA was used as the template by *Escherichia coli* DNA polymerase I. Initiation occurred at specific single-strand discontinuities, probably one-nucleotide gaps, found every few repeats on the C-C-C-C-A-A strand. At least one discontinuity is present on the G-G-G-G-T-T strand. Experiments with T4 DNA polymerase suggested that there are no free cohesive ends on the rDNA of the kind found in bacteriophage λ DNA. The orientation of the strands carrying the repeating hexanucleotide sequence was determined, and a model for the termini of the rDNA based on these findings is presented.

1. Introduction

The genes coding for ribosomal RNA (rDNA) are amplified to form extrachromosomal DNA in the developing oocytes of a number of animals (Brown & Dawid, 1968; Gall, 1968; for review see Tobler, 1975). Extrachromosomal rDNA is also found in the slime mold *Physarum* (Vogt & Braun, 1976) and in the ciliated protozoan *Tetrahymena*. rDNA from *Tetrahymena* can be extracted as extrachromosomal linear molecules of uniform length (Gall, 1974). Each molecule consists of two identical halves arranged in the form of a palindrome, or inverted repeat, with one copy of the ribosomal RNA genes on each half of the molecule (Karrer & Gall, 1976; Engberg *et al.*, 1976).

Tetrahymena has two different nuclei: a polyploid, metabolically active macronucleus which divides amitotically during vegetative cell division, and a diploid micronucleus, which divides mitotically and functions as a germinal nucleus. At conjugation the macronucleus is destroyed and replaced by a division product of the micronucleus (for review see Elliott, 1973). The macronucleus contains extrachromosomal rDNA molecules, approximately 200 copies per haploid genome (Engberg & Pearlman, 1972). By contrast the micronuclear genome contains only a single chromosomally integrated rDNA copy (Yao & Gall, 1977). Amplification of the rDNA presumably occurs after conjugation when the macronucleus is formed.

33

0022-2836/78/1201-33\$3.00/0

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Blackburn EH, Gall JG. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. *J Mol Biol.* 1978 Mar 25;120(1):33-53.

1984: Blackburn and Szostak

This paper established telomeres as essential components of chromosome biology and opened numerous new avenues for research into aging, cancer, and genome stability. The authors subsequently received the Nobel Prize for this work.

The 1984 paper by Shampay, Szostak, and Blackburn, titled "DNA Sequences of Telomeres Maintained in Yeast", published in *Nature*, provides critical insights into the structure and function of telomeres, the protective DNA sequences at the ends of chromosomes. The study demonstrated that telomeres from the ciliated protozoan *Tetrahymena* could be introduced into yeast and function effectively to stabilize linear DNA molecules. This cross-species functionality suggested that telomeres have a conserved role in protecting chromosome ends from degradation and fusion, highlighting their universal importance in maintaining genomic integrity. The researchers also identified the repetitive DNA sequences of telomeres, which were found to be similar across species, further supporting the idea that telomeres serve a fundamental biological function.

The findings of this study were foundational for understanding the mechanisms of chromosome stability and cellular aging. By showing that telomeres could be maintained and function in a heterologous system (yeast), the research provided evidence that telomere sequences and their protective role are evolutionarily conserved. This work laid the groundwork for subsequent discoveries, including the identification of telomerase, the enzyme responsible for synthesizing telomeric DNA, by Blackburn and Greider. The study also highlighted the importance of telomeres in preventing chromosomal damage and their role in cellular senescence and aging.

DNA sequences of telomeres maintained in yeast

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Telomeres, the ends of eukaryotic chromosomes, have long been recognized as specialized structures. Their stability compared with broken ends of chromosomes^{1,2} suggested that they have properties which protect them from fusion, degradation or recombination^{1,3,4}. Furthermore, a linear DNA molecule such as that of a eukaryotic chromosome must have a structure at its ends which allows its complete replication⁵⁻⁸, as no known DNA polymerase can initiate synthesis without a primer. At the ends of the relatively short, multi-copy linear DNA molecules found naturally in the nuclei of several lower eukaryotes, there are simple tandemly repeated sequences⁹⁻¹⁷ with, in the cases analysed, a specific array of single-strand breaks, on both DNA strands, in the distal portion of the block of repeats^{9,10,17}. In general, however, direct analysis of chromosomal termini presents problems because of their very low abundance in nuclei. To circumvent this problem, we have previously cloned a chromosomal telomere of the yeast *Saccharomyces cerevisiae* on a linear DNA vector molecule¹⁸. Here we show that yeast chromosomal telomeres terminate in a DNA sequence consisting of tandem irregular repeats of the general form C₁₋₃A. The same repeat units are added to the ends of *Tetrahymena* telomeres, in an apparently non-template-directed manner, during their replication on linear plasmids in yeast. Such DNA addition may have a fundamental role in telomere replication.

Figure 1a shows a map of the linear yeast plasmid pSZ219. One end is a telomere from a yeast chromosome and the other end is derived from the terminal restriction fragment of the naturally occurring linear ribosomal DNA molecule from the somatic nucleus of the ciliate *Tetrahymena*¹⁸. To obtain amounts of yeast telomeric DNA sufficient for sequence analysis, the yeast telomere on pSZ219 was subcloned in the circular *Escherichia coli* vector pBR322 as described in Fig. 1 legend. The resulting recombinant molecules containing the yeast

‡ To whom reprint requests should be addressed.

1985: Blackburn & Greider

Greider and Blackburn discovered telomerase, an enzyme which adds repetitive DNA sequences to the ends of chromosomes (telomeres). They demonstrated that telomerase has an RNA component that serves as a template for synthesizing telomeric DNA.

In their seminal 1985 paper published in *Cell*, Carol Greider and Elizabeth Blackburn reported the discovery of telomerase, an enzyme that adds repetitive DNA sequences to the ends of chromosomes (telomeres). Using extracts from the ciliate *Tetrahymena*, they identified a novel enzymatic activity capable of elongating telomeric DNA in vitro.

This enzyme, which they named telomerase, was shown to synthesize the TTGGGG repeat sequence characteristic of *Tetrahymena* telomeres. Importantly, they demonstrated that telomerase contains an RNA component that serves as a template for the addition of these repeats, solving the long-standing "end-replication problem" — the inability of DNA polymerase to fully replicate the ends of linear chromosomes.

The discovery of telomerase was a landmark in molecular biology, providing a mechanism for how cells maintain telomere length and chromosome stability. Greider and Blackburn's work revealed that telomerase is essential for counteracting the progressive shortening of telomeres that occurs with each cell division, linking it to cellular aging and longevity.

This paper laid the foundation for understanding the role of telomerase in aging, cancer, and stem cell biology, as well as its potential as a target for therapeutic interventions.

References: Morley p. 1133; Ramakrishnan p. 76.

Identification of a Specific Telomere Terminal Transferase Activity in *Tetrahymena* Extracts

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Summary

We have found a novel activity in *Tetrahymena* cell free extracts that adds tandem TTGGGG repeats onto synthetic telomere primers. The single-stranded DNA oligonucleotides (TTGGGG), and TGTGTGGGTGTGTG-GGTGTGTGGG, consisting of the *Tetrahymena* and yeast telomeric sequences respectively, each functioned as primers for elongation, while (CCCCAA), and two nontelomeric sequence DNA oligomers did not. Efficient synthesis of the TTGGGG repeats depended only on addition of micromolar concentrations of oligomer primer, dGTP, and dTTP to the extract. The activity was sensitive to heat and proteinase K treatment. The repeat addition was independent of both endogenous *Tetrahymena* DNA and the endogenous α -type DNA polymerase; and a greater elongation activity was present during macronuclear development, when a large number of telomeres are formed and replicated, than during vegetative cell growth. We propose that the novel telomere terminal transferase is involved in the addition of telomeric repeats necessary for the replication of chromosome ends in eukaryotes.

Introduction

Telomeres, the ends of eukaryotic chromosomes, are essential elements that stabilize chromosome ends (Muller, 1938; McClintock, 1941) and allow the complete replication of linear DNA molecules (reviewed in Blackburn and Szostak, 1984). A common feature of telomeres is a terminal DNA region consisting entirely of tandemly repeated units of simple, G+C-rich sequences. All of the known repeat units conform to the general formula $C_n(A/T)_m(T/A)_nG_n$, where $n = 1-8$ and $m = 1-4$ (reviewed in Blackburn, 1984). The orientation of these repeats with respect to the chromosome end is always the same; the C-rich strand runs 5' to 3' from the end of the chromosome toward the interior. Structural and functional studies of chromosomes and linear plasmids in yeast have shown that the only DNA elements essential for telomere function are the simple G+C-rich telomeric sequence repeats, in the correct orientation (Szostak and Blackburn, 1982; Murray and Szostak, 1983; Shampay et al., 1984; J. W. Szostak, personal communication).

Despite the conserved nature of telomeric sequences, the number of tandem sequence repeats on a given telomere is not fixed. Telomeric restriction fragments are commonly variable in length, forming diffuse bands upon gel electrophoresis (Blackburn and Gall, 1978; Johnson, 1980; Emery and Weiner, 1981; Shampay et al., 1984). The

length variability of these fragments lies entirely within the region of telomeric repeats (Blackburn and Gall, 1978; Emery and Weiner, 1981; Blackburn et al., 1983). In addition to this variability, a net increase in telomere length occurs during long term logarithmic phase growth of trypanosomes and the ciliate *Tetrahymena*. In both *Trypanosoma brucei* and *Tetrahymena thermophila* the telomeres lengthen steadily, by 4–10 base pairs per cell generation, over the course of 200–300 cell generations (Bernards et al., 1983; D. Larson and E. Spangler, unpublished results). The length increase of the total population of macronuclear telomeres in *Tetrahymena* is entirely attributable to an increase in the number of telomeric sequence repeats on the telomeres (D. Larson and E. Spangler, unpublished results).

Together, these findings show that telomeres are dynamic structures capable of a net increase in length. However, DNA polymerases function in the 5' to 3' direction and require a template and primer for DNA synthesis, which means that the ends of chromosomal DNA should become progressively shortened over the course of many rounds of DNA replication (Cavalier-Smith, 1974). The fact that chromosome length is maintained, and even increased, strongly suggests that the replication of telomeric ends is not accomplished solely by the action of conventional DNA replication enzymes.

The telomeric sequences of the ciliates *Tetrahymena* and *Oxytricha* stabilize the ends of linear DNA molecules in yeast, allowing them to be maintained and replicated in this organism (Szostak and Blackburn, 1982; Pluta et al., 1984). Yeast repeats are added onto the ends of *Tetrahymena* telomeres after maintenance and replication of a linear plasmid in yeast; this is evident because the $C_{1-3}A \cdot TG_{1-3}$ telomeric repeat units of yeast are distinct from the telomeric $CCCCAA \cdot TTGGGG$ repeats of *Tetrahymena* (Shampay et al., 1984). Recombination between the *Tetrahymena* repeats and resident yeast telomeres is not likely to account for these findings because of the lack of sequence homology of the repeats and the lack of a requirement for the *RAD52* recombination function in yeast (Dunn et al., 1984). Based on all these considerations, the proposal was made that telomere replication involves a terminal transferase-like activity which adds the host cell telomeric sequence repeats onto recognizable telomeric ends (Shampay et al., 1984). In this model, shown schematically in Figure 1, a telomeric sequence of G-rich DNA is added *de novo* onto the preexisting telomeric end. Once this protruding G-rich strand is formed, it can serve as the template for the synthesis of the complementary C-rich strand by conventional primase and DNA polymerase activities. Removal of RNA primer and incomplete synthesis or ligation of the most recently added terminal repeats would account for the single-strand breaks found in the distal part of this strand in several organisms (Blackburn and Gall, 1978; Johnson, 1980; Katzen et al., 1981; Szostak and Blackburn, 1982; Blackburn and Challoner, 1984).

During macronuclear development in mated *Tetra-*

Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell*. 1985 Dec;43(2 Pt 1):405-13.

1988: Lichtsteiner and Wright

Showed that telomerase could prevent cells from taking on a senescent phenotype. This is the fifth most cited paper in the history of aging research.

Serge Lichtsteiner studied gene regulation in *C. elegans* before leading a research group at Geron which focused on extending the lifespan of human cells. This scientific breakthrough resulted in the publication at right that became one of the most cited papers of all time in aging research. Working with collaborator Woodring “Woody” Wright, they found that introducing telomerase to human cells prevented them from taking on a senescent phenotype, allowing them to extend their lifespan dramatically.

Both authors worked for Geron Corporation, an early aging biotech. Woody Wright spent most of his career as a cancer and aging researcher at UT Southwestern.

Specifically, the authors introduced the catalytic subunit of human telomerase (hTERT) into normal human fibroblasts and retinal pigment epithelial cells, which normally lack telomerase activity.

This intervention resulted in the maintenance of telomere length, extended cellular lifespan, and a delay in the onset of senescence, suggesting that telomere shortening is a key determinant of cellular aging. The study demonstrated that the expression of telomerase in normal human cells could bypass the Hayflick limit.

Ned Sharpless, former director of the NCI wrote of this paper: “This landmark paper provides direct evidence that experimental restoration of catalytically active telomerase in cultured human cells bypasses replicative senescence without causing oncogenic transformation.”*

* Sharpless, N., Sherr, C. Forging a signature of *in vivo* senescence. *Nat Rev Cancer* 15, 397–408 (2015).

RESEARCH ARTICLES

Extension of Life-Span by Introduction of Telomerase into Normal Human Cells

Andrea G. Bodnar,* Michel Ouellette,* Maria Frolkis, Shawn E. Holt, Choy-Pik Chiu, Gregg B. Morin, Calvin B. Harley, Jerry W. Shay, Serge Lichtsteiner,† Woodring E. Wright†

Normal human cells undergo a finite number of cell divisions and ultimately enter a nondividing state called replicative senescence. It has been proposed that telomere shortening is the molecular clock that triggers senescence. To test this hypothesis, two telomerase-negative normal human cell types, retinal pigment epithelial cells and foreskin fibroblasts, were transfected with vectors encoding the human telomerase catalytic subunit. In contrast to telomerase-negative control clones, which exhibited telomere shortening and senescence, telomerase-expressing clones had elongated telomeres, divided vigorously, and showed reduced staining for β -galactosidase, a biomarker for senescence. Notably, the telomerase-expressing clones have a normal karyotype and have already exceeded their normal life-span by at least 20 doublings, thus establishing a causal relationship between telomere shortening and *in vitro* cellular senescence. The ability to maintain normal human cells in a phenotypically youthful state could have important applications in research and medicine.

Normal human diploid cells placed in culture have a finite proliferative life-span and enter a nondividing state termed senescence, which is characterized by altered gene expression (1, 2). Replicative senescence is dependent upon cumulative cell divisions and not chronologic or metabolic time, indicating that proliferation is limited by a “mitotic clock” (3). The reduction in proliferative capacity of cells from old donors and patients with premature aging syndromes (4), and the accumulation *in vitro* of senescent cells with altered patterns of gene expression (5, 6), implicate cellular senescence in aging and age-related pathologies (1, 2).

Telomere loss is thought to control entry into senescence (7–10). Human telomeres consist of repeats of the sequence TTAGGG(XXX)TAA at chromosome ends; these repeats are synthesized by the ribonucleoprotein enzyme telomerase (11, 12). Telomerase is active in germ-line cells and, in humans, telomeres in these cells are maintained at about 15 kilobase pairs (kbp). In contrast, telomerase is not

expressed in most human somatic tissues (13, 14), and telomere length is significantly shorter (15). The telomere hypothesis of cellular aging (16) proposes that cells become senescent when progressive telomere shortening during each division produces a threshold telomere length.

The human telomerase reverse transcriptase subunit (hTERT) has been cloned (17). We recently demonstrated that telomerase activity can be reconstituted by transient expression of hTERT in normal human diploid cells, which express low levels of the template RNA component of telomerase (hTR) but do not express hTERT (18). This provided the opportunity to manipulate telomere length and test the hypothesis that telomere shortening

causes cellular senescence.

Introduction of telomerase into normal human cells. To determine if telomerase expression increases cell life-span, we transfected hTERT⁺ normal cells with two different hTERT expression constructs. One construct was engineered for increased translational efficiency by removal of the 5' and 3' untranslated regions of hTERT and creation of a Kozak consensus sequence. This engineered hTERT cDNA was cloned downstream of the MPSV promoter (19). The second construct consisted of the complete (native) hTERT cDNA cloned downstream of the SV40 promoter in pZeoSV (19). In the first experiments, we compared the life-span of stable clones transfected with MPSV-hTERT versus “vector only” clones, and in the second, we compared the life-span of activity-positive and activity-negative stable clones containing integrated SV40-hTERT constructs.

hTERT⁺ normal retinal pigment epithelial cells (RPE-J40) were transfected with the MPSV-hTERT vector at population doubling (PD) 37, and 27 of the 39 resultant stable clones (69%) expressed telomerase activity. BJ foreskin fibroblasts were transfected with the MPSV-hTERT vector at PD 58, and 3 of the 22 stable clones (14%) expressed telomerase activity. Reverse transcriptase-polymerase chain reaction experiments demonstrated that the hTERT mRNA originated from the transfected cDNA and not the endogenous gene (20). Telomerase activity, measured relative to that in the lung cancer-derived human cell line H1299, ranged from 65 to 360% in the RPE clones (Fig. 1) and 86 to 95% in the BJ clones. This range of telomerase activity is similar to that observed for tumor cell lines (13). Thirty-three RPE clones and 24 BJ clones transfected with the control plasmid were also isolated; RPE clones that generated sufficient cells for the TRAP assay ($n = 15$) (Fig. 1) and control BJ clones ($n = 15$)

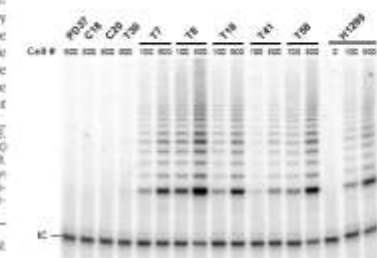


Fig. 1. Telomerase activity in stable RPE clones. Stable human RPE clones, obtained by transfection with a control vector (done numbers prefixed with “IC”) or with a vector expressing the hTERT cDNA (“T” clones) were analyzed for telomerase activity by the TRAP assay (19). “IC” represents the cell population at the time of transfection. The number of cells assayed for each clone is indicated above each lane. “IC” is the internal control in the TRAP assay. The positive control was the telomerase activity extracted from H1299 cells (20).

Bodnar, A. G. et al. 1998, Extension of life-span by introduction of telomerase into normal human cells. *Science* 279, 349–352.

1990: Harley, Futcher & Greider

Demonstrated that telomere shortening correlates with cellular aging in human cells. This is the fourth most cited paper in aging research.

In their seminal 1990 paper published in *Nature*, Calvin Harley, Bruce Futcher, and Carol Greider provided the first direct evidence linking telomere shortening to cellular aging in human cells.

The study focused on human fibroblasts, a type of connective tissue cell, and demonstrated that telomeres—the protective caps at the ends of chromosomes—progressively shorten with each cell division.

Using Southern blot analysis, the authors measured telomere length in fibroblasts at different population doublings and found a consistent decline in telomere length as the cells aged in culture. This observation supported the hypothesis that telomere erosion acts as a "mitotic clock," limiting the replicative lifespan of somatic cells and contributing to cellular senescence.

The findings of Harley et al. had profound implications for understanding the biology of aging and the mechanisms underlying cellular lifespan. The study provided experimental evidence for the "telomere hypothesis of aging," which posits that telomere shortening serves as a molecular timer for cellular aging. By linking telomere dynamics to replicative senescence, the paper laid the groundwork for subsequent research into the role of telomerase (the enzyme that maintains telomere length) in aging.

References: JSG 34.

Telomeres shorten during ageing of human fibroblasts

**Calvin B. Harley*, A. Bruce Futcher†
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THE terminus of a DNA helix has been called its Achilles' heel¹. Thus to prevent possible incomplete replication² and instability^{3,4} of the termini of linear DNA, eukaryotic chromosomes end in characteristic repetitive DNA sequences within specialized structures called telomeres⁵. In immortal cells, loss of telomeric DNA due to degradation or incomplete replication is apparently balanced by telomere elongation⁶⁻¹⁰, which may involve *de novo* synthesis of additional repeats by a novel DNA polymerase called telomerase¹¹⁻¹⁴. Such a polymerase has been recently detected in HeLa cells¹⁵. It has been proposed that the finite doubling capacity

Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature*. May 31, 1990; 345(6274):458-60.

1995: Judith Campisi

This technical paper has turned out to be incredibly useful in aging research. It is the third most cited paper on aging in the last 75 years. Campisi and colleagues show that a specific β -galactosidase is found on senescent cells but not on other cells.

Normal somatic cells invariably enter a state of irreversibly arrested growth and altered function after a finite number of divisions. This process, termed replicative senescence, is thought to be a tumor-suppressive mechanism and an underlying cause of aging. There is ample evidence that escape from senescence, or immortality, is important for malignant transformation. By contrast, the role of replicative senescence in organismic aging is controversial. Studies on cells cultured from donors of different ages, genetic backgrounds, or species suggest that senescence occurs *in vivo*, and that organismic lifespan and cell replicative lifespan are under common genetic control. However, senescent cells cannot be distinguished from quiescent or terminally differentiated cells in tissues. Thus, evidence that senescent cells exist and accumulate with age *in vivo* is lacking. We show that several human cells express a β -galactosidase, detectable at pH 6, upon senescence in culture.

This marker was expressed by senescent, but not presenescent, fibroblasts and keratinocytes but was absent from quiescent fibroblasts and terminally differentiated keratinocytes. It was also absent from immortal cells but was induced by genetic manipulations that reversed immortality. In skin samples from human donors of different age, there was an age-dependent increase in this marker in dermal fibroblasts and epidermal keratinocytes. This marker provides *in situ* evidence that senescent cells may exist and accumulate with age *in vivo*.

References: JSG 12; Ramakrishnan p. 194.

A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*

(replicative senescence/tumor suppression/ β -galactosidase)

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ABSTRACT Normal somatic cells invariably enter a state of irreversibly arrested growth and altered function after a finite number of divisions. This process, termed replicative senescence, is thought to be a tumor-suppressive mechanism and an underlying cause of aging. There is ample evidence that escape from senescence, or immortality, is important for malignant transformation. By contrast, the role of replicative senescence in organismic aging is controversial. Studies on cells cultured from donors of different ages, genetic backgrounds, or species suggest that senescence occurs *in vivo* and that organismic lifespan and cell replicative lifespan are under common genetic control. However, senescent cells cannot be distinguished from quiescent or terminally differentiated cells in tissues. Thus, evidence that senescent cells exist and accumulate with age *in vivo* is lacking. We show that several human cells express a β -galactosidase, histochemically detectable at pH 6, upon senescence in culture. This marker was expressed by senescent, but not presenescent, fibroblasts and keratinocytes but was absent from quiescent fibroblasts and terminally differentiated keratinocytes. It was also absent from immortal cells but was induced by genetic manipulations that reversed immortality. In skin samples from human donors of different age, there was an age-dependent increase in this marker in dermal fibroblasts and epidermal keratinocytes. This marker provides *in situ* evidence that senescent cells may exist and accumulate with age *in vivo*.

Normal, somatic cells do not divide indefinitely. This property, termed the finite replicative lifespan of cells, leads to an eventual arrest of cell division by a process termed replicative or cell senescence. Replicative senescence is generally studied in culture, where cells can be grown and monitored—in a controlled fashion—until most or all cells in a population senesce (1–4). Indirect evidence suggests that replicative senescence occurs *in vivo* (see refs. 2 and 4).

There are several salient features of senescence in culture (2–4). (i) It is exceedingly stringent in human cells, which, unlike many rodent cells, rarely if ever spontaneously immortalize. (ii) Senescent cells arrest growth with a G₁ DNA content and do not enter S phase in response to physiologic mitogens. Many genes remain mitogen-inducible, but some that are needed for cell cycle progression are repressed. (iii) Multiple, dominant-acting genes control the finite replicative lifespan and growth arrest of senescent cells. (iv) Senescent cells show selected, cell-specific changes in function. (v) Senescent cells remain metabolically active, and resist apoptotic death, for long periods of time.

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The idea that cellular senescence is tumor suppressive stems from molecular, cellular, and *in vivo* data. Immortality greatly increases the susceptibility to malignant transformation, in culture and *in vivo* (5, 6). Indeed, many tumors contain immortal cells or cells with an extended replicative lifespan (7). In addition, some oncogenes act primarily to immortalize or extend the lifespan of cells. Finally, Rb and p53, tumor suppressors that commonly suffer loss-of-function mutations in human cancers, are critical for cell senescence (see refs. 4 and 8).

The idea that senescence contributes to aging, by contrast, stems largely from studies in culture. Cells cultured from old donors tend to senesce after fewer population doublings (PD) than cells from young donors (9–11). Thus, cells in renewable tissues may deplete their replicative potential during aging. Moreover, cells from short-lived species tend to senesce after fewer PD than cells from long-lived species (11, 12), and cells from humans with hereditary premature aging syndromes senesce more rapidly than age-matched controls (13, 14). Thus, cellular replicative lifespan and organismic lifespan may be under overlapping genetic control. Together, the data support the idea that replicative senescence occurs *in vivo*. It is assumed that senescent cells accumulate *in vivo*, where their altered phenotype contributes to age-related pathology.

Senescent cells are identified in culture by their failure to synthesize DNA under optimal conditions and exponential increase with passage. *In vivo*, however, cell growth is not easily manipulated or monitored, and DNA synthesis measurements do not distinguish senescent cells from quiescent or terminally differentiated cells. Senescence markers identified in culture (2–4) either are not specific or require single cell quantitation or manipulations (e.g., mitogen stimulation) that are difficult to do or control *in vivo*. Thus, the idea that senescent cells accumulate with age has remained speculative because senescent cells cannot be identified *in situ*.

We describe a simple biomarker for replicative senescence that provides *in situ* evidence that senescent cells may persist and accumulate with age *in vivo*.

MATERIALS AND METHODS

Cells. WI-38, AG09602, AG06234, AG07720, AG0439, AG00780, and AG06300 were from the Coriell Institute (Camden, NJ). HCA2, HT1080, HeLa, TE85, and CMV-MJ were from O.P.-S., and 88-6 and MC17326 were from J. Oshima and G. Martin (University of Washington, Seattle).

Abbreviations: PD, population doublings; NHEK, neonatal human epidermal keratinocytes; dex, dexamethasone; β -Gal, β -galactosidase; SA- β -Gal, senescence-associated β -Gal.

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9363

Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, et al. A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. *Proc Natl Acad Sci U S A*. Sep 26, 1995; 92(20):9363-7.

2001: Judith Campisi

Senescent cells secrete factors that stimulate cancer.

The 2001 paper by Judith Campisi and coauthors titled "Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging" explores the role of senescent fibroblasts in creating a microenvironment that supports epithelial cell proliferation and tumorigenesis. The researchers demonstrated that senescent fibroblasts, which accumulate with age, secrete a variety of growth factors, cytokines, and proteases, collectively known as the senescence-associated secretory phenotype (SASP). These secreted factors create a pro-inflammatory and growth-promoting environment that enhances the proliferation and transformation of nearby epithelial cells. Using in vitro and in vivo models, the study showed that senescent fibroblasts significantly increased the growth and tumorigenic potential of epithelial cells, suggesting that the presence of senescent cells in aged tissues could contribute to the increased incidence of cancer in older individuals.

The findings of this study established a critical link between aging and cancer, highlighting how age-related accumulation of senescent cells can create a tissue microenvironment conducive to tumor development. The authors proposed that the SASP from senescent fibroblasts not only promotes epithelial cell growth but also disrupts tissue homeostasis, facilitating the progression of pre-malignant cells to full-blown tumors. This work provided a mechanistic explanation for the observed increase in cancer risk with age and underscored the dual role of cellular senescence—while it acts as a tumor-suppressive mechanism by preventing the proliferation of damaged cells, it can also paradoxically promote cancer through its secretory phenotype. The study has significant implications for understanding the interplay between aging and cancer and suggests that targeting senescent cells or their secretory factors could be a potential strategy for cancer prevention and treatment in aging populations.

Senescent fibroblasts promote epithelial cell growth and tumorigenesis: A link between cancer and aging

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Edited by Robert A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, MA, and approved August 14, 2001 (received for review February 1, 2001)

Mammalian cells can respond to damage or stress by entering a state of arrested growth and altered function termed cellular senescence. Several lines of evidence suggest that the senescence response suppresses tumorigenesis. Cellular senescence is also thought to contribute to aging, but the mechanism is not well understood. We show that senescent human fibroblasts stimulate premalignant and malignant, but not normal, epithelial cells to proliferate in culture and form tumors in mice. In culture, the growth stimulation was evident when senescent cells comprised only 10% of the fibroblast population and was equally robust whether senescence was induced by replicative exhaustion, oncogenic RAS, p14^{ARF}, or hydrogen peroxide. Moreover, it was due at least in part to soluble and insoluble factors secreted by senescent cells. In mice, senescent, much more than presenescent, fibroblasts caused premalignant and malignant epithelial cells to form tumors. Our findings suggest that, although cellular senescence suppresses tumorigenesis early in life, it may promote cancer in aged organisms, suggesting it is an example of evolutionary antagonistic pleiotropy.

Multicellular organisms have evolved mechanisms to prevent the unregulated growth and malignant transformation of proliferating cells. One such mechanism is cellular senescence, which arrests proliferation—essentially irreversibly—in response to potentially oncogenic events. Cellular senescence appears to be a major barrier that cells must overcome to progress to full-blown malignancy (1–3).

Cellular senescence was first described as a process that limits the proliferation of cultured human fibroblasts (replicative senescence). Proliferating cells progressively lose telomeric DNA, and short telomeres, which are potentially oncogenic, elicit a senescence response. In addition, DNA damage, oncogene expression, and supraphysiological mitogenic signals cause cellular senescence. Cellular senescence is controlled by tumor suppressor genes and seems to be a checkpoint that prevents the growth of cells at risk for neoplastic transformation (2, 3). In this regard, cellular senescence is similar to apoptosis. However, whereas apoptosis kills and eliminates damaged or potential cancer cells, cellular senescence stably arrests their growth.

Cellular senescence is also thought to contribute to aging (4–7), although how it does so is poorly understood. In addition to arresting growth, senescent cells show changes in function (2, 6, 7). Because senescent cells accumulate with age (8–10), they may contribute to age-related declines in tissue function. If so, cellular senescence may be an example of antagonistic pleiotropy. Aging phenotypes are thought to result from the declining force of natural selection with age. Consequently, traits selected to maintain early life fitness can have unselected deleterious effects late in life, a phenomenon termed antagonistic pleiotropy (11). The senescence-induced growth arrest may suppress the development of cancer in young organisms. The functional changes, by contrast, may be unselected consequences of the growth arrest and thus compromise tissue function as senescent cells accumulate.

Cellular senescence has been extensively studied in stromal fibroblasts from humans and mice. Upon senescence, such cells show striking changes in gene expression (7, 12), some of which relate to the growth arrest and senescent morphology. Other changes, however, relate to fibroblast function. Senescent fibroblasts secrete growth factors, cytokines, extracellular matrix, and degradative enzymes (2, 7, 13), all of which can alter tissue microenvironments and affect nearby epithelial cells. Interestingly, this secretory phenotype resembles that of fibroblasts adjacent to some carcinomas, although senescent and tumor-associated fibroblasts differ in growth potential, morphology, and other traits. Tumor-associated fibroblasts can stimulate epithelial tumorigenesis (14).

Because senescent cells can alter the tissue microenvironment, we and others proposed that senescent cells may contribute to the exponential rise in cancer that occurs with age (2, 4, 13, 15). It is now clear that nonmutational events, such as telomere dysfunction or epigenetic changes in gene regulation or the stromal milieu, are important for the development of late-life cancers (15). Changes in the stroma, which supports and maintains epithelial functions, may be particularly important in humans, where most age-related cancers arise from epithelial cells (15).

Here, we show that senescent human fibroblasts promote the proliferation and tumorigenesis of mutant epithelial cells. Our data suggest that cellular senescence is antagonistically pleiotropic, protecting from cancer early in life, but promoting carcinogenesis in aged organisms.

Materials and Methods

Cells. WI-38 (American Type Culture Collection), 82-6 (J. Osima, University of Washington, Seattle), and 184 (M. Stampfer and P. Yaswen, Lawrence Berkeley National Laboratory) fibroblasts senesce after ~50, 40, and 25 doublings, respectively, and were cultured as described (8, 16). Presenescent and senescent cultures generally contained >70% and <10% proliferating cells and were <10% and >70% senescence-associated β -galactosidase positive, respectively (8, 16). HaCAT (A. Paller, Northwestern University, Evanston, IL), S1 (M. Bissell, Lawrence Berkeley National Laboratory), Ha(Pk) (L. Packer, University of California, Berkeley), and MDA231 (R. Lupu, Lawrence Berkeley National Laboratory) cells were cultured as described (17–20). Keratinocytes (Clonetics) were cultured as directed by the supplier and used 2–3 passages after receipt. 82-6 and WI-38 were immortalized with an hTERT-expressing retrovirus (21).

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Abbreviations: DAPI, 4,6-diamidino-2-phenylindole; EGFP, enhanced green fluorescence protein.

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Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci U S A*. 2001 Oct 9;98(21):12072-7

2002: Lawrence Donehower

The first to link P53, an essential DNA repair enzyme, to aging.

The 2002 paper "p53 Mutant Mice that Display Early Ageing-Associated Phenotypes" by Tyner, Venkatachalam, Choi, and colleagues in Lawrence Donehower's lab was a landmark study that established a direct link between the tumor suppressor gene p53 and the aging process.

The researchers generated a mouse model with a hyperactive p53 pathway, which exhibited enhanced tumor suppression but also displayed accelerated aging phenotypes.

These mice showed signs of premature aging, including reduced lifespan, osteoporosis, organ atrophy, and decreased stress tolerance. The study demonstrated that while p53 is critical for preventing cancer by inducing apoptosis or senescence in damaged cells, its overactivation can lead to the depletion of stem cells and impaired tissue regeneration, contributing to accelerated aging.

The findings highlighted the dual role of p53 in balancing tumor suppression and longevity, supporting the idea of a trade-off between cancer prevention and aging. This work was the first to provide direct evidence that p53, beyond its role in cancer, plays a significant role in regulating the aging process.

The study opened new avenues for research into how tumor suppressor genes influence cellular senescence, DNA damage response, and age-related decline in tissue function, laying the foundation for understanding the complex interplay between cancer and aging.

p53 mutant mice that display early ageing-associated phenotypes

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The p53 tumour suppressor is activated by numerous stressors to induce apoptosis, cell cycle arrest, or senescence. To study the biological effects of altered p53 function, we generated mice with a deletion mutation in the first six exons of the p53 gene that express a truncated RNA capable of encoding a carboxy-terminal p53 fragment. This mutation confers phenotypes consistent with activated p53 rather than inactivated p53. Mutant (p53^{Δ1-6}) mice exhibit enhanced resistance to spontaneous tumours compared with wild-type (p53^{+/+}) littermates. As p53^{Δ1-6} mice age, they display an early onset of phenotypes associated with ageing. These include reduced longevity, osteoporosis, generalized organ atrophy and a diminished stress tolerance. A second line of transgenic mice containing a temperature-sensitive mutant allele of p53 also exhibits early ageing phenotypes. These data suggest that p53 has a role in regulating organismal ageing.

The p53 tumour suppressor protein responds to a variety of cellular stresses, including DNA damage, hypoxia and aberrantly activated oncogenes, and may induce cell cycle arrest or apoptosis^{1,2}. Studies on cultured primary cells have linked p53 activation to cellular replicative senescence—a terminal cell cycle arrest state reached after a finite number of cell divisions³. When cultured human diploid fibroblasts senesce, the activation state of p53 increases^{4,5}. Introduction of viral oncoproteins, mutant p53 forms, or anti-p53 antibodies (which inactivate wild-type p53) delay senescence of human primary cells^{6–10}. Introduction of oncogenic Ras into primary murine fibroblasts is initially mitogenic, but then induces a premature senescence dependent on wild-type p53 (ref. 11). These data are consistent with hypotheses that cellular replicative senescence serves as a mechanism of tumour suppression^{11,12}.

Recent reports about genetically engineered mice with altered longevity phenotypes implicate p53 as a modulator of organismal senescence^{13–16}. Late-generation telomerase-deficient mice exhibit shortened telomeres, decreased longevity and early senescence-related phenotypes¹³. Cells from these mice showed high levels of activated p53 (ref. 15). Crossing of the telomerase-deficient mice with p53-deficient mice resulted in rescue of some senescence-related phenotypes¹⁷. Early senescence phenotypes were also attributed to a Ku80-deficient mouse that was defective for double-stranded DNA break repair¹⁸. Embryonic fibroblasts from these mice showed a premature senescence that was rescued by p53 deficiency¹⁷. p66^{lac} null mice displayed 30% enhanced longevity compared with wild-type mice¹⁹. p66^{lac} mediates a stress response to reactive oxygen species, and cells from the deficient mice exhibited a defective p53 apoptotic response¹⁹.

A direct test of the involvement of p53 in the longevity of organisms could involve modulation of p53 levels in the mouse. We, and others, have generated p53-deficient mice^{20–22}, but were prevented from studying ageing by early tumours. Conversely, attempts to generate mice that overexpress wild-type p53 have been unsuccessful, probably owing to deleterious effects of increased p53 on the developing embryo (A. Bernstein and J. Banet, personal communication). We report the serendipitous

development of a genetically altered mouse that may express a truncated form of p53 that augments wild-type p53 activity. Mice with this defective p53 allele (which we term the 'tr' allele) are highly resistant to tumours and display early onset of phenotypes associated with ageing. A second line of transgenic mice containing multiple copies of a temperature-sensitive mutant p53 allele²³ also displayed some of the phenotypes of early ageing observed in p53^{tr} mice. The observation of such early ageing phenotypes in two distinct p53 mutant lines implicates p53 as a regulator of organismal ageing.

Generation of p53^{tr} mice

The p53^{tr} mouse was obtained following an aberrant gene-targeting event in embryonic stem cells. Initially, a p53 targeting construct containing a mutation at codon 245 (Arg245Trp) in p53 was introduced into embryonic stem cells (Fig. 1a). In one embryonic stem cell clone, the 3' segment of the targeting construct recombined with the endogenous p53 allele. Southern hybridization and polymerase chain reaction (PCR) assays revealed that the modified allele retained p53 exons 7–11 (including the codon 245 mutation) while deleting p53 exons 1–6 and an undefined region upstream of p53 (Fig. 1a, b). Preliminary mapping experiments indicate that the m-allele deletion extends over 20 kilobases (kb) upstream of p53. The deletion extends beyond the nearest p53 proximal gene, Efnh3 (S.D.T. and X.L., unpublished data).

We generated mice containing the p53 m allele (p53^{tr} mice). When the p53^{tr} mice began to show unexpected phenotypes (see below), we determined that the p53 m allele was expressed as a messenger RNA. Using the 5' rapid amplification of complementary DNA ends (RACE) procedure, we identified a discrete amplified fragment in tissues of p53^{tr} mice that was not found in tissues of p53^{+/+} mice. The sequence revealed a chimaeric message containing a 55-bp leader of unknown origin spliced into exon 7 of p53 (Fig. 1c). The foreign leader sequence contained no AUG codons, whereas the p53 exon 7 portion of the message contained three in-frame AUG codons; the third (Met codon 243) being adjacent to a viable translation initiation signal (Fig. 1c). Thus, the chimaeric

Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, Lu X, Soron G, Cooper B, Brayton C, Park SH, Thompson T, Karsenty G, Bradley A, Donehower LA. p53 mutant mice that display early ageing-associated phenotypes. *Nature*. 2002 Jan 3;415(6867):45–53.

2008: Judith Campisi

The authors provide a quantitative assessment of factors secreted by senescent cells (called SASP). They show that human cells induced to senesce by genotoxic stress secrete myriad factors associated with inflammation and malignancy.

The heavily cited 2008 paper by Campisi et al., entitled "Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the p53 Tumor Suppressor", published in *PLoS Biology*, explores how senescent cells influence their microenvironment through the secretion of various factors, known as the senescence-associated secretory phenotype (SASP). The study demonstrates that oncogenic RAS-induced senescence and p53-mediated senescence both trigger the secretion of a complex mix of cytokines, chemokines, growth factors, and proteases. These secreted factors can have profound effects on neighboring cells, promoting inflammation, tissue remodeling, and even tumorigenesis. The researchers found that the SASP is regulated by the NF- κ B and C/EBP β signaling pathways, which are activated in senescent cells. This secretory phenotype highlights the dual role of cellular senescence: while it acts as a cell-autonomous tumor-suppressive mechanism by halting the proliferation of damaged cells, it can also have cell-nonautonomous effects that may contribute to aging and cancer progression.

By showing that senescent cells can create a pro-inflammatory and growth-promoting microenvironment, this paper underscores the potential negative consequences of senescence for the first time, particularly in the context of aging and cancer. The findings suggest that the SASP can drive paracrine effects, such as stimulating the proliferation of pre-malignant cells or disrupting tissue homeostasis, which may explain the increased cancer risk associated with aging.

Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the p53 Tumor Suppressor

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Cellular senescence suppresses cancer by arresting cell proliferation, essentially permanently, in response to oncogenic stimuli, including genotoxic stress. We modified the use of antibody arrays to provide a quantitative assessment of factors secreted by senescent cells. We show that human cells induced to senesce by genotoxic stress secrete myriad factors associated with inflammation and malignancy. This senescence-associated secretory phenotype (SASP) developed slowly over several days and only after DNA damage of sufficient magnitude to induce senescence. Remarkably similar SASPs developed in normal fibroblasts, normal epithelial cells, and epithelial tumor cells after genotoxic stress in culture, and in epithelial tumor cells in vivo after treatment of prostate cancer patients with DNA-damaging chemotherapy. In cultured premalignant epithelial cells, SASPs induced an epithelial-mesenchyme transition and invasiveness, hallmarks of malignancy, by a paracrine mechanism that depended largely on the SASP factors interleukin (IL)-6 and IL-8. Strikingly, two manipulations markedly amplified, and accelerated development of, the SASP: oncogenic RAS expression, which causes genotoxic stress and senescence in normal cells, and functional loss of the p53 tumor suppressor protein. Both loss of p53 and gain of oncogenic RAS also exacerbated the promalignant paracrine activities of the SASPs. Our findings define a central feature of genotoxic stress-induced senescence. Moreover, they suggest a cell-nonautonomous mechanism by which p53 can restrain, and oncogenic RAS can promote, the development of age-related cancer by altering the tissue microenvironment.

Citation: Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, et al. (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 6(12): e301. doi:10.1371/journal.pbio.0060301

Introduction

Cancer is a multistep disease in which cells acquire increasingly malignant phenotypes. These phenotypes are acquired in part by somatic mutations, which derange normal controls over cell proliferation (growth), survival, invasion, and other processes important for malignant tumorigenesis [1]. In addition, there is increasing evidence that the tissue microenvironment is an important determinant of whether and how malignancies develop [2,3]. Normal tissue environments tend to suppress malignant phenotypes, whereas abnormal tissue environments such as those caused by inflammation can promote cancer progression.

Cancer development is restrained by a variety of tumor suppressor genes. Some of these genes permanently arrest the growth of cells at risk for neoplastic transformation, a process termed cellular senescence [4–6]. Two tumor suppressor pathways, controlled by the p53 and p16INK4a/pRB proteins, regulate senescence responses. Both pathways integrate multiple aspects of cellular physiology and direct cell fate towards survival, death, proliferation, or growth arrest, depending on the context [7,8].

Several lines of evidence indicate that cellular senescence is a potent tumor-suppressive mechanism [4,9,10]. Many potentially oncogenic stimuli (e.g., dysfunctional telomeres, DNA

damage, and certain oncogenes) induce senescence [6,11]. Moreover, mutations that dampen the p53 or p16INK4a/pRB pathways confer resistance to senescence and greatly increase cancer risk [12,13]. Most cancers harbor mutations in one or both of these pathways [14,15]. Lastly, in mice and humans, a senescence response to strong mitogenic signals, such as those delivered by certain oncogenes, prevents premalignant lesions from progressing to malignant cancers [16–19].

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Abbreviations: CM, conditioned medium; DDR, DNA damage response; BSLA, enzyme-linked immunosorbent assay; EMT, epithelial-mesenchymal transition; GSE, genetic suppressor element; IL, interleukin; MMT, melaninoma; FRC, fibroblast; FRC, normal human prostate epithelial cell; RFP, replicative exhaustion; SASP, senescence-associated secretory phenotype; SN, senescent; shRNA, short hairpin RNA; KRA, X-mediator.

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2011: Baker, Kirkland and van Deursen

This was the first paper to show that removing senescent cells could extend the lifespan of an animal.

Cellular senescence, a stress-induced irreversible growth arrest often characterized by expression of p16Ink4a (encoded by the Ink4a/Arf locus, also known as Cdkn2a) and a distinctive secretory phenotype, prevents the proliferation of preneoplastic cells and has beneficial roles in tissue remodelling during embryogenesis and wound healing. Senescent cells accumulate in various tissues and organs over time and have been speculated to have a role in ageing.

To explore the physiological relevance and consequences of naturally occurring senescent cells, here we use a previously established transgene, INK-ATTAC, to induce apoptosis in p16Ink4a-expressing cells of wild-type mice by injection of AP20187 twice a week starting at one year of age. We show that compared to vehicle alone, AP20187 treatment extended median lifespan in both male and female mice of two distinct genetic backgrounds.

The clearance of p16Ink4a-positive cells delayed tumorigenesis and attenuated age-related deterioration of several organs without apparent side effects, including kidney, heart and fat, where clearance preserved the functionality of glomeruli, cardio-protective KATP channels and adipocytes, respectively. Thus, p16Ink4a-positive cells that accumulate during adulthood negatively influence lifespan and promote age-dependent changes in several organs, and their therapeutic removal may be an attractive approach to extend healthy lifespan.

Ramakrishnan p. 184.

Clearance of p16^{Ink4a}-positive senescent cells delays ageing-associated disorders

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Advanced age is the main risk factor for most chronic diseases and functional deficits in humans, but the fundamental mechanisms that drive ageing remain largely unknown, impeding the development of interventions that might delay or prevent age-related disorders and maximize healthy lifespan. Cellular senescence, which halts the proliferation of damaged or dysfunctional cells, is an important mechanism to constrain the malignant progression of tumour cells^{1,2}. Senescent cells accumulate in various tissues and organs with ageing³ and have been hypothesized to disrupt tissue structure and function because of the components they secrete^{4,5}. However, whether senescent cells are causally implicated in age-related dysfunction and whether their removal is beneficial has remained unknown. To address these fundamental questions, we made use of a biomarker for senescence, p16^{Ink4a}, to design a novel transgene, INK-ATTAC, for inducible elimination of p16^{Ink4a}-positive senescent cells upon administration of a drug. Here we show that in the *BubR1* hypomorphic mouse background, INK-ATTAC removes p16^{Ink4a}-positive senescent cells upon drug treatment. In tissues—such as adipose tissue, skeletal muscle and eye—in which p16^{Ink4a} contributes to the acquisition of age-related pathologies, life-long removal of p16^{Ink4a}-expressing cells delayed onset of these phenotypes. Furthermore, late-life clearance attenuated progression of already established age-related disorders. These data indicate that cellular senescence is causally implicated in generating age-related phenotypes and that removal of senescent cells can prevent or delay tissue dysfunction and extend healthspan.

To examine the role of cellular senescence in ageing and age-related pathologies, we designed a transgenic strategy for the clearance of senescent cells in mice. We based our approach on an earlier mouse model, termed FAT-ATTAC (fat apoptosis through targeted activation of caspase), in which adipocytes were selectively killed by apoptosis upon the administration of AP20187, a synthetic drug that induces dimerization of a membrane-bound myristoylated FK506-binding-protein-caspase 8 (FKBP-Casp8) fusion protein expressed specifically in adipocytes via the minimal *Fabp4* promoter⁶. Although a universal marker that is widely expressed in senescent cells has not been identified, most senescent cells seem to express p16^{Ink4a}, a cyclin-dependent kinase inhibitor and tumour suppressor that enforces growth arrest by activating Rb⁷. Additionally, the expression of p16^{Ink4a} is known to increase with ageing in several rodent and human tissues⁸. We replaced the *Fabp4* promoter with a 2.617-kb fragment of the p16^{Ink4a} gene promoter that is transcriptionally active in senescent, but not non-senescent cells (Fig. 1a)⁹. We added an internal ribosome entry site (IRES) followed by an open reading frame (ORF) coding for enhanced green fluorescence protein (EGFP) to allow for detection and collection of p16^{Ink4a}-positive senescent cells. Injection of the resulting construct into fertilized eggs yielded nine transgenic INK-ATTAC founder lines. To examine whether removal of p16^{Ink4a}-expressing cells is technically feasible and whether this affects age-associated deficits in mice,

we bred each of the founder lines into a *BubR1* hypomorphic (*BubR1*^{h3}) genetic background. *BubR1* encodes a key member of the mitotic checkpoint, a surveillance mechanism that ensures accurate chromosome segregation in mitosis by inhibiting the ubiquitin ligase activity of Cdc20-activated anaphase-promoting complex (APC^{Cdh20}). In the presence of unattached chromosomes^{10,11}, *BubR1*^{h3} mice have a markedly shortened lifespan and exhibit a variety of age-related phenotypes, including infertility, lordokyphosis, osteopenia, cataracts, fat loss, cardiac arrhythmias, arterial wall stiffening, impaired wound healing and dermal thinning^{12–14}. It has been proposed that *BubR1* is a determinant of natural ageing, because levels of *BubR1* decline markedly with age^{15–16}. *BubR1*^{h3} mice selectively accumulate p16^{Ink4a}-positive cells in certain tissues in which age-associated pathologies develop, including adipose tissue, skeletal muscle and eye¹⁷. Inactivation of p16^{Ink4a} in these mice is known to delay the onset of age-related phenotypes selectively in these tissues¹⁷. To screen for INK-ATTAC transgene activity in p16^{Ink4a}-positive cells, we collected samples of inguinal adipose tissue (IAT) from each of the nine *BubR1*^{h3}/INK-ATTAC strains at 5 months of age and analysed them for GFP expression by fluorescence microscopy. We observed GFP fluorescence in two of these strains, *BubR1*^{h3}/INK-ATTAC-3 and -5 (Fig. 1b and Supplementary Fig. 1a). Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of various tissues from *BubR1*^{h3}/INK-ATTAC-3 and -5 mice demonstrated that INK-ATTAC and GFP transcript levels were significantly elevated in adipose tissue, skeletal muscle and eye, but not in tissues in which endogenous p16^{Ink4a} is not induced, including liver and heart (Fig. 1c and Supplementary Fig. 1b).

To confirm that transgenic INK-ATTAC and endogenous p16^{Ink4a} are under the same transcriptional control mechanism outside the context of *BubR1* hypomorphism, we harvested bone marrow cells from 2-month-old wild-type (WT)/INK-ATTAC-3 and -5 mice and cultured them in the absence or presence of rosiglitazone, a drug that can induce cellular senescence and p16^{Ink4a} expression through activation of PPAR γ ¹⁸. Immunofluorescence microscopy revealed that a high proportion of cells expressed Flag-tagged FKBP-Casp8 in the presence of rosiglitazone, but not in its absence (Fig. 1d). Furthermore, we observed selective INK-ATTAC transgene induction in tissues of WT/INK-ATTAC-3 mice showing elevated expression of endogenous p16^{Ink4a} upon chronological ageing (Supplementary Fig. 2). Together, these data indicate that INK-ATTAC gene activity in founder lines 3 and 5 overlaps with endogenous p16^{Ink4a} expression.

Next, we tested whether INK-ATTAC is expressed in senescent cells in *BubR1* hypomorphic tissue. Fat tissue of aged *BubR1*^{h3}/INK-ATTAC mice stained strongly for senescence-associated β -galactosidase (SA- β -Gal) (Fig. 1e). qRT-PCR analysis demonstrated that INK-ATTAC expression correlates with expression of senescence markers in IAT (Fig. 1f and Supplementary Fig. 3a). Skeletal muscle and lens tissue of aged *BubR1*^{h3}/INK-ATTAC mice are SA- β -Gal negative (data not shown), but both these tissues expressed other markers of senescence.

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222 | NATURE | VOL 479 | 10 NOVEMBER 2011

Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM, “Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders,” *Nature*, Nov 2, 2011; 479(7372):232-6.

2013: Jesús Gil

Senescence has long been considered a cell autonomous arrest program restricting the propagation of damaged cells. Gil and colleagues provide evidence that senescent cells can communicate with their environment. This is relevant for both senolytic and parabiotic anti-aging strategies.

Oncogene-induced senescence (OIS) is crucial for tumour suppression. Senescent cells implement a complex pro-inflammatory response termed the senescence-associated secretory phenotype (SASP). The SASP reinforces senescence, activates immune surveillance and paradoxically also has pro-tumorigenic properties. Here, we present evidence that the SASP can also induce paracrine senescence in normal cells both in culture and in human and mouse models of OIS *in vivo*.

The authors coupled quantitative proteomics with small-molecule screens to identify multiple SASP components mediating paracrine senescence, including TGF- β family ligands, VEGF, CCL2 and CCL20. Amongst them, TGF- β ligands play a major role by regulating p15^{INK4b} and p21^{CIP1}. They further showed that expression of the SASP is controlled by inflammasome-mediated IL-1 signalling.

The inflammasome and IL-1 signalling are activated in senescent cells and IL-1 α expression can reproduce SASP activation, resulting in senescence. Ned Sharpless, former NCI Director, wrote of this paper: “This paper details an elegant study describing the paracrine spreading of SASP during OIS and control of SASP expression by inflammasome-mediated IL-1 expression.”*

* Sharpless, N., Sherr, C. Forging a signature of *in vivo* senescence. *Nat Rev Cancer* **15**, 397–408 (2015).

A complex secretory program orchestrated by the inflammasome controls paracrine senescence

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Oncogene-induced senescence (OIS) is crucial for tumour suppression. Senescent cells implement a complex pro-inflammatory response termed the senescence-associated secretory phenotype (SASP). The SASP reinforces senescence, activates immune surveillance and paradoxically also has pro-tumorigenic properties. Here, we present evidence that the SASP can also induce paracrine senescence in normal cells both in culture and in human and mouse models of OIS *in vivo*. Coupling quantitative proteomics with small-molecule screens, we identified multiple SASP components mediating paracrine senescence, including TGF- β family ligands, VEGF, CCL2 and CCL20. Amongst them, TGF- β ligands play a major role by regulating p15^{INK4b} and p21^{CIP1}. Expression of the SASP is controlled by inflammasome-mediated IL-1 signalling. The inflammasome and IL-1 signalling are activated in senescent cells and IL-1 α expression can reproduce SASP activation, resulting in senescence. Our results demonstrate that the SASP can cause paracrine senescence and impact on tumour suppression and senescence *in vivo*.

Senescence protects damaged cells from neoplastic transformation by inducing a stable growth arrest^{1–3}. Concomitant with this arrest, cells secrete a complex mixture of factors referred to as the SASP or senescence-messaging secretome^{4,5}. The SASP can exert opposing and contradictory effects⁴. Initial studies focused on the pro-tumorigenic properties of the SASP (refs 5–7) but the SASP also mediates important tumour suppressive effects⁸. Specific components of the SASP such as IGFBP-7, PAI-1, IL-6 and CXCL2-binding chemokines (such as IL-8 or GRO α) can reinforce senescence^{9–11}. The SASP also contributes to the surveillance and elimination of senescent cells by the immune system^{12–14}.

It is unclear whether pro-senescence effects can be exerted in a non-cell-autonomous fashion (paracrine) in addition to a cell-autonomous fashion (autocrine) and whether senescence can be transmitted to normal cells. Early experiments where young and old fibroblasts were mixed suggested that senescence was exclusively cell intrinsic^{15,16}

although more recently a bystander senescence response has been suggested¹⁷. However, although some factors secreted by senescent cells, such as IL-6, reinforce senescence in an intracrine fashion⁹, others such as IGFBP-7 can exhibit paracrine effects¹¹. In this investigation, we present unequivocal evidence supporting that senescence can be transmitted in a paracrine fashion, and provide insights into the pathways regulating and mediating paracrine senescence.

RESULTS

Paracrine transmission of senescence by cells undergoing OIS

To understand whether cells undergoing OIS can transmit senescence in a non-cell-autonomous manner, we established co-cultures of senescent and normal human IMR90 fibroblasts. Cells were distinguished by expressing an mCherry fluorescent marker (Cherry) in the normal IMR90 cells. The Cherry-positive and -negative populations were monitored using high content analysis microscopy

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2016: Baker, Kirkland and van Deursen

This was the follow-on paper from the Mayo team that showed that removing senescent cells could extend the lifespan of an animal.

In this follow-on paper to their 2011 work, Jan van Deursen's team at Mayo investigated the role of senescent cells marked by p16^{Ink4a} expression in aging and lifespan.

The study used a genetically engineered mouse model that allowed for the selective elimination of p16^{Ink4a}-positive senescent cells. The removal of these cells in middle-aged mice delayed the onset of age-related pathologies, improved organ function, and extended healthspan without causing significant side effects. These findings suggest that naturally occurring senescent cells actively contribute to the deterioration of tissues and the shortening of a healthy lifespan.

Unlike the 2011 paper which used progeroid mice, this study use wild-type mice and the findings were the same. Highly impressive.

To state the obvious, this study's findings had and continued to have broad implications for aging research and potential therapeutic interventions. By demonstrating that the targeted removal of senescent cells improves physiological function and extends healthspan, the study provides strong evidence supporting the development of senolytic therapies—drugs designed to selectively clear senescent cells. This research suggests that such interventions could mitigate age-related diseases, improve overall health, and possibly extend lifespan.

Ramakrishnan p. 184.

Naturally occurring p16^{Ink4a}-positive cells shorten healthy lifespan

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Cellular senescence, a stress-induced irreversible growth arrest often characterized by expression of p16^{Ink4a} (encoded by the *Ink4a/Arf* locus, also known as *Cdkn2a*) and a distinctive secretory phenotype, prevents the proliferation of preneoplastic cells and has beneficial roles in tissue remodelling during embryogenesis and wound healing. Senescent cells accumulate in various tissues and organs over time, and have been speculated to have a role in ageing. To explore the physiological relevance and consequences of naturally occurring senescent cells, here we use a previously established transgene, *INK-ATTAC*, to induce apoptosis in p16^{Ink4a}-expressing cells of wild-type mice by injection of AP20187 twice a week starting at one year of age. We show that compared to vehicle alone, AP20187 treatment extended median lifespan in both male and female mice of two distinct genetic backgrounds. The clearance of p16^{Ink4a}-positive cells delayed tumorigenesis and attenuated age-related deterioration of several organs without apparent side effects, including kidney, heart and fat, where clearance preserved the functionality of glomeruli, cardio-protective K_{ATP} channels and adipocytes, respectively. Thus, p16^{Ink4a}-positive cells that accumulate during adulthood negatively influence lifespan and promote age-dependent changes in several organs, and their therapeutic removal may be an attractive approach to extend healthy lifespan.

Cellular senescence is a well-established cancer defence mechanism that has also been proposed to have roles in ageing and age-associated diseases, presumably through the depletion of stem and progenitor cells, and the adverse actions of the senescence-associated secretory phenotype, which consists of many proinflammatory cytokines and chemokines, matrix metalloproteinases and growth factors^{1–3}. Consistent with this idea is the observation that interference with senescent cell accumulation in *FlubR1* progeroid mice delays several of the ageing-associated disorders that these animals develop^{4,5}. However, because progeroid syndromes do not mimic the complex degenerative changes of ageing completely, the relevance of these findings has remained unclear. Furthermore, recent studies showing that senescent cells have beneficial effects in injury repair and tissue remodelling^{6–10} have called into question the simplistic view of senescence as only a driver of age-dependent pathologies, raising the specter that senescent cell clearance might remove useful cells in addition to detrimental ones. Here we investigated the identity and physiological effect of naturally occurring senescent cells using *INK-ATTAC* (hereafter termed *ATTAC*)⁴, a transgenic mouse model that expresses the FKBP-binding protein–caspase 8 (FKBP–Casp8) fusion protein and green fluorescent protein (GFP) under the control of a minimal *Ink4a* (also known as *Ink4a/Arf* or *Cdkn2a*) promoter fragment transcriptionally active in senescent cells^{4,11}. Earlier we have shown that, in *FlubR1* progeroid mice, *ATTAC* ablates p16^{Ink4a}-positive senescent cells upon administration of AP20187 (AP), a dimerizer that activates FKBP-fused Casp8 (ref. 4). Our first objective was to validate the properties of *ATTAC* in naturally occurring p16^{Ink4a}-positive senescent cells.

ATTAC clears senescent adipocyte progenitor cells

Our initial validation was focused on fat. We collected GFP⁺ and GFP[−] cell populations from inguinal white adipose tissue (IWAT) of

12-month-old *ATTAC* mice by FACS (Fig. 1a). GFP⁺ cells expressed much higher levels of *Ink4a* and *FKBP-Casp8* than GFP[−] cells, as well as a broad panel of senescence markers (Fig. 1b). GFP⁺ cells, but not GFP[−] cells, were also highly positive for senescence-associated- β -galactosidase (SA- β -Gal; Fig. 1c). Furthermore, intact IWAT from aged, but not young *ATTAC* mice had SA- β -Gal activity, but less than IWAT of *FlubR1* progeroid mice, a difference also reflected in *Ink4a*, *FKBP-Casp8* and GFP transcript levels (Extended Data Fig. 1a, b).

IWAT of 18-month-old *ATTAC* mice treated bi-weekly with AP from 12 months onwards had eightfold less GFP⁺ adipocyte progenitors than vehicle-injected controls, although total progenitor cell numbers remained unchanged (Fig. 1d and Extended Data Fig. 1c). SA- β -Gal staining and quantitative reverse transcriptase PCR (qRT-PCR) analysis of senescence markers confirmed that *Ink4a*-positive senescent cells in IWAT increased between 12 and 18 months, and that AP eliminated most of these cells (Fig. 1e and Extended Data Fig. 1d). Consistent with senescence of progenitor cells, transmission electron microscopy (TEM) on SA- β -Gal-stained IWAT showed that X-Gal crystals were present in small perivascular cells rather than endothelium, white blood cells or adipocytes (Fig. 1f). X-Gal crystals were found in 0.2% and 1.6% of total IWAT cells from AP-treated and control mice, respectively (Fig. 1g).

Clearance of *Ink4a*-positive cells prevented loss of fat mass occurring between 12 and 18 months (Fig. 1h, i and Extended Data Fig. 1e). Age-dependent fat tissue dysfunction is characterized by decreased adipogenesis and adipocyte atrophy¹². Consistent with this, adipocyte size decreased between 12 and 18 months of age (Fig. 1j), as did transcript levels of two key transcriptional regulators of adipogenesis, *Pparg* and *Cebpa* (Fig. 1k). AP treatment of *ATTAC* mice prevented these decreases. Collectively, these data indicate that senescence contributes to age-dependent fat tissue alterations.

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184 | NATURE | VOL 530 | 11 FEBRUARY 2016

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2020: Scott Lowe and Michel Sadelain

In their 2020 pathbreaking paper, Sadelain and Lowe engineered CAR T cells to specifically recognize and eliminate senescent cells, which accumulate with age and contribute to tissue dysfunction and age-related diseases.

The 2020 *Nature* paper by Sadelain and Lowe, "Senolytic CAR T Cells Reverse Senescence-Associated Pathologies," introduces a novel and innovative approach to targeting senescent cells using chimeric antigen receptor (CAR) T-cell therapy. By targeting senescent cells, this approach aims to address the root cause of many aging-related pathologies, offering a potential therapeutic strategy to improve healthspan and treat age-related conditions.

Sadelain, Lowe and their colleagues note that cellular senescence is characterized by stable cell-cycle arrest and a secretory program that generates an inflammatory milieu that leads to chronic tissue damage and contributes to chronic diseases. Accordingly, eliminating senescent cells from damaged tissues in mice ameliorates the symptoms of these pathologies and even promotes longevity. In this paper, the authors test chimeric antigen receptor (CAR) T cells that target senescent cells can be effective senolytic agents. The CAR T cells target the urokinase-type plasminogen activator receptor (uPAR) as a cell-surface protein that is broadly induced during senescence and show that uPAR-specific CAR T cells efficiently ablate senescent cells in vitro and in vivo. These results establish the therapeutic potential of senolytic CAR T cells for senescence-associated diseases.

Article

Senolytic CAR T cells reverse senescence-associated pathologies

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Cellular senescence is characterized by stable cell-cycle arrest and a secretory program that modulates the tissue microenvironment^{1,2}. Physiologically, senescence serves as a tumour-suppressive mechanism that prevents the expansion of premalignant cells^{3,4} and has a beneficial role in wound-healing responses^{5,6}. Pathologically, the aberrant accumulation of senescent cells generates an inflammatory milieu that leads to chronic tissue damage and contributes to diseases such as liver and lung fibrosis, atherosclerosis, diabetes and osteoarthritis^{7,8}. Accordingly, eliminating senescent cells from damaged tissues in mice ameliorates the symptoms of these pathologies and even promotes longevity^{9,10}. Here we test the therapeutic concept that chimeric antigen receptor (CAR) T cells that target senescent cells can be effective senolytic agents. We identify the urokinase-type plasminogen activator receptor (uPAR)¹¹ as a cell-surface protein that is broadly induced during senescence and show that uPAR-specific CAR T cells efficiently ablate senescent cells in vitro and in vivo. CAR T cells that target uPAR extend the survival of mice with lung adenocarcinoma that are treated with a senescence-inducing combination of drugs, and restore tissue homeostasis in mice in which liver fibrosis is induced chemically or by diet. These results establish the therapeutic potential of senolytic CAR T cells for senescence-associated diseases.

Given the contribution of senescence to tissue damage, there is growing interest in the development of 'senolytic' agents that selectively eliminate senescent cells¹². Several small molecules exhibit senolytic activity, but most lack potency and produce substantial side effects^{13,14}. An alternative approach could involve CAR T cells directed against senescence-specific surface antigens. CARs are synthetic receptors that redirect T cell specificity, effector potential and other functions¹⁵. CAR T cells that target CD19 have shown notable efficacy in patients with refractory B cell malignancies¹⁶, and other cell-surface antigens show promise as targets for CAR therapy in different contexts^{17–19}. Here we investigate whether CAR T cells could serve as senolytic agents.

Upregulation of uPAR during senescence

To identify cell-surface proteins that are broadly and specifically upregulated in senescent cells, we compared RNA-sequencing (RNA-seq) datasets derived from three independent and robust models of senescence: 1) therapy-induced senescence in mouse lung adenocarcinoma

Kras^{G12S}/p53^{-/-} (KP) cells (p53 is also known as *Tp53*) that are triggered to senesce by a combination of MEK inhibition and CDK4 and CDK6 (CDK4/6) inhibition²⁰; 2) oncogene-induced senescence in mouse hepatocytes, mediated by the *in vivo* delivery of *Nras*^{G12V} through hydrodynamic tail vein injection (HTVI)²¹; and 3) culture-induced senescence in mouse hepatic stellate cells (HSCs) (Extended Data Fig. 1a). We focused on transcripts that encode molecules that are located in the plasma membrane (as determined by UniProtKB) and that were upregulated in all datasets (Extended Data Fig. 1b, c). Eight transcripts were identified, which encode proteins related to extracellular matrix remodelling or the coagulation cascade (Extended Data Fig. 1d).

Given that ideal antigens for the engagement of CAR T cells should be highly expressed on target cells but not in vital tissues, we ranked each transcript according to its magnitude of upregulation (log₂ expression in senescent cells/expression in non-senescent cells), and then excluded those that were highly expressed in vital tissues (as determined by the Human Protein Atlas and the Human Proteome Map²²). This process identified *PLAUR*, which encodes the

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2024: Scott Lowe and Michel Sadelain

Sadelain, Lowe and colleagues at Memorial Sloan Kettering show that using CAR T to remove senescent cells is safe and effective in reducing chronic diseases of aging in mice. This followed their 2020 *Nature* paper.

Sadelain, Lowe and colleagues previously showed that senescent cells have elevated expression of uPAR and leveraged CAR T cell technology to engineer T cells with an anti-mouse uPAR scFv to kill uPAR-expressing cells in mouse liver fibrosis models. These findings provided crucial proof of concept, but only examined senescence cell clearance in young animals and did not study long-term effects. In the current paper, the authors performed an extensive characterization of uPAR expression in 3-month-old and 20-month-old C57BL/6 mice. They determined that uPAR expression increased with age in multiple tissues. Next, the authors generated uPAR-targeting CAR T cells from six-week-old CD45.1 mice, as they previously developed⁵. Aged C57BL/6 mice were then intravenously infused with uPAR-targeting CAR T cells or negative controls.

uPAR-targeting CAR T cells reduced uPAR-positive and SA- β -Gal-positive cells in multiple tissues, as well as resulted in reduced systemic inflammation and improved glucose tolerance and overall metabolic function. The authors next showed that the uPAR-targeting CAR T cells could be used prophylactically to delay the onset of aging in younger mice.

Article

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Prophylactic and long-lasting efficacy of senolytic CAR T cells against age-related metabolic dysfunction

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Senescent cells, which accumulate in organisms over time, contribute to age-related tissue decline. Genetic ablation of senescent cells can ameliorate various age-related pathologies, including metabolic dysfunction and decreased physical fitness. While small-molecule drugs that eliminate senescent cells ('senolytics') partially replicate these phenotypes, they require continuous administration. We have developed a senolytic therapy based on chimeric antigen receptor (CAR) T cells targeting the senescence-associated protein urokinase plasminogen activator receptor (uPAR), and we previously showed these can safely eliminate senescent cells in young animals. We now show that uPAR-positive senescent cells accumulate during aging and that they can be safely targeted with senolytic CAR T cells. Treatment with anti-uPAR CAR T cells improves exercise capacity in physiological aging, and it ameliorates metabolic dysfunction (for example, improving glucose tolerance) in aged mice and in mice on a high-fat diet. Importantly, a single administration of these senolytic CAR T cells is sufficient to achieve long-term therapeutic and preventive effects.

Cellular senescence is a stress response program characterized by stable cell cycle arrest^{1,2} and the production of the senescence-associated secretory phenotype (SASP), which includes pro-inflammatory cytokines and matrix remodeling enzymes³. In physiological conditions

in young individuals (for example, wound healing, tumor suppression), the SASP contributes to the recruitment of immune cells, whose role is to clear the senescent cells and facilitate restoration of tissue homeostasis⁴. However, during aging, the combination of increased tissue

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336

Amor, C., Fernández-Maestre, I., Chowdhury, S. et al. Prophylactic and long-lasting efficacy of senolytic CAR T cells against age-related metabolic dysfunction. *Nat Aging* 4, 336–349 (2024)

Section 8.E: Autophagy & Aging

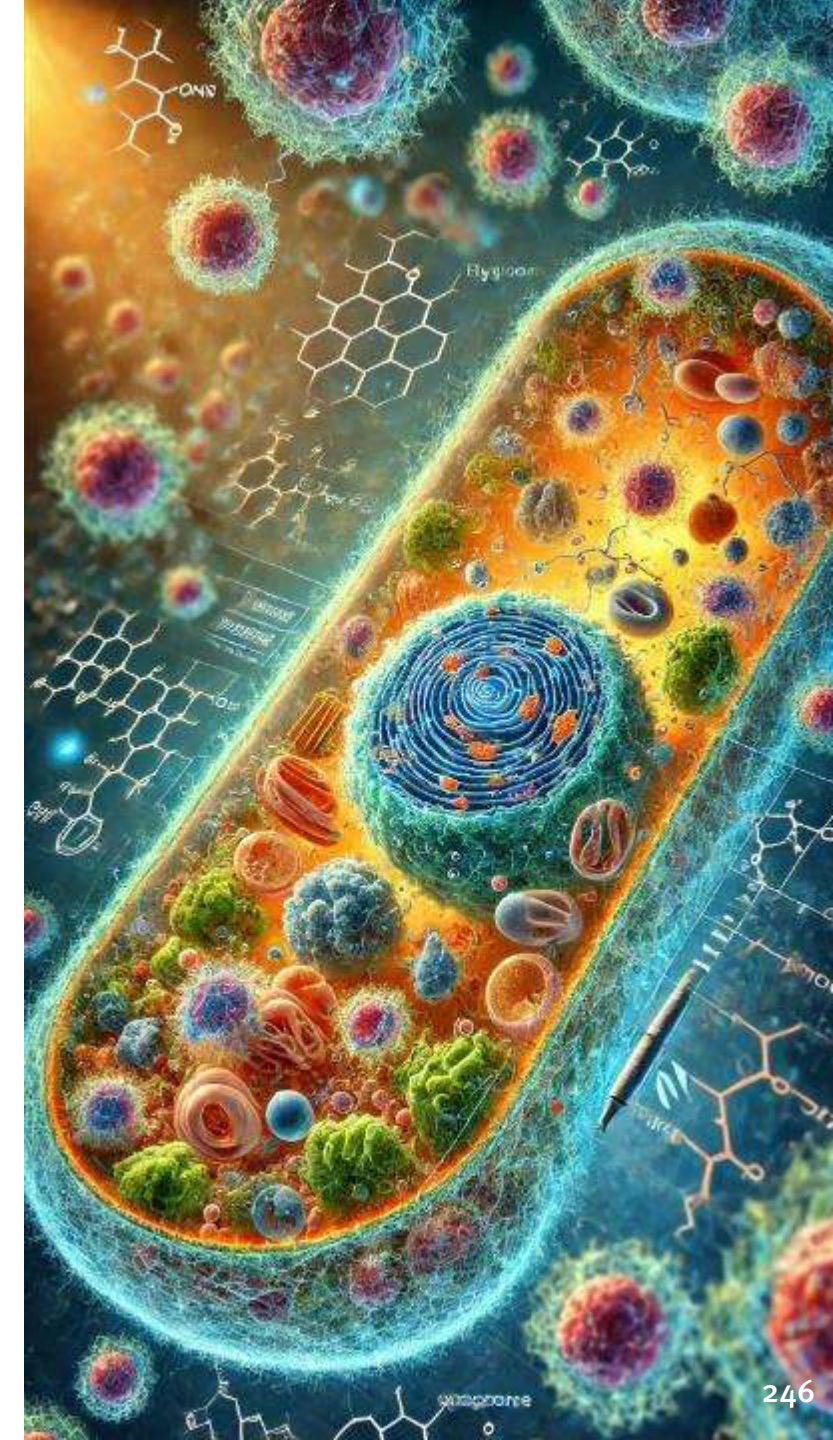
Autophagy, the process by which cells degrade and recycle damaged organelles and proteins, has emerged as a central mechanism in aging research. In the late 1990's, Yoshinori Ohsumi's groundbreaking work on autophagy in yeast laid the foundation for understanding its role in cellular homeostasis.

Unlike the oxidative stress and caloric restriction theories, there was less historic anticipation of the role of autophagy in aging.

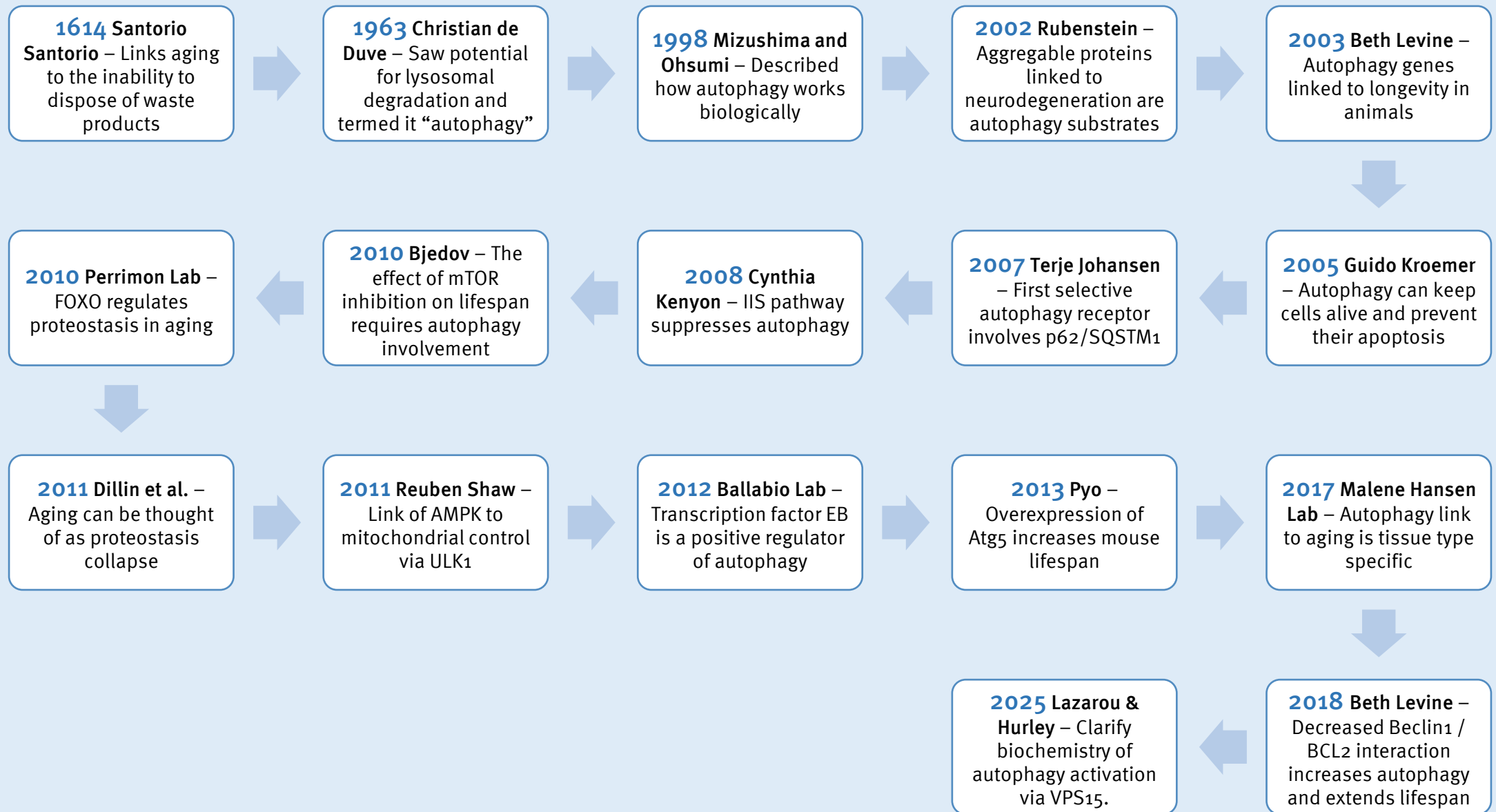
It is true that Santorio Santorio attributed aging to the build up of waste in the body in 1616. And, it's also true that Francis Bacon picked up on this idea and argued that waste buildup in the body caused death. But because the existence of the cell, much less the lysosome, were unknown, any anticipation at best could be said to have been metaphorical.

The evidence linking autophagy breakdown to cellular damage and death obtained in the last two decades has been remarkably strong. Over the past two decades, studies have shown that autophagy declines with age, contributing to the accumulation of cellular damage and the progression of age-related diseases. Research in model organisms, such as nematodes and mice, has demonstrated that enhancing autophagy can extend lifespan and improve health.

Importantly, caloric restriction, a well-known longevity intervention, has been shown to upregulate autophagy, thereby reducing cellular stress and damage. Pharmacological agents like rapamycin, which inhibits mTOR (a key regulator of autophagy), have also been shown to promote autophagy and extend lifespan in animal models. These findings have spurred interest in developing autophagy-enhancing therapies for age-related conditions.



Progression of the Autophagy and Aging Literature



1998: Mizushima and Ohsumi

First to describe the protein system that drives autophagy – a key protein breakdown process, later linked to longevity. Ohsumi awarded Nobel Prize for this work.

In the landmark 1998 study published in *Nature*, Yoshinori Ohsumi and his team elucidated the molecular mechanisms underlying autophagy, a critical cellular process for degrading and recycling damaged organelles and proteins. The study identified a protein conjugation system essential for autophagy in yeast, which involves the covalent attachment of Atg12 to Atg5, mediated by the E1-like enzyme Atg7 and the E2-like enzyme Atg10. This conjugation system was found to be crucial for the formation of autophagosomes, the double-membrane vesicles that sequester cellular components for degradation. The researchers demonstrated that mutations in any of these genes (Atg5, Atg7, or Atg10) disrupted autophagy, highlighting their indispensable roles in the process. This discovery provided the first detailed molecular framework for understanding how cells initiate and execute autophagy, a process now recognized as vital for maintaining cellular homeostasis and responding to stress.

Ohsumi's work laid the foundation for understanding autophagy as a highly regulated and conserved pathway across eukaryotes, including humans. The study revealed that the Atg12-Atg5 conjugate forms a complex with Atg16, which localizes to the pre-autophagosomal structure and is essential for autophagosome formation. This discovery not only advanced the field of cell biology but also had profound implications for understanding human diseases, including cancer, neurodegenerative disorders, and infections.

A protein conjugation system essential for autophagy

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Autophagy is a process for the bulk degradation of proteins, in which cytoplasmic components of the cell are enclosed by double-membrane structures known as autophagosomes for delivery to lysosomes or vacuoles for degradation¹⁻⁴. This process is crucial for survival during starvation and cell differentiation. No molecules have been identified that are involved in autophagy in higher eukaryotes. We have isolated 14 autophagy-defective (*apg*) mutants of the yeast *Saccharomyces cerevisiae*⁵ and examined the autophagic process at the molecular level⁶⁻⁹. We show here that a unique covalent-modification system is essential for autophagy to occur. The carboxy-terminal glycine residue of Apg12, a 186-amino-acid protein, is conjugated to a lysine at residue 149 of Apg5, a 294-amino-acid protein. Of the *apg* mutants, we found that *apg7* and *apg10* were unable to form an Apg5/Apg12 conjugate. By cloning *APG7*, we discovered that Apg7 is a ubiquitin-E1-like enzyme. This conjugation can be reconstituted *in vitro*

Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, Klionsky DJ, Ohsumi M, Ohsumi Y. A protein conjugation system essential for autophagy. *Nature*. 1998 Sep 24;395(6700):395-8.

2002: David Rubensztein

The 2002 study by Ravikumar et al. demonstrates that autophagy degrades toxic aggregate-prone proteins with polyglutamine and polyalanine expansions, offering a potential therapeutic strategy for neurodegenerative diseases caused by protein aggregation. This was the first paper that we are aware of linking protein aggregation / neurodegenerative diseases like Alzheimer's to autophagy.

The 2002 paper by Ravikumar, Duden, and Rubinsztein, titled "Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy", published in *Human Molecular Genetics*, investigates the role of autophagy in the clearance of aggregate-prone proteins associated with neurodegenerative diseases. The study focuses on proteins with polyglutamine (polyQ) and polyalanine (polyA) expansions, which are known to form toxic aggregates in conditions such as Huntington's disease and certain forms of muscular dystrophy. The researchers demonstrated that these aggregate-prone proteins are selectively degraded by macroautophagy, a cellular process that involves the engulfment of cytoplasmic components by autophagosomes and their subsequent degradation in lysosomes. They found that enhancing autophagy, either through pharmacological induction (e.g., using rapamycin) or genetic manipulation, significantly reduced the accumulation of these toxic protein aggregates.

The study also highlights the therapeutic potential of modulating autophagy to treat neurodegenerative diseases caused by protein aggregation. By showing that autophagy can effectively clear polyQ and polyA expansion proteins, the research provides a mechanistic basis for targeting autophagy as a strategy to mitigate the toxicity of these aggregates. The findings suggest that enhancing autophagy could be a viable approach to delay or prevent the progression of diseases like Huntington's disease and other proteinopathies.

Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy

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Protein conformational disorders (PCDs), such as Alzheimer's disease, Huntington's disease (HD), Parkinson's disease and oculopharyngeal muscular dystrophy, are associated with proteins that misfold and aggregate. Here we have used exon 1 of the HD gene with expanded polyglutamine [poly(Q)] repeats and enhanced green fluorescent protein tagged to 19 alanines as models for aggregate-prone proteins, to investigate the pathways mediating their degradation. Autophagy is involved in the degradation of these model proteins, since they accumulated when cells were treated with different inhibitors acting at distinct stages of the autophagy–lysosome pathway, in two different cell lines. Furthermore, rapamycin, which stimulates autophagy, enhanced the clearance of our aggregate-prone proteins. Rapamycin also reduced the appearance of aggregates and the cell death associated with the poly(Q) and polyalanine [poly(A)] expansions. Since rapamycin is used clinically, this drug or related analogues may be suitable candidates for therapeutic investigation in HD and related diseases. We have also re-examined the role of the proteasome, since previous studies in poly(Q) diseases have used lactacystin as an inhibitor – recent studies have shown that lactacystin may also affect lysosomal function. Both lactacystin and the specific proteasomal inhibitor epoxomicin increased soluble protein levels of the poly(Q) constructs, suggesting that these are also cleared by the proteasome. However, while poly(Q) aggregation was enhanced by lactacystin in our inducible PC12 cell model, aggregation was reduced by epoxomicin, suggesting that some other protein(s) induced by epoxomicin may regulate poly(Q) aggregation.

INTRODUCTION

Proteinopathies or protein conformational disorders (PCDs), such as Alzheimer's disease, Huntington's disease (HD), Parkinson's disease and oculopharyngeal muscular dystrophy (OPMD), are associated with particular proteins or sets of proteins that misfold and aggregate in specific tissues (1). However, it is controversial and unclear whether the aggregates (also known as inclusions) seen in these diseases or the monomeric proteins are directly toxic, or whether toxicity is mediated by the aggregation process itself.

Some PCDs are caused by codon reiteration mutations, where protein misfolding is mediated by the abnormal expansion of a tract of repeated amino acids. These include polyglutamine [poly(Q)] expansion diseases, exemplified by HD. HD is characterized by expansions of a poly(Q) stretch in exon 1 of the Huntingtin gene to more than 37 glutamines, and a short N-terminal fragment encoding the polyglutamine stretch is sufficient to cause aggregates in mice (2,3) and in cell models (4–6). Indeed, many believe that the mutant protein acquires its toxicity and its propensity to aggregate after cleavage, forming a short (so far incompletely defined)

N-terminal fragment containing the polyglutamine stretch (5). More recently, polyalanine [poly(A)] expansion mutations in the polyadenine-binding protein 2 gene have been shown to cause OPMD, which is associated with aggregates in muscle cell nuclei (7). This disease has been modelled in cell culture systems, where aggregate formation is associated with cell death (8). We have recently shown that polyA expansions of 19 or more repeats tagged with enhanced green fluorescent protein are sufficient to cause intracytoplasmic aggregate formation and cell death in cultured cells (9).

Many of the codon reiteration diseases (and other PCDs) are dominantly inherited, and genetic and transgenic studies suggest that they are generally due to gain-of-function mutations [e.g. in poly(Q) diseases] (reviewed in 10). In many cases, disease severity correlates with the expression levels of the mutant protein (reviewed in 10). Thus, it is important to understand the factors regulating clearance of these aggregate-prone proteins and of the aggregates themselves. The traditional view is that such proteins are expected to be cleared by the ubiquitin–proteasome pathway, where misfolded proteins are tagged with ubiquitin, which serves to route them to the proteasome for degradation. A role for this pathway in

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Ravikumar B, Duden R, Rubinsztein DC. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Hum Mol Genet.* May 2002; 11(9):1107–17.

2003: Beth Levine

First study to show an explicit *in vivo* link between autophagy genes and longevity. Also links autophagy to daf-2 / IGF-1 signalling.

The 2003 *Science* paper by Beth Levine and colleagues, titled "Autophagy Genes Are Essential for Dauer Development and Life-Span Extension in *C. elegans*" explores the role of autophagy in the development and longevity of the nematode *Caenorhabditis elegans*.

In the first part of the study, the authors investigate the role of autophagy in the formation of the dauer larva, a stress-resistant developmental stage in *C. elegans* that occurs under unfavorable environmental conditions, such as limited food or overcrowding. They demonstrate that autophagy genes, including *bec-1* (the *C. elegans* homolog of the yeast autophagy gene ATG6/VPS30), are essential for dauer development. When autophagy genes are knocked down, the worms fail to form dauers and instead continue normal development or die.

The second part of the study links autophagy to lifespan extension. The authors show that autophagy genes are required for the increased longevity observed in *C. elegans* mutants with reduced insulin/IGF-1 signaling (e.g., *daf-2* mutants), a well-known pathway that regulates aging. When autophagy genes are inhibited in these long-lived mutants, their lifespan is significantly shortened. This suggests that autophagy is a key mechanism through which reduced insulin/IGF-1 signaling promotes longevity. The study provides some of the first direct evidence that autophagy is not only crucial for survival under stress but also plays a central role in aging and lifespan extension.

Autophagy Genes Are Essential for Dauer Development and Life-Span Extension in *C. elegans*

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Eeva-Liisa Eskelinen,³ David H. Hall,⁴ Beth Levine^{1*}

Both dauer formation (a stage of developmental arrest) and adult life-span in *Caenorhabditis elegans* are negatively regulated by insulin-like signaling, but little is known about cellular pathways that mediate these processes. Autophagy, through the sequestration and delivery of cargo to the lysosomes, is the major route for degrading long-lived proteins and cytoplasmic organelles in eukaryotic cells. Using nematodes with a loss-of-function mutation in the insulin-like signaling pathway, we show that *bec-1*, the *C. elegans* ortholog of the yeast and mammalian autophagy gene *APG6/VPS30/beclin1*, is essential for normal dauer morphogenesis and life-span extension. Dauer formation is associated with increased autophagy and also requires *C. elegans* orthologs of the yeast autophagy genes *APG1*, *APG7*, *APG8*, and *AUT10*. Thus, autophagy is a cellular pathway essential for dauer development and life-span extension in *C. elegans*.

Under conditions of high population density, limited food, or increased temperature, *Caenorhabditis elegans* nematodes reversibly arrest in an alternate third larval stage, the dauer diapause, which is specialized to survive in an unfavorable environment (1). Entry into dauer is regulated by different signaling pathways, including transforming growth factor (TGF- β), cyclic guanosine monophosphate, and insulin-like signaling. Single-gene mutations in the insulin-like signaling pathway that promote constitutive dauer phenotypes also prolong adult life-span (2). Despite extensive characterization of regulatory signals, the cellular pathways involved in dauer morphogenesis and longevity are not well understood.

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The regulation of dauer entry in *C. elegans* shares similarities with the induction of autophagy in other eukaryotes. The environmental cues that promote dauer morphogenesis in *C. elegans* are also potent stimulators of autophagy in yeast and mammalian cells (3, 4). Through autophagy, cells generate sufficient pools of amino acids to synthesize proteins that are essential for survival when the environmental food supply is limited. Components of the insulin-like signaling pathway that positively (e.g., *daf-18*) and negatively (e.g., *age-1* and *akt1/akt2*) regulate dauer entry in *C. elegans* may also regulate autophagy in mammalian cells (5, 6). Furthermore, autophagy may be involved in life-span extension induced by dietary restriction in mammals (7), in delaying leaf senescence (8, 9), and in developmental processes that require extensive cellular and tissue remodeling (10). We therefore postulated that autophagy is essential in both dauer development and life-span extension in *C. elegans*.

To test this, we used RNA interference (RNAi) (11) to inhibit autophagy genes in *C. elegans* that carry a loss-of-function mutation in *daf-2*, the *C. elegans* insulin-like

tyrosine kinase receptor (12). *daf-2(e1370)* animals are temperature-sensitive *daf-c* class mutants (13), with inappropriate constitutive dauer entry at 25°C and extended adult life-span at lower temperatures (such as 15° to 22.5°C) (12, 14) that allow reproductive growth. *C. elegans* contains candidate homologs of several yeast autophagy (*APG*) genes. We focused on *bec-1*, the *C. elegans* ortholog (T19E7.3) of yeast *APG6/VPS30*, because its mammalian counterpart, *beclin 1*, is important in autophagy, embryonic development, and tumor suppression (15, 16). In both yeast and mammalian cells, *ApG6/Vps30/Beclin 1* is part of a Class III phosphatidylinositol 3-kinase complex that is thought to be important in mediating localization of other *ApG* proteins to preautophagosomal structures (17, 18).

C. elegans bec-1 encodes a predicted 375–amino acid, coiled-coil protein that shares 28% homology with yeast *ApG6/Vps30p* and 31% homology with human *Beclin 1*. To determine whether *bec-1* is a functional homolog of yeast *APG6/VPS30*, we tested its ability to restore autophagy and vacuolar protein sorting in *APG6/VPS30*-disrupted (*ΔapG6/vps30*) yeast. Using differential interference contrast microscopy, we found that *ΔapG6/vps30* yeast transformed with *C. elegans bec-1* or human *beclin 1*, but not with empty vector, showed an increase in nitrogen starvation-induced autophagy similar to that of *ΔapG6/vps30* yeast transformed with yeast *APG6/VPS30* (Fig. 1A). In contrast, *ΔapG6/vps30* yeast transformed with either *C. elegans bec-1* or human *beclin 1* were unable to properly sort and mature the vacuolar protein carboxypeptidase Y (CPY) (Fig. 1B, top). This defect was not due to a lack of stable *C. elegans* BEC-1 or human Beclin 1 protein expression in yeast (Fig. 1B, bottom) and could not be overcome by overexpression of the *ApG6/Vps30p* binding partner, *Vps38p*, that is part of the yeast vacuolar protein sorting complex (18) (Fig. 1B, middle). Thus, similar to human *beclin 1* (15), *C. elegans bec-1* complements the autophagy but not the vacuolar protein sorting function of *APG6/VPS30* in yeast. In addition, we examined the *C. elegans bec-1* expression pattern using a reporter gene in which the *bec-1* promoter was fused to green fluorescent protein (GFP) coding sequences

Beth Levine et al., Autophagy Genes Are Essential for Dauer Development and Life-Span Extension in *C. elegans*. *Science*, September 5, 2003, p. 1367.

2005: Guido Kroemer

Showed that autophagy can keep cells alive and prevent their apoptosis.

The 2005 paper by Guido Kroemer and colleagues, titled "Inhibition of Macroautophagy Triggers Apoptosis" (in *Molecular and Cellular Biology*), explores the relationship between autophagy (specifically macroautophagy) and apoptosis, two critical cellular processes.

The authors show that macroautophagy acts as a protective mechanism for cells under stress, such as nutrient deprivation or other cytotoxic conditions. Autophagy allows cells to recycle damaged organelles and proteins, providing energy and maintaining cellular homeostasis.

When autophagy is inhibited (e.g., through genetic or pharmacological means), cells become more susceptible to apoptosis (programmed cell death). This suggests that autophagy serves as a critical survival pathway, and its failure can trigger cell death.

The paper provides convincing evidence that the inhibition of autophagy results in the accumulation of damaged cellular components and increased oxidative stress, which in turn activates the intrinsic (mitochondrial) pathway of apoptosis. This highlights a direct mechanistic link between autophagy and apoptosis.

The findings suggest that modulating autophagy could have therapeutic potential. For example, enhancing autophagy might protect cells in degenerative diseases, while inhibiting autophagy could sensitize cancer cells to apoptosis.

Inhibition of Macroautophagy Triggers Apoptosis†

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Mammalian cells were observed to die under conditions in which nutrients were depleted and, simultaneously, macroautophagy was inhibited either genetically (by a small interfering RNA targeting *Atg5*, *Atg6/Beclin 1-1*, *Atg10*, or *Atg12*) or pharmacologically (by 3-methyladenine, hydroxychloroquine, bafilomycin A1, or monensin). Cell death occurred through apoptosis (type 1 cell death), since it was reduced by stabilization of mitochondrial membranes (with *Bcl-2* or *vMLA*, a cytomegalovirus-derived gene) or by caspase inhibition. Under conditions in which the fusion between lysosomes and autophagosomes was inhibited, the formation of autophagic vacuoles was enhanced at a preapoptotic stage, as indicated by accumulation of LC3-II protein, ultrastructural studies, and an increase in the acidic vacuolar compartment. Cells exhibiting a morphology reminiscent of (autophagic) type 2 cell death, however, recovered, and only cells with a disrupted mitochondrial transmembrane potential were beyond the point of no return and inexorably died even under optimal culture conditions. All together, these data indicate that autophagy may be cytoprotective, at least under conditions of nutrient depletion, and point to an important cross talk between type 1 and type 2 cell death pathways.

Type 1 (apoptotic) cell death and type 2 (autophagic) cell death are viewed as clearly distinct subroutines of cellular demise (9, 42, 47). Apoptosis, which is currently viewed as the quantitatively most important death modality, is morphologically defined by cellular and nuclear shrinkage (pyknosis), chromatin condensation, blebbing, nuclear fragmentation (karyorrhexis), and formation of apoptotic bodies (32). At the biochemical level, apoptosis of mammalian cells is characterized by mitochondrial membrane permeabilization (MMP) and/or massive caspase activation (1, 21, 68). Autophagic cell death is characterized by the accumulation of autophagic vacuoles (AV) (see below). Although less frequent, type 2 cell death is pathophysiologically relevant. For example, type 2 cell death affects degenerating neurons in some pathologies (17, 39), participates in retinal degeneration (24), seals the fate of *Salmonella*-infected macrophages (26), and sometimes mediates chemotherapy-induced tumor killing (30, 31, 55). Moreover, an important autophagy-regulatory gene such as *Beclin 1* functions as a haploinsufficient tumor suppressor gene (60, 76), further underscoring the likely clinical importance of type 2 cell death. Nonetheless, the biochemical mechanisms accounting for cell killing remain largely unexplored in type 2 cell death, and the mutual relationship between apoptotic and au-

tophagic death is currently debated (20, 27, 41–43, 48, 62, 70, 72, 75).

Autophagy is a regulated process of degradation and recycling of cellular constituents, participating in organelle turnover and in the bioenergetic management of starvation (37). During autophagy, part of the cytoplasm or entire organelles are sequestered into double-membraned vesicles, called AV or autophagosomes. Autophagosomes ultimately fuse with lysosomes, thereby generating single-membraned autophagolysosomes and degrading their content (73). Autophagy has been extensively studied in *Saccharomyces cerevisiae*, especially at the genetic level, leading to the discovery of autophagy-relevant *Apg* and *Aut* genes, now renamed *Atg* genes (36, 61). Several *Atg* proteins have been implicated in autophagosome formation. The ubiquitination of *Atg5* and *Atg12* by the E1-like enzymes *Atg7* and *Atg10* is required to recruit other proteins to the autophagosomal membrane and to form the autophagic vacuole in a pathway, which was first elucidated for yeast and then confirmed for mammalian cells (51, 53). LC3 is the mammalian equivalent of yeast *Atg8*. It exists in two forms, LC3-I and its proteolytic derivative LC3-II (18 and 16 kDa, respectively), which are localized in the cytosol (LC3-I) or in autophagosomal membranes (LC3-II). LC3-II thus can be used to estimate the abundance of autophagosomes before they are destroyed through fusion with lysosomes (29, 51). Similarly, LC3-green fluorescent protein (GFP) fusion protein redistributes from a diffuse to a vacuolar pattern when AV are formed (29, 51). Finally, *Beclin 1* is the mammalian orthologue of yeast *Atg6* (45). *Beclin 1* localizes to the trans-Golgi network, belongs to the class III phosphatidylinositol 3-kinase complex, and participates in autophagosome formation (33, 45). *Beclin 1* is monoalleli-

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2007: Terje Johansen

First selective autophagy receptor involves p62/SQSTM1.

The 2007 paper by Pankiv et al., titled "p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy", published in the *Journal of Biological Chemistry*, elucidates the molecular mechanism by which the adaptor protein p62/SQSTM1 mediates the selective autophagy of ubiquitinated protein aggregates. The study demonstrates that p62 directly interacts with Atg8/LC3, a key protein located on the autophagosome membrane, through a specific sequence called the LC3-interacting region (LIR). This interaction is crucial for the recruitment of ubiquitinated protein aggregates to autophagosomes, where they are subsequently degraded. The researchers also showed that p62 itself is degraded by autophagy, indicating that it acts as both a cargo receptor and a substrate in the autophagy pathway. This dual role of p62 ensures the efficient clearance of toxic protein aggregates and maintains cellular homeostasis.

The findings of this study provide critical insights into the selective autophagy process, highlighting the importance of p62 as a bridge between ubiquitinated cargo and the autophagic machinery. By elucidating the direct binding of p62 to Atg8/LC3, the research clarifies how cells selectively target damaged or misfolded proteins for degradation, a process essential for preventing the accumulation of toxic aggregates associated with neurodegenerative diseases like Alzheimer's and Parkinson's. The study also underscores the role of p62 in regulating autophagy and its potential as a therapeutic target for diseases characterized by protein aggregation.

p62/SQSTM1 Binds Directly to Atg8/LC3 to Facilitate Degradation of Ubiquitinated Protein Aggregates by Autophagy^{*[5]}

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Protein degradation by basal constitutive autophagy is important to avoid accumulation of polyubiquitinated protein aggregates and development of neurodegenerative diseases. The polyubiquitin-binding protein p62/SQSTM1 is degraded by autophagy. It is found in cellular inclusion bodies together with polyubiquitinated proteins and in cytosolic protein aggregates that accumulate in various chronic, toxic, and degenerative diseases. Here we show for the first time a direct interaction between p62 and the autophagic effector proteins LC3A and -B and the related γ -aminobutyrate receptor-associated protein and γ -aminobutyrate receptor-associated-like proteins. The binding is mediated by a 22-residue sequence of p62 containing an evolutionarily conserved motif. To monitor the autophagic sequestration of p62- and LC3-positive bodies, we developed a novel pH-sensitive fluorescent tag consisting of a tandem fusion of the red, acid-insensitive mCherry and the acid-sensitive green fluorescent proteins. This approach revealed that p62- and LC3-positive bodies are degraded in autolysosomes. Strikingly, even rather large p62-positive inclusion bodies (2 μ m diameter) become degraded by autophagy. The specific interaction between p62 and LC3, requiring the motif we have mapped, is instrumental in mediating autophagic degradation of the p62-positive bodies. We also demonstrate that the previously reported aggresome-like induced structures containing ubiquitinated proteins in cytosolic bodies are dependent on p62 for their formation. In fact, p62 bodies and these structures are indistinguishable. Taken together, our results clearly suggest that p62 is required both for the formation and the degradation of polyubiquitin-containing bodies by autophagy.

All eukaryotic cells use the following two systems for protein degradation: the ubiquitin-proteasome system and the lyso-

somes. The proteasome is used for selective degradation of short lived and abnormal/misfolded proteins following labeling with Lys-48-linked polyubiquitin chains (1). The lysosome degrades extracellular and plasma membrane proteins brought there by endocytosis and cytoplasmic components delivered by autophagy. Various categories of autophagy have been defined differing in the delivery route of cytoplasmic material. These include macroautophagy (hereafter called autophagy), microautophagy, and chaperone-mediated autophagy (2–4). Macroautophagy is regarded as the main pathway. This process involves the sequestration of a region of the cytoplasm within a double or multiple membrane-bounded autophagosome. Autophagosomes then undergo a maturation process, including fusion events with endosomes and/or lysosomes forming structures called amphisomes and autolysosomes, respectively (3–5). Autophagy is thought to be mainly a nonselective, bulk degradation pathway responsible for degradation of the majority of long lived proteins and some organelles. Two evolutionarily conserved protein conjugation systems are necessary for the formation of the autophagosome, the Atg12-Atg5- and the Atg8-phosphatidylethanolamine conjugation systems (6). The best characterized mammalian Atg8 homologue is light chain 3 (LC3).³ After synthesis pro-LC3 is cleaved by Atg4B to expose a C-terminal glycine residue (7, 8). This represents the cytosolic LC3-I form. Conjugation of phosphatidylethanolamine to the C terminus of LC3-I defines the LC3-II form that is tightly associated with the autophagosomal membrane (7, 9). The LC3-II form is involved during the late steps of autophagy after the isolation membrane has formed (3). In humans, three LC3 isoforms (LC3A, -B, and -C) and four additional human Atg8 homologues have been identified (GABARAP, GEC1/GABARAPL1, GATE16/GABARAPL2, and GABARAPL3) (10, 11). The role(s) of the GABARAP isoforms in autophagy is not known.

A number of studies have identified autophagy as a crucial cellular process to avoid accumulation of abnormal proteins in different neurodegenerative diseases (reviewed in Ref. 12). Mice carrying neuron-specific knock-outs of Atg5 or Atg7 dis-

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^[5] The on-line version of this article (available at <http://www.jbc.org/>) contains supplemental Figs. 1–5.

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³ The abbreviations used are: LC3, light chain 3; ALIS, aggresome-like induced structures; GFP, green fluorescent protein; EGFP, enhanced GFP; mCherry, monomeric red fluorescent protein; GABARAP, γ -aminobutyrate receptor-associated protein; siRNA, small interfering RNA; GST, glutathione S-transferase; MBP, maltose-binding protein; LIR, LC3-interacting region; MAP, microtubule-associated protein; MEF, murine embryo fibroblasts; Ab, antibody; mAb, monoclonal antibody.

2008: Cynthia Kenyon

The effect of dietary restriction on lifespan depends on autophagy and the insulin pathway – at least in nematodes.

The 2008 paper by Hansen et al., titled "A Role for Autophagy in the Extension of Lifespan by Dietary Restriction in *C. elegans*", published in *PLoS Genetics*, investigates the role of autophagy in mediating the lifespan extension observed under dietary restriction (DR) in *Caenorhabditis elegans* (nematodes). The study demonstrates that dietary restriction significantly extends lifespan in *C. elegans* and that this effect is dependent on the induction of autophagy, a cellular process that degrades and recycles damaged organelles and misfolded proteins. The researchers found that DR upregulates autophagy-related genes and increases autophagic activity, and that inhibiting autophagy through genetic mutations or RNA interference (RNAi) abolishes the lifespan extension conferred by DR.

They also identified the transcription factor PHA-4/FoxA as a key regulator of autophagy under DR, linking nutrient sensing to autophagic activation. Additionally, the study showed that autophagy is required for the longevity benefits of DR across different genetic backgrounds and dietary conditions, highlighting its central role in mediating the effects of reduced caloric intake. The study also suggests that enhancing autophagy could be a potential strategy to mimic the benefits of dietary restriction and promote healthy aging. Overall, this work advances our understanding of the interplay between nutrient availability, autophagy, and lifespan regulation, offering insights into the molecular mechanisms underlying the anti-aging effects of dietary restriction.

A Role for Autophagy in the Extension of Lifespan by Dietary Restriction in *C. elegans*

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In many organisms, dietary restriction appears to extend lifespan, at least in part, by down-regulating the nutrient-sensor TOR (Target Of Rapamycin). TOR inhibition elicits autophagy, the large-scale recycling of cytoplasmic macromolecules and organelles. In this study, we asked whether autophagy might contribute to the lifespan extension induced by dietary restriction in *C. elegans*. We find that dietary restriction and TOR inhibition produce an autophagic phenotype and that inhibiting genes required for autophagy prevents dietary restriction and TOR inhibition from extending lifespan. The longevity response to dietary restriction in *C. elegans* requires the PHA-4 transcription factor. We find that the autophagic response to dietary restriction also requires PHA-4 activity, indicating that autophagy is a transcriptionally regulated response to food limitation. In spite of the rejuvenating effect that autophagy is predicted to have on cells, our findings suggest that autophagy is not sufficient to extend lifespan. Long-lived *daf-2* insulin/IGF-1 receptor mutants require both autophagy and the transcription factor DAF-16/FOXO for their longevity, but we find that autophagy takes place in the absence of DAF-16. Perhaps autophagy is not sufficient for lifespan extension because although it provides raw material for new macromolecular synthesis, DAF-16/FOXO must program the cells to recycle this raw material into cell-protective longevity proteins.

Citation: Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, et al. (2008) A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. *PLoS Genet* 4(2): e24. doi:10.1371/journal.pgen.0040024

Introduction

Dietary restriction, the reduced intake of food without malnutrition, increases the lifespan of many organisms, from yeast to mammals [1]. Dietary restriction increases lifespan, at least in part, by reducing the activities of pathways involved in growth and nutrient processing, including the TOR (Target Of Rapamycin) pathway. Inhibition of the TOR pathway extends lifespan in yeast, worms and flies [2–5], and dietary restriction cannot further extend the lifespans of yeast, worms or flies in which the TOR pathway has been inhibited [3,4,6]. This suggests that down-regulation of the TOR pathway plays an important role in the longevity response to food limitation.

TOR regulates several processes that could be involved in the longevity response to dietary restriction. For instance, TOR stimulates protein synthesis in yeast and in mammals by modulating key components of the translation machinery, including the ribosomal-protein S6 kinase (S6K) and the translation initiation factor 4E-binding protein (4E-BP). Inhibition of positive regulators of translation, including S6K, extends lifespan in both worms and flies [3, 6–10] and inhibition of the negative regulator 4E-BP shortens lifespan in flies [11]. One could imagine that TOR inhibition extends lifespan solely by inhibiting protein synthesis. However, another process regulated by TOR, autophagy [12], could also potentially influence the longevity of animals subjected to dietary restriction.

Macroautophagy (hereafter referred to as autophagy) is a process in which portions of the cytoplasm, including mitochondria and other organelles, are degraded under

conditions of nutrient limitation, allowing cellular macromolecules to be catabolized and recycled. During autophagy, large double-membrane vesicles, called autophagosomes, are generated and degraded in lysosomes, together with their contents. The breakdown products are subsequently recycled to the cytoplasm [13]. The regulation of autophagy has been studied extensively in yeast [14]. In this organism, autophagy is controlled by the ATG genes, many of which have functional homologs in other organisms [13,15]. In yeast, TOR inhibits the protein kinase Atg1, which would otherwise mediate an early activation step in the autophagic process [16]. In response to Atg1 activity, the Vps34 complex, which contains the Class III phosphatidylinositol-3-kinase Vps34 as well as Atg6/Vps30, the ortholog of the mammalian protein Beclin1, stimulates and nucleates the formation of autophagosomes [14,17,18].

Autophagy is induced under conditions of stress, including nutrient limitation. For instance, dietary restriction stim-

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2010: Linda Partridge

The effect of mTOR inhibition on lifespan requires autophagy involvement.

The 2010 paper by Bjedov et al., titled "Mechanisms of Life Span Extension by Rapamycin in the Fruit Fly *Drosophila melanogaster*", published in *Cell Metabolism*, explores how the drug rapamycin extends lifespan in the fruit fly *Drosophila melanogaster*.

The study demonstrates that rapamycin, an inhibitor of the TOR pathway, significantly extends lifespan in flies when administered in adulthood, suggesting that TOR signaling plays a critical role in aging. The researchers found that rapamycin's lifespan-extending effects are mediated through multiple mechanisms, including reduced protein synthesis, improved stress resistance, and enhanced autophagy, a cellular process that degrades damaged organelles and misfolded proteins.

They also observed that rapamycin delays age-related pathologies, such as intestinal stem cell dysfunction and cardiac aging, further supporting its role in promoting healthy aging. The study highlights the importance of the TOR pathway in regulating lifespan and suggests that modulating this pathway could be a viable strategy to extend lifespan and improve healthspan.

Additionally, the researchers noted that the effects of rapamycin are influenced by dietary composition, with the drug being more effective in extending lifespan under certain dietary conditions.

Mechanisms of Life Span Extension by Rapamycin in the Fruit Fly *Drosophila melanogaster*

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SUMMARY

The target of rapamycin (TOR) pathway is a major nutrient-sensing pathway that, when genetically downregulated, increases life span in evolutionarily diverse organisms including mammals. The central component of this pathway, TOR kinase, is the target of the inhibitory drug rapamycin, a highly specific and well-described drug approved for human use. We show here that feeding rapamycin to adult *Drosophila* produces the life span extension seen in some TOR mutants. Increase in life span by rapamycin was associated with increased resistance to both starvation and paraquat. Analysis of the underlying mechanisms revealed that rapamycin increased longevity specifically through the TORC1 branch of the TOR pathway, through alterations to both autophagy and translation. Rapamycin could increase life span of weak insulin/Igf signaling (IIS) pathway mutants and of flies with life span maximized by dietary restriction, indicating additional mechanisms.

INTRODUCTION

The TOR (target of rapamycin) pathway is a highly conserved nutrient-sensing pathway that functions in such evolutionarily distinct organisms as yeast and mammals to regulate growth and metabolism in response to growth factors, amino acids, various stresses, and changes in cellular energy status. It has also been implicated in the control of protein translation and ribosome biogenesis, upregulation of which is required for growth (for detailed review, see Wullschlegel et al., 2006). More recently, the TOR pathway has emerged as an important modulator of aging, an effect firmly established in several invertebrate models (Kaeberlein and Kennedy, 2008).

The central component of the TOR pathway is TOR kinase, which participates in two different multiprotein complexes: TORC1 and TORC2. TORC1 regulates translation and growth through phosphorylation of two key downstream effectors, S6K and 4E-BP. Thus, under favorable conditions, such as an amino acid-rich diet, when TORC1 is active, phosphorylated S6K functions as a positive mediator of the TOR pathway by promoting

cellular and organismal growth, and additionally by altering metabolism (Montagne et al., 1999; Um et al., 2006). Accordingly, S6K-deficient animals are smaller, and their metabolism replicates conditions of low-calorie diet, although the exact mechanisms responsible are not completely understood (Um et al., 2006). TORC1-dependent phosphorylation of 4E-BP disrupts its association with the translation initiation factor 4E (eIF4E), allowing the latter to promote cap-dependent translation. All nuclear-encoded eukaryotic mRNAs possess a cap structure consisting of 5' 7-methylguanosine, which allows them to be recruited to the ribosome and translated in a cap-dependent manner when conditions are favorable (Sonenberg and Hinnebusch, 2009). However, under stress, such as lack of nutrients or growth factors, and in hypoxia, cap-independent translation increases (Sonenberg and Hinnebusch, 2009), allowing a switch to synthesis of other proteins, such as growth factors and heat shock proteins (Baird et al., 2006), which enables the cell to respond to stress and to survive, rather than to initiate apoptosis.

Under starvation, TORC1 additionally promotes autophagy, a critical process for supplying starving cells with bioenergetic components (Um et al., 2005). During autophagy, cytosolic contents targeted for degradation, including proteins, lipids, nucleic acid, and carbohydrates, are enclosed by a double-membrane structure, the autophagosome, which ultimately fuses with a lysosome for degradation of its cargo (Klionsky, 2007). This not only provides the cell with nutrients and energy but also removes damaged cellular components and thus has an important cytoprotective function.

In contrast to the plethora of known TORC1 functions, the TORC2 complex seems to be mainly involved in actin organization and, additionally, to upregulate the IIS pathway by activating phosphorylation of Akt, the main kinase in the IIS pathway (Wullschlegel et al., 2006). Akt can then phosphorylate and inactivate TSC2, a TOR pathway suppressor. Contrary to the positive effects of TORC2 on the IIS pathway, S6K, a downstream component of TORC1, inhibits IIS by negatively regulating the insulin receptor substrate (IRS) at the level of transcription, degradation and phosphorylation (Um et al., 2006; Wullschlegel et al., 2006). Thus, there is a complex cross-wiring between the two signaling pathways, the outcome of which may depend on cell type and on the intensity and duration of the signal (Sarbasov et al., 2006).

TOR kinase is inhibited by rapamycin, a natural macrolide compound isolated from bacteria, analogs of which are

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2010: Perrimon Laboratory



FOXO and its downstream target 4E-BP (a regulator of protein synthesis) are key mediators of proteostasis in muscle tissue.

The 2010 paper by Demontis and Perrimon, titled "FOXO/4E-BP Signaling in *Drosophila* Muscles Regulates Organism-Wide Proteostasis During Aging", published in *Cell*, explores the role of muscle-specific signaling pathways in regulating systemic protein homeostasis (proteostasis) and aging in *Drosophila melanogaster* (fruit flies). The study identifies the transcription factor FOXO and its downstream target 4E-BP (a regulator of protein synthesis) as key mediators of proteostasis in muscle tissue.

The researchers found that activating FOXO/4E-BP signaling in muscles reduces age-related protein aggregation not only in the muscles themselves but also in distant tissues, such as the brain. This systemic effect is mediated by the secretion of factors from muscles that enhance proteostasis throughout the organism, highlighting the central role of muscle tissue in coordinating organism-wide responses to aging.

The study also demonstrates that FOXO/4E-BP signaling in muscles improves lifespan and healthspan in *Drosophila*. By reducing protein synthesis and enhancing autophagy, this signaling pathway mitigates the accumulation of damaged proteins, a hallmark of aging. The researchers showed that muscle-specific activation of FOXO/4E-BP delays age-related functional decline and extends lifespan, underscoring the importance of maintaining proteostasis in muscle tissue for overall organismal health. These findings reveal a novel mechanism by which muscle tissue acts as a signaling hub to regulate systemic aging and proteostasis, offering insights into the interplay between tissue-specific and organism-wide aging processes.

FOXO/4E-BP Signaling in *Drosophila* Muscles Regulates Organism-wide Proteostasis during Aging

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SUMMARY

The progressive loss of muscle strength during aging is a common degenerative event of unclear pathogenesis. Although muscle functional decline precedes age-related changes in other tissues, its contribution to systemic aging is unknown. Here, we show that muscle aging is characterized in *Drosophila* by the progressive accumulation of protein aggregates that associate with impaired muscle function. The transcription factor FOXO and its target 4E-BP remove damaged proteins at least in part via the autophagy/lysosome system, whereas foxo mutants have dysfunctional proteostasis. Both FOXO and 4E-BP delay muscle functional decay and extend life span. Moreover, FOXO/4E-BP signaling in muscles decreases feeding behavior and the release of insulin from producing cells, which in turn delays the age-related accumulation of protein aggregates in other tissues. These findings reveal an organism-wide regulation of proteostasis in response to muscle aging and a key role of FOXO/4E-BP signaling in the coordination of organismal and tissue aging.

INTRODUCTION

Aging of multicellular organisms involves distinct pathogenic events that include higher mortality, the progressive loss of organ function, and susceptibility to degenerative diseases, some of which arise from protein misfolding and aggregation. Recent genetic studies in the mouse, the nematode *Caenorhabditis elegans*, and the fruitfly *Drosophila melanogaster* have expanded our understanding of the evolutionarily conserved signaling pathways regulating aging, with the identification of several mutants that have prolonged or shortened life spans (Kenyon, 2005). Manipulation of longevity-regulating pathways in certain tissues is sufficient to extend life expectancy, indicating that some tissues have a predominant role in life span extension (Libina et al., 2003; Wang et al., 2005; Wolkow et al., 2000). For example, foxo overexpression in the *Drosophila* fat

body extends life span, indicating a key role of this tissue in the regulation of longevity (Giannakou et al., 2004; Hwangbo et al., 2004). In addition, because most tissues undergo progressive deterioration during aging (Gargan et al., 2002), it is thought that organismal life span may be linked to tissue senescence. However, our understanding of the mechanisms regulating tissue aging and their interconnection to life span is limited. For example, analysis in *Drosophila* has revealed that the prevention of age-dependent changes in cardiac performance does not alter life span (Wessells et al., 2004), raising the possibility that functional decline in distinct tissues may have different outcomes on the systemic regulation of aging.

The Insulin/IGF-1 signaling pathway has been implicated in the control of aging across evolution via its downstream signaling component FOXO (DAF-16 in *C. elegans*), a member of the fork-head box O transcription factor family (Salk and Brunet, 2008). FOXO regulates the expression of a series of target genes involved in metabolism, cell growth, cell proliferation, stress resistance, and differentiation via direct binding to target gene promoter regions (Salk and Brunet, 2008). Mutations in foxo/daF-16 reduce life span and stress resistance in both *C. elegans* and flies, indicating a key role in organism aging (Jung et al., 2003; Salk and Brunet, 2008). In addition to regulating life span, FOXO has been reported to prevent the pathogenesis of some age-related diseases. For example, FOXO reduces the toxicity associated with aggregation-prone human mutant Alzheimer's and Huntington's disease proteins (proteotoxicity) in *C. elegans* and mice, suggesting that regulating protein homeostasis (proteostasis) during aging may have a direct effect on the pathogenesis of human neurodegenerative diseases (Cohen et al., 2006; Hsu et al., 2003; Morley et al., 2002). However, little is known on the protective mechanisms induced in response to FOXO signaling and whether they vary in different aging tissues and disease contexts.

Among the plethora of age-related pathological conditions, the gradual decay in muscle strength is one of the hallmarks of aging in many organisms, including *Drosophila*, *C. elegans*, mice and, importantly, humans (Augustin and Partridge, 2009; Hemdon et al., 2002; Nair, 2005; Zheng et al., 2006). However, despite its medical relevance, the mechanisms underlying muscle aging are incompletely understood. Functional changes in skeletal muscles temporally precede the manifestation of

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Demontis F, Perrimon N. FOXO/4E-BP signaling in *Drosophila* muscles regulates organism-wide proteostasis during aging. *Cell*. Nov 24, 2010;143(5):813–25.

2011: Reuben Shaw

AMPK phosphorylates ULK1 (hATG1) to connect energy sensing to mitophagy.

This heavily cited 2011 *Science* paper by Reuben Shaw, Malene Hansen and team explores the molecular mechanisms linking cellular energy sensing to the process of mitophagy, the selective degradation of damaged mitochondria.

The researchers identify the kinase ULK1 (unc-51 like autophagy activating kinase 1), a key initiator of autophagy, as a direct target of AMP-activated protein kinase (AMPK), a central regulator of cellular energy homeostasis. Under conditions of energy stress, such as glucose deprivation or mitochondrial dysfunction, AMPK phosphorylates ULK1 at specific sites, activating its function.

This phosphorylation event is critical for ULK1's role in initiating mitophagy, as it enables the recruitment of downstream autophagy machinery to damaged mitochondria. The study demonstrates that AMPK-mediated activation of ULK1 is essential for maintaining mitochondrial quality control and cellular survival during energy stress, highlighting a direct connection between energy sensing and autophagy regulation.

Furthermore, the researchers show that this AMPK-ULK1 axis is conserved across species, emphasizing its fundamental role in cellular physiology. They also reveal that the mTORC1 complex, a well-known inhibitor of autophagy, negatively regulates ULK1 by phosphorylating it at different sites, creating a regulatory balance between AMPK and mTORC1 in response to nutrient availability. This work links how cells integrate energy status with mitochondrial quality control, offering a mechanistic understanding of how autophagy is fine-tuned to maintain cellular health.

Phosphorylation of ULK1 (hATG1) by AMP-Activated Protein Kinase Connects Energy Sensing to Mitophagy

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Adenosine monophosphate-activated protein kinase (AMPK) is a conserved sensor of intracellular energy activated in response to low nutrient availability and environmental stress. In a screen for conserved substrates of AMPK, we identified ULK1 and ULK2, mammalian orthologs of the yeast protein kinase Atg1, which is required for autophagy. Genetic analysis of AMPK or ULK1 in mammalian liver and *Caenorhabditis elegans* revealed a requirement for these kinases in autophagy. In mammals, loss of AMPK or ULK1 resulted in aberrant accumulation of the autophagy adaptor p62 and defective mitophagy. Reconstitution of ULK1-deficient cells with a mutant ULK1 that cannot be phosphorylated by AMPK revealed that such phosphorylation is required for mitochondrial homeostasis and cell survival during starvation. These findings uncover a conserved biochemical mechanism coupling nutrient status with autophagy and cell survival.

A highly conserved sensor of cellular nutrient status found in all eukaryotes is the adenosine monophosphate (AMP)-activated protein kinase (AMPK). In response to decreases in intracellular ATP, AMPK is activated and serves as a metabolic checkpoint, restoring ATP levels through acute regulation of metabolic enzymes and inhibition of pro-growth anabolic pathways (1). Inactivation of LKB1, the upstream kinase necessary for activation of AMPK under low-energy conditions, is a frequent event in sev-

eral forms of human cancer (2). In addition, LKB1 signaling is required in the liver for the therapeutic effect of metformin, the most prevalent type 2 diabetes drug worldwide, and LKB1 inactivation in mouse liver results in a type 2 diabetes-like metabolic disease (3). Thus the LKB1-AMPK pathway provides a direct link between tumor suppression and control of cellular and organismal metabolism.

Similar to AMPK activation, the cellular process of autophagy is initiated under nutrient-poor

456

28 JANUARY 2011 VOL 331 SCIENCE www.sciencemag.org

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2012: Andrea Ballabio Lab

TFEB (Transcription Factor EB) is a master regulator that coordinates autophagy and lysosomal biogenesis.

The 2011 paper by Settembre et al., titled "TFEB Links Autophagy to Lysosomal Biogenesis", published in *Science*, identifies the transcription factor TFEB (Transcription Factor EB) as a master regulator that coordinates autophagy and lysosomal biogenesis. The study demonstrates that TFEB directly controls the expression of genes involved in both autophagy and lysosomal function, creating a coordinated cellular response to nutrient deprivation and stress. Under conditions of starvation or lysosomal stress, TFEB translocates from the cytoplasm to the nucleus, where it activates the transcription of genes encoding autophagy-related proteins and lysosomal enzymes.

This dual role of TFEB enhances the cell's capacity to degrade and recycle cellular components, thereby maintaining cellular homeostasis. The researchers also found that overexpression of TFEB in cultured cells and in vivo models increases autophagic flux and lysosomal activity, while its inhibition impairs these processes. These findings reveal a critical regulatory network that integrates autophagy and lysosomal function, highlighting TFEB as a central player in cellular clearance pathways. The study has significant implications for understanding and potentially treating diseases characterized by impaired autophagy and lysosomal dysfunction, such as neurodegenerative disorders and lysosomal storage diseases. Overall, this work establishes TFEB as a key regulator of cellular homeostasis and provides a foundation for therapeutic strategies aimed at modulating autophagy and lysosomal activity.

TFEB Links Autophagy to Lysosomal Biogenesis

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Autophagy is a cellular catabolic process that relies on the cooperation of autophagosomes and lysosomes. During starvation, the cell expands both compartments to enhance degradation processes. We found that starvation activates a transcriptional program that controls major steps of the autophagic pathway, including autophagosome formation, autophagosome-lysosome fusion, and substrate degradation. The transcription factor EB (TFEB), a master gene for lysosomal biogenesis, coordinated this program by driving expression of autophagy and lysosomal genes. Nuclear localization and activity of TFEB were regulated by serine phosphorylation mediated by the extracellular signal-regulated kinase 2, whose activity was tuned by the levels of extracellular nutrients. Thus, a mitogen-activated protein kinase-dependent mechanism regulates autophagy by controlling the biogenesis and partnership of two distinct cellular organelles.

Macro-autophagy is an evolutionarily conserved mechanism that targets intracytoplasmic material to lysosomes, thus providing an energy supply during nutrient starvation (1, 2). Autophagy activation during starvation is negatively regulated by mammalian target of rapamycin complex 1 (mTORC1), whose activity is dependent on cellular energy needs (3). The observation that starvation induced the transcription of several autophagy genes, whereas inhibition of mTORC1 did not, suggests the presence of alternative transcriptional mTORC1-independent regulation of autophagy (4, 5) (fig. S1, A and B).

We tested whether TFEB, a transcription factor ("EB") that controls lysosomal biogenesis by positively regulating genes belonging to the Co-

ordinated Lysosomal Expression and Regulation (CLEAR) network, also regulated autophagy (6, 7). Stable TFEB overexpression in HeLa cells significantly increased the number of autophagosomes detected by immunofluorescence and immunoblotting of the LC3 protein, which specifically associates with autophagosomes (8) (Fig. 1, A and B). Similar data were obtained by transient overexpression of TFEB in HeLa and COS7 (monkey kidney fibroblast) cells (fig. S2, A to C) and from electron microscopy on mouse embryonic fibroblasts (MEFs) infected with a lentivirus overexpressing TFEB (fig. S3, A to D). This increase persisted in cells treated with bafilomycin and pepstatin, as well as the cysteine proteinase inhibitor E64, which are lysosomal inhibitors of autophagosome and LC3-II degradation; the sustained increase indicated that TFEB activates the formation of autophagosomes (Fig. 1A and fig. S4, A and B).

RNA interference (RNAi) of *TFEB* in HeLa cells resulted in decreased levels of LC3-II both in normal and starved conditions, in either the presence or absence of bafilomycin (Fig. 1, C and D). The decrease of LC3-II correlated with the levels of *TFEB* down-regulation achieved by the different RNAi oligomers (fig. S4, C and D). These gain- and loss-of-function data suggest

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Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, Erdin SU, Huynh T, Medina D, Colella P, Sardiello M, Rubinsztein DC, Ballabio A. TFEB links autophagy to lysosomal biogenesis. *Science*, Jun 17, 2011; 332(6036):1429-33.

2013: Yong-Keun Jung

Overexpression of Atg5 increases mouse lifespan by activating autophagy.

The 2013 paper by Pyo et al., titled "Overexpression of Atg5 in mice activates autophagy and extends lifespan", published in *Nature Communications*, investigates the effects of enhancing autophagy on lifespan and health in mice. The researchers generated transgenic mice that overexpressed Atg5, a key protein involved in the formation of autophagosomes, the structures responsible for engulfing and degrading cellular debris.

They found that increased Atg5 expression led to enhanced autophagy, as evidenced by higher levels of autophagic flux and reduced accumulation of damaged proteins and organelles. Importantly, the study demonstrated that these mice exhibited a significant extension in lifespan compared to wild-type controls, along with improved metabolic health and reduced age-related pathologies. These findings suggest that boosting autophagy can delay aging and promote longevity in mammals.

The study also provides mechanistic insights into how autophagy contributes to lifespan extension. Enhanced autophagy in Atg5-overexpressing mice was associated with reduced oxidative stress, improved mitochondrial function, and decreased levels of cellular damage, all of which are hallmarks of aging. The researchers observed that the mice maintained better physical performance and metabolic homeostasis as they aged, further supporting the role of autophagy in preserving cellular and organismal health.



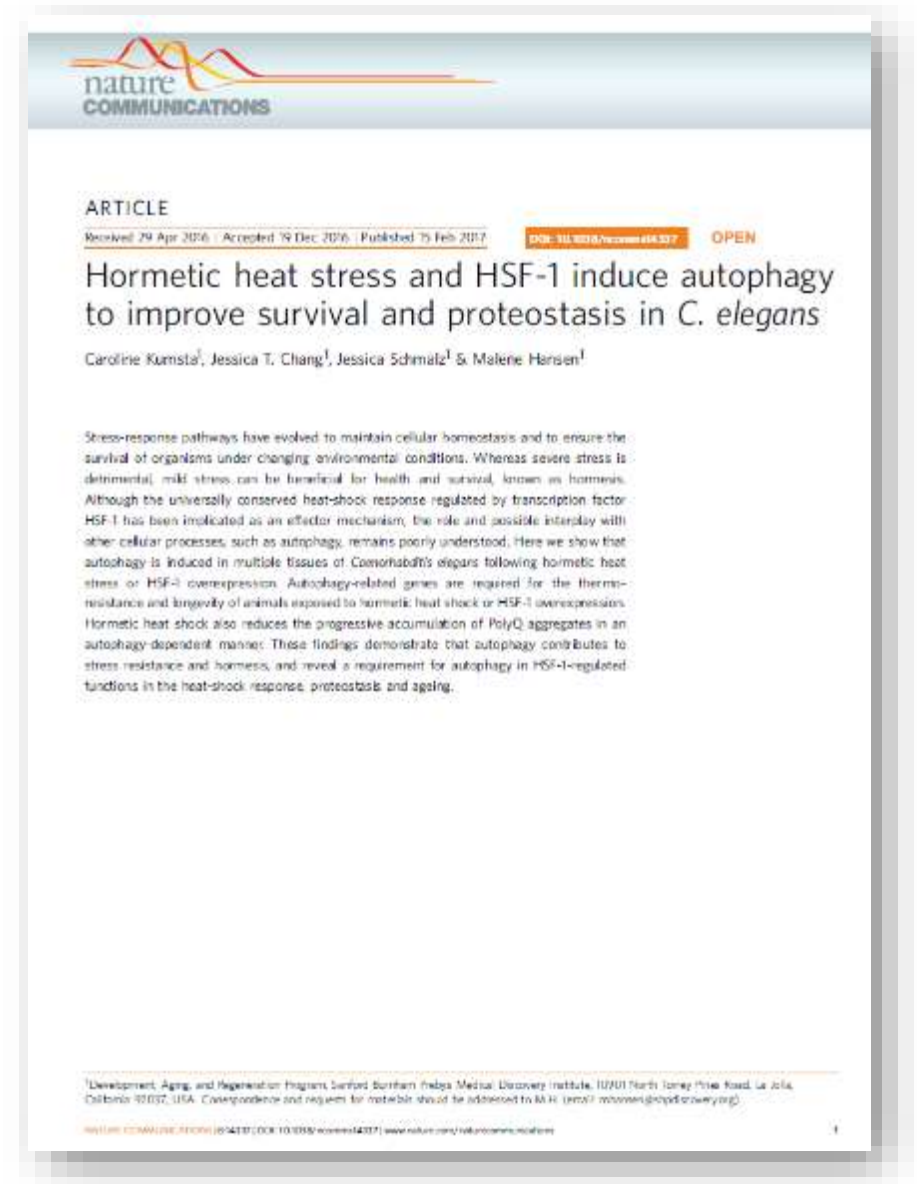
Pyo JO, Yoo SM, Ahn HH, Nah J, Hong SH, Kam TI, Jung S, Jung YK. Overexpression of Atg5 in mice activates autophagy and extends lifespan. Nat Commun. 2013;4:2300.

2017: Malene Hansen Lab

Autophagy contributes to stress resistance and hormesis. This paper identifies a requirement for autophagy in HSF-1-regulated functions in the heat-shock response, proteostasis and aging.

The 2017 paper by Kumsta et al., titled "Hormetic Heat Stress and HSF-1 Induce Autophagy to Improve Survival and Proteostasis in *C. elegans*", published in *Nature Communications*, investigates how mild heat stress, a form of hormesis, activates autophagy to improve survival and protein homeostasis (proteostasis) in *Caenorhabditis elegans* (nematodes). The researchers found that exposure to mild heat stress induces the heat shock factor **HSF-1**, a key regulator of the heat shock response, which in turn activates autophagy. This activation of autophagy enhances the clearance of damaged proteins and aggregates, improving cellular proteostasis and increasing survival under stress conditions. The study demonstrates that autophagy is essential for the beneficial effects of hormetic heat stress, as blocking autophagy abolishes the improvements in survival and proteostasis. These findings highlight the interplay between the heat shock response and autophagy in maintaining cellular health under stress.

The study also reveals that HSF-1 directly regulates autophagy by promoting the expression of autophagy-related genes, providing a mechanistic link between heat stress, proteostasis, and longevity. The researchers showed that the activation of autophagy by HSF-1 is conserved across species, suggesting that this pathway plays a fundamental role in stress responses and aging. By demonstrating that mild heat stress can improve proteostasis and survival through HSF-1-mediated autophagy, the study underscores the potential of hormetic interventions to promote healthy aging.



Kumsta C, Chang JT, Schmalz J, Hansen M. Hormetic heat stress and HSF-1 induce autophagy to improve survival and proteostasis in *C. elegans*. *Nat Commun*. Feb 15, 2017;8:14337.

2018: Beth Levine

Decreased Beclin1 / BCL2 interaction increases autophagy and extends lifespan. Importantly, this study was done in mammals.

The 2018 paper from the Beth Levine autophagy group at UT Southwestern titled "Disruption of the Beclin 1-BCL2 Autophagy Regulatory Complex Promotes Longevity in Mice", published in *Nature*, explores how modulating the interaction between Beclin 1 and BCL2—a key regulatory complex that controls autophagy—can influence lifespan and healthspan in mice. The study demonstrates that disrupting the Beclin 1-BCL2 complex enhances autophagy, a cellular process that degrades damaged organelles and misfolded proteins, thereby maintaining cellular homeostasis.

Mice with a mutation that prevents Beclin 1 from binding to BCL2 exhibited increased autophagic activity, improved metabolic health, and extended lifespan. These mice also showed reduced age-related pathologies, such as spontaneous tumors and heart and kidney dysfunction, highlighting the role of autophagy in promoting healthy aging. The findings suggest that the Beclin 1-BCL2 complex acts as a critical brake on autophagy, and its disruption can unleash the anti-aging potential of enhanced autophagy.

The study provides mechanistic insights into how autophagy regulation impacts longevity and age-related diseases. By preventing the inhibitory interaction between Beclin 1 and BCL2, the researchers were able to boost autophagy without causing detrimental effects, demonstrating that controlled enhancement of autophagy can be beneficial. The findings also suggest that pharmacological interventions targeting the Beclin 1-BCL2 interaction could mimic the effects of the mutation, offering a potential therapeutic strategy to promote healthy aging.

Disruption of the beclin 1–BCL2 autophagy regulatory complex promotes longevity in mice

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Autophagy increases the lifespan of model organisms; however, its role in promoting mammalian longevity is less well-established^{1,2}. Here we report lifespan and healthspan extension in a mouse model with increased basal autophagy. To determine the effects of constitutively increased autophagy on mammalian health, we generated targeted mutant mice with a Phe121Ala mutation in beclin 1 (*Becn1*^{F121A/F121A}) that decreases its interaction with the negative regulator BCL2. We demonstrate that the interaction between beclin 1 and BCL2 is disrupted in several tissues in *Becn1*^{F121A/F121A} knock-in mice in association with higher levels of basal autophagic flux. Compared to wild-type littermates, the lifespan of both male and female knock-in mice is significantly increased. The healthspan of the knock-in mice also improves, as phenotypes such as age-related renal and cardiac pathological changes and spontaneous tumorigenesis are diminished. Moreover, mice deficient in the anti-aging protein klotho³ have increased beclin 1 and BCL2 interaction and decreased autophagy. These phenotypes, along with premature lethality and infertility, are rescued by the beclin 1(F121A) mutation. Together, our data demonstrate that disruption of the beclin 1–BCL2 complex is an effective mechanism to increase autophagy, prevent premature aging, improve healthspan and promote longevity in mammals.

Autophagy, an evolutionarily conserved lysosomal degradation pathway, has a key role in tissue homeostasis, health and disease⁴. In 2003, we showed that the *Caenorhabditis elegans* autophagy gene *bec-1* (orthologue of yeast *ATG6*, mammalian beclin 1) was required for lifespan extension in nematodes with a loss of function in the insulin signalling pathway⁵. Subsequently, numerous loss-of-function studies in *C. elegans* and *Drosophila* have confirmed an essential role for the autophagy machinery in longevity^{1,2}, and tissue-specific deletion of core autophagy genes have shown that autophagy delays age-related changes in mouse tissues, including kidney and heart^{6,7}. Moreover, physiological inducers (such as caloric restriction) as well as pharmacological inducers (such as spermidine) of autophagy increase lifespan in mice^{1,8,9}. Despite these clues that autophagy may be a longevity pathway in mammals, definitive evidence that increased basal autophagy extends mammalian healthspan and lifespan is lacking.

An earlier study¹⁰ demonstrated an increase in lifespan of mice that transgenically overexpress *ATG5*. However, it is unclear how overexpression of *ATG5*, a protein necessary for autophagy but not directly involved in the regulation of autophagy levels, results in increased autophagy. Moreover, *ATG5* has other key functions, such as the regulation of inflammation¹¹, and these roles are not shared by other genes in the autophagy pathway. Therefore, it is imperative to use a more direct and specific genetic approach to assess the effects of enhanced basal autophagy on mammalian lifespan and healthspan. To do so,

we focused on the mammalian autophagy protein, beclin 1 (encoded by *Becn1*)¹², which is part of an autophagy-specific class III phosphatidylinositol-3-OH kinase (PI3K) complex¹³ that has a key role in the regulation of the initiation of autophagosome formation¹⁴.

We recently reported the construction of mice with a Phe-to-Ala knock-in substitution mutation in the BH3 domain of beclin 1 (F121A; corresponding to F123A in human beclin 1)¹⁵ that decreases the binding of two negative regulators of autophagy (BCL2 and BCL-XL) to beclin 1 in vitro^{16,17}. Using these mice, we performed co-immunoprecipitation of endogenous beclin 1 with BCL2 in muscle, heart, kidney and liver of two-month-old wild-type and homozygous knock-in mice. We observed a marked reduction in beclin 1 co-immunoprecipitation with BCL2 in the tissues of the knock-in mice (Fig. 1a, b). In parallel, we analysed autophagic flux by crossing wild-type or knock-in mice with animals that transgenically express green fluorescent protein (GFP)-tagged LC3¹⁸, a fluorescent marker of autophagosomes. In skeletal muscle, heart, renal glomeruli, proximal convoluted tubules and liver, knock-in mice had significantly increased numbers of GFP-LC3 puncta compared to wild-type control littermates (Fig. 1c, d; Extended Data Fig. 1). In all tissues except for the liver, there was a further increase in GFP-LC3 puncta after treatment with chloroquine, an inhibitor of lysosomal acidification and autophagosome-lysosomal fusion, indicating that the increased numbers of GFP-LC3 puncta in knock-in mice represents a true increase in basal autophagic flux, rather than a block in autophagosomal maturation. We further confirmed that knock-in mice had increased autophagic flux by western blot analyses. Both hearts and kidneys had increased conversion of LC3-I to LC3-II (the lipidated, autophagosome-associated form of LC3), decreased levels of total LC3 and decreased levels of the autophagy substrate p62¹⁹ (Fig. 1e, f). Similar findings were also observed in the hearts and kidneys of six- to eight-month-old mice (Extended Data Fig. 2), indicating that the effects of the knock-in mutation are sustained over time in adulthood.

We further evaluated the effect of the knock-in mutation on autophagy using mouse embryonic fibroblasts (MEFs) derived from knock-in or wild-type littermate controls. In knock-in MEFs, there was decreased co-immunoprecipitation of beclin 1 with BCL2, increased numbers of GFP-LC3 puncta, decreased levels of p62 and total LC3, and increased numbers of autophagic structures (autophagosomes and autolysosomes) observed by quantitative electron microscopy (Extended Data Fig. 3a–d). Treatment with the lysosomal inhibitor bafilomycin A1 indicated a bona fide increase in autophagic flux in knock-in MEFs. To evaluate possible effects of the beclin 1 knock-in mutation other than autophagy, we examined endocytosis, a process thought to involve the beclin 1–VPS34 complex²⁰. We did not observe any significant differences in endocytosis between knock-in and

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2025: Lazarou, Hummer and Hurley

The cross-national team solved for the co-crystal structure of PI3-Kinase with its subunit partner VPS15, a pseudo-kinase.

You will recall that other researchers have already discovered that ATG14 is required for autophagy as is Beclin1 is required. Michael Lazarou from WEHI in Australia, Gerhard Hummer of the Max Planck and Jim Hurley of UC Berkeley to try to figure out how yet another gene/protein pair VPS15 comes into play. While this all sounds like meaningless alphabet soup, the biochemistry clarified in this *tour de force* paper in the Feb 6, 2025 issue of *Science* is anything but. Turning on macro-autophagy is not to be done lightly given that it is a fundamentally destructive biology. We would only like to see unwanted proteins get eliminated by this process.

The authors of this paper note that there are multiple subassemblies that need to bind and signal for autophagy to be activated. A key subassembly involves the binding of PI3K α 1 to its partners and PI3K α 3 to its partners. The authors show that the binding of PI3-Kinase to three related pseudo-kinases, VPS15, VPS34 and VPS15 is mediated by tight binding to guanosine triphosphate (GTP). They solve for the co-crystal structure of PI3K binding to the pseudo-kinases, particularly VPS15. This turns out to be a non-trivial exercise and this is because VPS15 is quite mobile and thus somewhat hard to pin down for a proper cryo-EM study. The binding of VPS15 to GTP as part of the PI3K complex in turn is shown to activate autophagy and all the various things that this implies.

This is all scientifically highly interesting because a number of diseases such as Parkinson's appear to partly involve breakdown of autophagy. Our blueprint of how the mechanism works is getting sufficiently complete and detailed to permit the design of drugs to repair specific causes of autophagic breakdown.

Science

RESEARCH ARTICLE

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Structural pathway for PI3-kinase regulation by VPS15 in autophagy

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The class III phosphatidylinositol-3 kinase complexes I and II (PI3KC3-C1 and -C2) have vital roles in macroautophagy and endosomal maturation, respectively. We elucidated a structural pathway of enzyme activation through cryo-EM analysis of PI3KC3-C1. The inactive conformation of the VPS15 pseudokinase stabilizes the inactive conformation, sequestering its N-myristate in the N-lobe of the pseudokinase. Upon activation, the myristate is liberated such that the VPS34 lipid kinase catalyzes PI3P production on membranes. The VPS15 pseudokinase domain binds tightly to guanosine triphosphate (GTP), and stabilizes a web of interactions to autoinhibit the cytosolic complex and to promote the activation upon membrane binding. These findings show in atomic detail how the VPS34 lipid kinase is activated in the context of a complete PI3K complex.

Macroautophagy (hereafter autophagy) is the primary eukaryotic catabolic response to insufficient supplies of amino acids, and the main cellular mechanism for clearing toxic aggregates and dysfunctional organelles (1). Defects in autophagy are implicated as drivers of many human diseases (2), such as Parkinson's disease in which the genes *Parkin* and *PINK1* initiate damage-induced mitophagy (3). Autophagy is initiated by the concerted action of several core complexes (4), including the phosphatidylinositol 3-kinase class III complex I (PI3KC3-C1). PI3KC3-C1 is a heterotetrameric complex that comprises one subunit each of the VPS34 lipid kinase, the regulatory scaffold VPS15 (vacuolar protein sorting 15, also known as PIK3R4; phosphoinositide-3-kinase regulatory subunit 4), BECN1 (beclin 1, autophagy-related), and the autophagy-specific subunit ATG14 (autophagy-related 14) (5–9). The related complex II (PI3KC3-C2) which functions in both endosome maturation and autophagy, shares the three subunits VPS34, VPS15, and BECN1, and has the same overall architecture. In PI3KC3-C2, UVRAG (UV radiation resistance associated) replaces ATG14 as the fourth subunit (5). PI3KC3-C1 produces phosphatidylinositol (PI) 3-phosphate (PI3P) on the phagophore, which is the precursor to the autophagosome. PI3P recruits WIP1 (WD repeat domain, phosphoinositide-interacting) proteins, which are in turn critical for all subsequent steps in autophagosome expansion (10). In endosome maturation, PI3P generated by PI3KC3-C2 (11, 12)

recruits the early endosome fusion (13) and ESCRT machinery (14) and other essential factors. In both the autophagic and endosomal pathways, PI3KC3 activation is a decisive step. Because of their central role in autophagy and endosomal sorting, the structure and dynamics of PI3KC3-C1 and -C2 complexes have been intensively studied (15–21). Yet a near-atomic picture of the activating transition has not been described, likely because of the dynamic character of the complex. The VPS34 lipid kinase domain (VPS34^{LD}) is highly mobile relative to the rest of PI3KC3-C1 (15, 17), and its mobility is necessary for function (17). This has made it challenging to determine the structures of conformational substates at high resolution. We obtained a structure of human PI3KC3-C1 at a local resolution of up to 3.5 Å. Building on the insight that the small GTPase RAB1A binds and recruits PI3KC3-C1 (22), we reconstructed the complex in the presence of RAB1A at a local resolution of nearly 2.5 Å in the best-resolved regions, and to classify and refine inactive, transitional, and active conformations, defining the major conformational states along the PI3KC3 activation pathway. We describe a large conformational change in VPS34^{LD} relative to the VPS15 pseudo-kinase domain (VPS15^{PK}). We discovered that an Arg residue at the gatekeeper position of VPS15^{PK}, not detected in other members of the kinase, confers GTP binding. We mapped the position of the covalent N-

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Cook ASI, Chen M, Nguyen TN, Cabezas AC, Khun G, Rao S, Garcia SN, Yang M, Iavarone AT, Ren X, Lazarou M, Hummer G, Hurley JH., “Structural pathway for PI3-kinase regulation by VPS15 in autophagy,” *Science*, Feb 6, 2025: eadl3787.

Section 8.F: Parabiosis

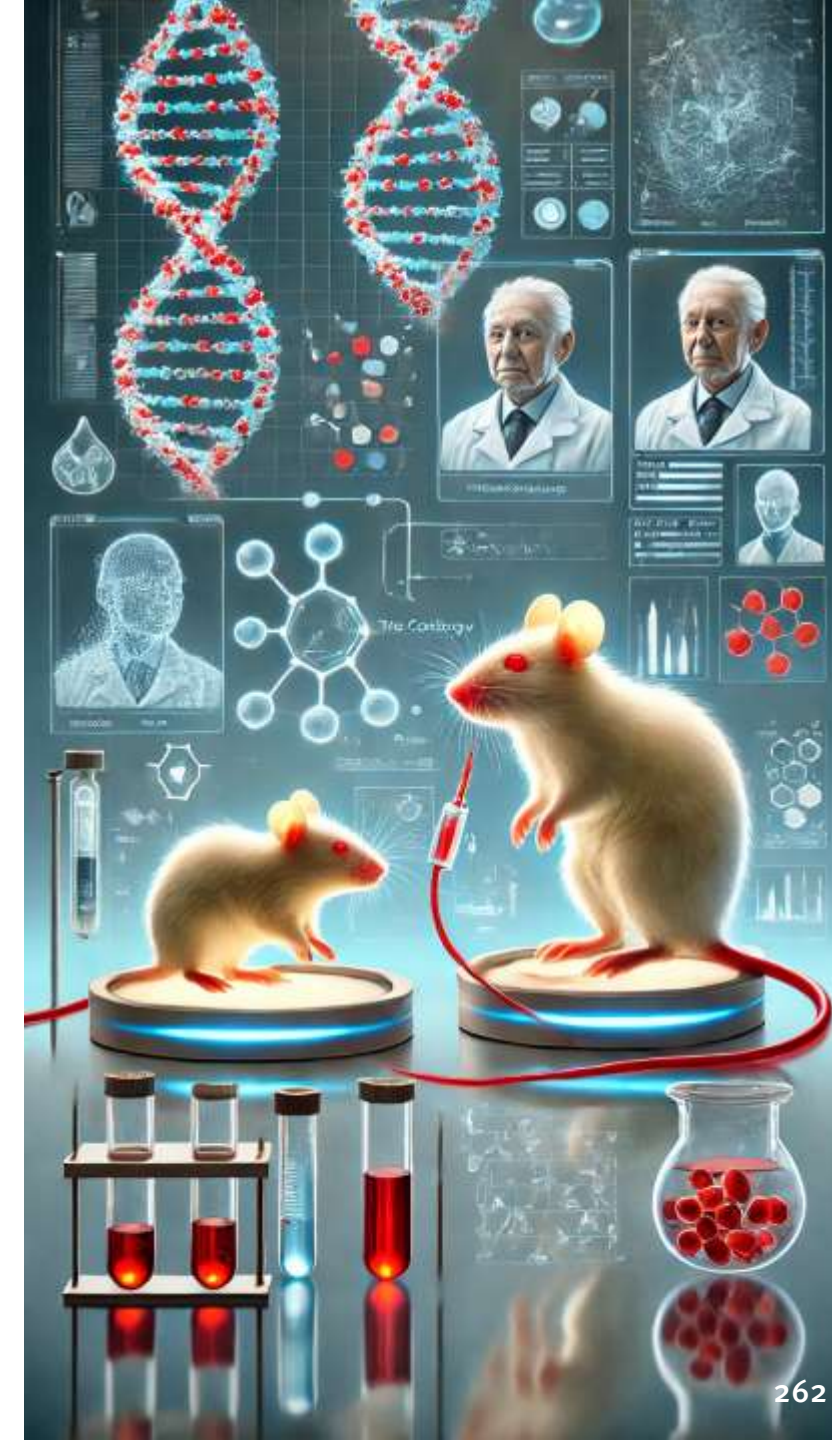
The basic idea of the parabiosis literature is that young animal biology is somewhat transferrable to older animals in a manner that would confer youthfulness.

A related idea has been around for millennia and is featured in the Bible where King David is rejuvenated after lying down between two younger women.

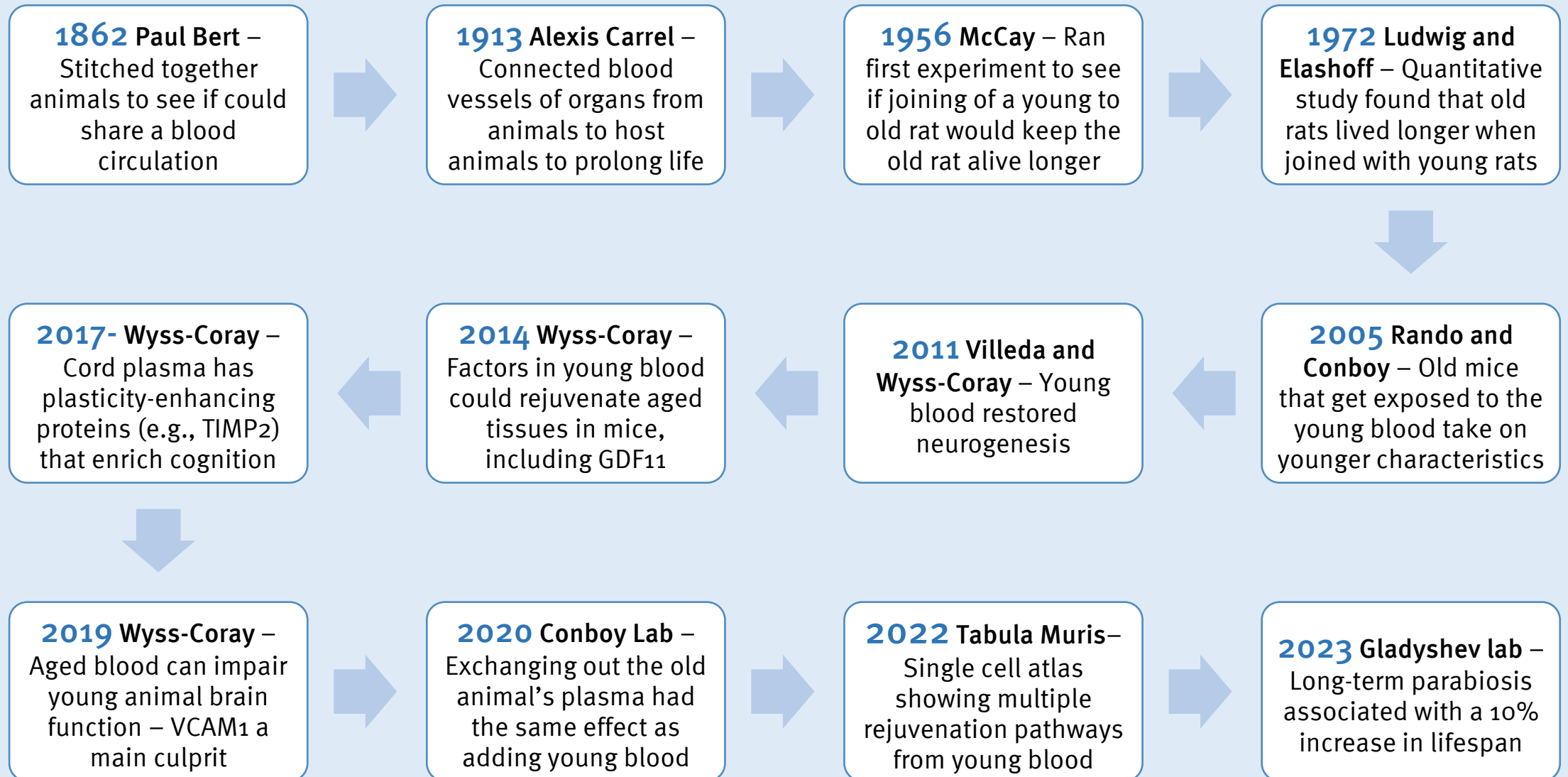
Fast forward to the present. A series of research labs, including those of Tom Rando, the Conboys, Gladyshev and Tony Wyss-Coray, have been exploring the potential of parabiosis, in which blood of younger animals is shared with older animals to achieve a level of rejuvenation.

While this literature sounds vaguely suspect the evidence generated about the underlying biology is anything but. A series of papers have shown convincingly that parabiosis can rejuvenate mice to some degree. Larger mammals have not yet been tested although the ideas of parabiosis have been tried by humans for decades.

The literature addresses all the right questions including how does this happen, what is the form of rejuvenation etc. Obviously, the idea remains exploratory, and we have yet to see rigorous clinical data in primates, humans, dogs etc.



Milestones Publications in the Parabiosis Literature



2005: Rando and Conboy

Thomas Rando, Irina Conboy and Michael Conboy showed that when old mice get exposed to the blood of young mice, they take on younger characteristics. This harkens back to Cohausen's *Hermippus* of 1742.

The 2005 *Nature* paper "*Rejuvenation of Aged Progenitor Cells by Exposure to a Young Systemic Environment*," demonstrated that aging-related declines in stem cell function could be reversed by exposure to a youthful systemic environment. Using a heterochronic parabiosis model—where the circulatory systems of young and old mice were joined—they observed that aged progenitor cells, particularly muscle stem cells (satellite cells), regained a more youthful regenerative capacity. This suggested that age-related declines in tissue repair were not solely due to intrinsic cellular aging but were significantly influenced by systemic, extracellular factors in the blood, such as circulating proteins and signaling molecules.

Their findings challenged the traditional view that aging is an irreversible, cell-autonomous process and highlighted the role of age-related changes in the systemic environment. Specifically, they found that exposure to young blood restored Notch signaling, a key pathway in muscle regeneration, while reducing inhibitory factors associated with aging. This work had profound implications for regenerative medicine and aging research, inspiring later studies on "young blood" therapies, plasma exchange, and the potential of systemic interventions to reverse aspects of aging and age-related diseases.

This paper set off "an extensive search for specific factors in blood that could be related to aging". Today, the field of parabiosis continues to capture the imagination of scientists and entrepreneurs alike.

References: Ramakrishnan p. 192.

Rejuvenation of aged progenitor cells by exposure to a young systemic environment

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The decline of tissue regenerative potential is a hallmark of ageing and may be due to age-related changes in tissue-specific stem cells^{1–5}. A decline in skeletal muscle stem cell (satellite cell) activity due to a loss of Notch signalling results in impaired regeneration of aged muscle^{1,6}. The decline in hepatic progenitor cell proliferation owing to the formation of a complex involving cEBP- α and the chromatin remodelling factor brahma (Brm) inhibits the regenerative capacity of aged liver⁷. To examine the influence of systemic factors on aged progenitor cells from these tissues, we established parabiotic pairings (that is, a shared circulatory system) between young and old mice (heterochronic parabioses), exposing old mice to factors present in young serum. Notably, heterochronic parabiosis restored the activation of Notch signalling as well as the proliferation and regenerative capacity of aged satellite cells. The exposure of satellite cells from old mice to young serum enhanced the expression of the Notch ligand (Delta), increased Notch activation, and enhanced proliferation *in vitro*. Furthermore, heterochronic parabiosis increased aged hepatocyte proliferation and restored the cEBP- α complex to levels seen in young animals. These results suggest that the age-related decline of progenitor cell activity can be modulated by systemic factors that change with age.

Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*, Feb 17 2005; 433(7027):760–4.

2011: Villeda and Wyss-Coray

Young blood restored neurogenesis in older mice.

The 2011 paper by Villeda et al., titled "The ageing systemic milieu negatively regulates neurogenesis and cognitive function", published in *Nature*, explores how systemic factors in aged blood contribute to the decline in neurogenesis and cognitive function during aging. Using a heterochronic parabiosis model, where the circulatory systems of young and old mice are connected, the researchers demonstrated that exposure to aged blood reduces the proliferation and differentiation of neural precursor cells (NPCs) in the hippocampus, a brain region essential for learning and memory. Conversely, young blood was found to enhance neurogenesis and improve cognitive function in aged mice. The study identified specific factors in aged blood, such as elevated levels of CCL11 (eotaxin), that negatively impact neurogenesis and cognitive performance. These findings highlight the role of systemic factors in regulating brain aging and suggest that the aging blood environment plays a critical role in cognitive decline.

The study also provides mechanistic insights into how systemic aging influences brain function. The researchers found that aged blood promotes inflammation and alters signaling pathways in the brain, leading to reduced neurogenesis and impaired cognitive abilities. By identifying specific factors like CCL11, the study offers potential targets for therapeutic interventions aimed at mitigating age-related cognitive decline. The findings underscore the importance of understanding the systemic regulation of brain aging and suggest that modulating systemic factors could be a strategy to promote neurogenesis and cognitive resilience in older individuals. Overall, this work advances our understanding of the interplay between systemic aging and brain function, providing a foundation for developing therapies to counteract age-related cognitive decline and improve brain health.

The ageing systemic milieu negatively regulates neurogenesis and cognitive function

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In the central nervous system, ageing results in a precipitous decline in adult neural stem/progenitor cells and neurogenesis, with concomitant impairments in cognitive functions¹. Interestingly, such impairments can be ameliorated through systemic perturbations such as exercise². Here, using heterochronic parabiosis we show that blood-borne factors present in the systemic milieu can inhibit or promote adult neurogenesis in an age-dependent fashion in mice. Accordingly, exposing a young mouse to an old systemic environment or to plasma from old mice decreased synaptic plasticity, and impaired contextual fear conditioning and spatial learning and memory. We identify chemokines—including CCL11 (also known as eotaxin)—the plasma levels of which correlate with reduced neurogenesis in heterochronic parabionts and aged mice, and the levels of which are increased in the plasma and cerebrospinal fluid of healthy ageing humans. Lastly, increasing peripheral CCL11 chemokine levels *in vivo* in young mice decreased adult neurogenesis and impaired learning and memory. Together our data indicate that the decline in neurogenesis and cognitive impairments observed during ageing can be in part attributed to changes in blood-borne factors.

Adult neurogenesis occurs in local microenvironments, or neurogenic niches, in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus^{3,4}. Permissive cues within the neurogenic niche are thought to drive the production of new neurons and their subsequent integration into the neurocircuitry of the brain^{5,6}, directly contributing to cognitive processes including learning and memory^{7–9}. Importantly, the neurogenic niche is localized around blood vessels^{4,11}, allowing for potential communication with the systemic environment. Therefore, the possibility arises that diminished neurogenesis during ageing may be modulated by the balance of two independent forces: intrinsic central nervous system (CNS)-derived cues^{2,12–14} and cues extrinsic to the CNS delivered by blood. Thus we hypothesized that age-related systemic molecular changes could cause a decline in neurogenesis and impair cognitive function during ageing.

We first characterized cellular, electrophysiological and behavioural changes associated with the neurogenic niche in the dentate gyrus of the hippocampus in an ageing cohort of mice. We observed cellular changes consistent with markedly decreased adult neurogenesis¹ and increased neuroinflammation with age¹⁵ (Supplementary Fig. 2a–c). Additionally, we detected deficits in synaptic plasticity (Supplementary Fig. 3a–c), and behavioural deficits in contextual fear conditioning

(Supplementary Fig. 4a–c) and radial arm water maze (RAWM; Supplementary Fig. 4d–f) paradigms in old animals, consistent with decreased cognitive function during ageing¹⁶.

Next we investigated the contribution of peripheral systemic factors to the age-related decline in neurogenesis in the dentate gyrus of the hippocampus in the setting of isochronic (young–young and old–old) and heterochronic (young–old) parabiosis (Fig. 1a). Remarkably, the number of doublecortin (Dcx)-positive newly born neurons (Fig. 1b, c), BrdU-positive cells (Fig. 1e, f) and Sox2-positive progenitors (Supplementary Fig. 5a, b) decreased in young heterochronic parabionts. In contrast, we observed an increase in the number of Dcx-positive (Fig. 1b, d), BrdU-positive (Fig. 1e, g) and Sox2-positive (Supplementary Fig. 5a, c) cells in the old heterochronic parabionts. The number of Dcx-positive neurons between unpaired age-matched animals and isochronic animals showed no difference (Supplementary Fig. 6a, b). As a control, flow cytometry analysis confirmed a shared vasculature in a subset of parabiotic pairs, in which one parabiont was transgenic for green fluorescent protein (GFP; Supplementary Fig. 7a–d). Together our findings suggest that global age-dependent systemic changes can modulate neurogenesis in both the young and aged neurogenic niche, potentially contributing to the decline in regenerative capacity observed in the normal ageing brain.

As previously reported by others¹⁷, we rarely detected peripherally derived GFP cells in the CNS of wild-type mice, and these numbers did not differ between isochronic and heterochronic pairings (Supplementary Fig. 7e), suggesting that the observed effects are mediated by soluble factors in plasma. To confirm that circulating factors within aged blood contribute to reduced neurogenesis with age, we intravenously injected plasma isolated from young or old mice into young adult mice (Fig. 2a). The number of Dcx-positive cells in the dentate gyrus decreased in animals receiving old plasma compared to animals receiving young plasma (Fig. 2b, c), indicating that soluble factors present in old blood inhibit adult neurogenesis.

To investigate the functional effect of the ageing systemic milieu, extracellular electrophysiological recordings were done on hippocampal slices prepared from young isochronic and heterochronic parabionts (Fig. 1h, i) and Supplementary Fig. 6c). A decrease in long-term potentiation (LTP) in the dentate gyrus of heterochronic parabionts was detected (Fig. 1h, i). These data indicate that age-related systemic changes can elicit deficits in synaptic plasticity. As LTP is considered to be a correlate of learning and memory¹⁸, these findings suggest that age-related systemic changes may also affect cognitive functions during ageing.

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90 | NATURE | VOL 477 | 1 SEPTEMBER 2011

Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A, Lucin KM, Czirr E, Park JS, Couillard-Després S, Aigner L, Li G, Peskind ER, Kaye JA, Quinn JF, Galasko DR, Xie XS, Rando TA, Wyss-Coray T. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature*. 2011 Aug 31;477(7362):90–4.

2014: Tony Wyss-Coray

Demonstrated that factors in young blood could rejuvenate aged tissues, sparking interest in plasma-based therapies for aging including GDF11.

This 2014 study by Tony Wyss-Coray and colleagues published in *Nature Medicine*, demonstrates that exposure to young blood can reverse age-related declines in brain function in mice. Using a model known as heterochronic parabiosis—where the circulatory systems of young and old mice are surgically connected—the researchers observed significant molecular and structural changes in the brains of aged mice. Specifically, they found increased expression of genes related to synaptic plasticity in the hippocampus, an area crucial for memory. Additionally, dendritic spine density increased in aged neurons, and electrophysiological measurements showed enhanced synaptic plasticity.

Importantly, these biological changes translated into improved cognitive performance. Aged mice that received young plasma exhibited better memory and learning, as evidenced by improvements in contextual fear conditioning and spatial navigation tasks.

Mechanistically, the effects were partly mediated by activation of the transcription factor CREB (cyclic AMP response element-binding protein), known for its role in promoting memory formation. This work suggests that circulating factors in young blood can rejuvenate aging brains and potentially offer a therapeutic avenue for age-related cognitive decline.

Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice

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As human lifespan increases, a greater fraction of the population is suffering from age-related cognitive impairments, making it important to elucidate a means to combat the effects of aging^{1,2}. Here we report that exposure of an aged animal to young blood can counteract and reverse pre-existing effects of brain aging at the molecular, structural, functional and cognitive level. Genome-wide microarray analysis of heterochronic parabionts—in which circulatory systems of young and aged animals are connected—identified synaptic plasticity-related transcriptional changes in the hippocampus of aged mice. Dendritic spine density of mature neurons increased and synaptic plasticity improved in the hippocampus of aged heterochronic parabionts. At the cognitive level, systemic administration of young blood plasma into aged mice improved age-related cognitive impairments in both contextual fear conditioning and spatial learning and memory. Structural and cognitive enhancements elicited by exposure to young blood are mediated, in part, by activation of the cyclic AMP response element binding protein (Creb) in the aged hippocampus. Our data indicate that exposure of aged mice to young blood late in life is capable of rejuvenating synaptic plasticity and improving cognitive function.

Aging drives cognitive impairments and susceptibility to degenerative disorders in healthy individuals³⁻⁶ by structurally and functionally changing the adult brain^{3,7-13}. Considering the increase in the proportion of elderly humans^{1,2}, it is important to identify a means for maintaining cognitive integrity by protecting against, or even counteracting, the aging process. In aged animals, exposure to young blood through heterochronic parabiosis improves stem cell function in muscle^{14,15}, liver¹⁴, spinal cord¹⁶ and the brain¹² and ameliorates cardiac hypertrophy¹⁷. However, whether enhancements of young

blood extend beyond regeneration in the aged brain is unknown, raising the question of whether young blood can counteract aging and rejuvenate cognitive processes.

In humans and mice, the hippocampus is particularly vulnerable to aging, exhibiting downregulation of plasticity-related genes, reduced spine density, decreased synaptic plasticity and impairments in associated cognitive functions^{3-13,18-20}. We first performed genome-wide microarray analysis of hippocampi from aged (18 months) isochronic (aged-aged) and aged (18 months) heterochronic (aged-young) parabionts (Fig. 1a). We observed a distinct gene expression profile between the two parabiont groups (Fig. 1c and Supplementary Table 1) and identified synaptic plasticity regulation as one of the top gene ontology enrichment categories associated with heterochronic parabiosis. Furthermore, Ingenuity Pathway Analysis (IPA) detected prominent involvement of plasticity-related signaling pathways, including Creb²¹, in the top-signaling network (Fig. 1b). Together our data reveal a transcriptional profile that is indicative of plasticity changes in heterochronic parabionts.

We then used immunohistochemistry to examine a subset of identified genes in a second cohort of parabionts (Fig. 1d-g). We observed increased numbers of cells expressing the immediate early genes Egr1 (Fig. 1d,e) and c-Fos (Fig. 1d,f) and a corresponding increase in phosphorylated Creb (Fig. 1d,g) in the dentate gyrus (DG) of heterochronic compared to isochronic parabionts. Although we observed increased phosphorylated Creb in the CA1 region, we detected no changes in immediate early genes in heterochronic parabionts (Supplementary Fig. 1a-c). We confirmed age-related differences in immediate early gene expression and Creb phosphorylation between young and aged unpaired animals (Supplementary Fig. 2). Molecular changes were not elicited by the parabiosis procedure (Supplementary Fig. 1d-i), and we observed no differences in general health, maintenance behavior or stress responses between

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2017: Tony Wyss-Coray

Wyss-Coray and colleagues show that human cord plasma contains plasticity-enhancing proteins, including TIMP2, of high translational value for targeting aging, particularly with parabiosis strategies.

Aging leads to changes in cognitive function that can lead to neurological disorders. Tony Wyss-Coray and colleagues show that human umbilical cord plasma is able to revitalize the hippocampus and improve cognitive function in aged mice. They find that tissue inhibitor of metalloproteinases 2 (TIMP2), a blood-borne factor, is enriched in human cord plasma, young mouse plasma and young mouse hippocampi.

They find that umbilical cord plasma revitalized hippocampal function in older mice. Further, when aged mice were treated with cord plasma, they showed improved synaptic plasticity and enhanced learning and memory.

It enters the brain following systemic administration and is necessary for the cognitive benefits conferred by cord plasma.

Systemic TIMP2 is also essential for spatial memory in young mice, while treatment of brain slices with TIMP2 antibody prevents long-term potentiation, suggesting that TIMP2 has a role in normal hippocampal function.

Human umbilical cord plasma proteins revitalize hippocampal function in aged mice

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Ageing drives changes in neuronal and cognitive function, the decline of which is a major feature of many neurological disorders. The hippocampus, a brain region subserving roles of spatial and episodic memory and learning, is sensitive to the detrimental effects of ageing at morphological and molecular levels. With advancing age, synapses in various hippocampal subfields exhibit impaired long-term potentiation¹, an electrophysiological correlate of learning and memory. At the molecular level, immediate early genes are among the synaptic plasticity genes that are both induced by long-term potentiation^{2–4} and downregulated in the aged brain^{5–8}. In addition to revitalizing other aged tissues^{9–13}, exposure to factors in young blood counteracts age-related changes in these central nervous system parameters^{14–16}, although the identities of specific cognition-promoting factors or whether such activity exists in human plasma remains unknown¹⁷. We hypothesized that plasma of an early developmental stage, namely umbilical cord plasma, provides a reservoir of such plasticity-promoting proteins. Here we show that human cord plasma treatment revitalizes the hippocampus and improves cognitive function in aged mice. Tissue inhibitor of metalloproteinases 2 (TIMP2), a blood-borne factor enriched in human cord plasma, young mouse plasma, and young mouse hippocampi, appears in the brain after systemic administration and increases synaptic plasticity and hippocampal-dependent cognition in aged mice. Depletion experiments in aged mice revealed TIMP2 to be necessary for the cognitive benefits conferred by cord plasma. We find that systemic pools of TIMP2 are necessary for spatial memory in young mice, while treatment of brain slices with TIMP2 antibody prevents long-term potentiation, arguing for previously unknown roles for TIMP2 in normal hippocampal function. Our findings reveal that human cord plasma contains plasticity-enhancing proteins of high translational value for targeting ageing- or disease-associated hippocampal dysfunction.

To begin to characterize differences in plasma proteins during different periods of life, we measured 66 common proteins in blood plasma from elderly individuals (aged 61–82 years), young adults (aged 19–24 years), and umbilical cord plasma from neonates (Extended Data Fig. 1a). Unsupervised clustering revealed distinct separation by age group in plasma proteins (Extended Data Fig. 1b). Proteins expected to be elevated in cord plasma, including human chorionic gonadotropin-β and α-fetoprotein, were increased (Extended Data Fig. 1c, d), and we detected age-related increases in immunoglobulin M (Extended Data Fig. 1e). To assess whether human plasma alters the hippocampus, a brain region critical for memory and influenced by young blood^{15,16}, we used immunodeficient 'NOD/SCID' (NSG) mice that could receive intravenous infusions of human plasma without adverse immune consequences¹⁸. Immunohistochemical comparison of hippocampi from young and aged NSG mice revealed reduced activation of the

synaptic plasticity marker c-Fos, impaired neurogenesis, and increased microgliosis with age (Extended Data Fig. 2a–f), consistent with observations in wild-type (WT) mice^{15,16,19}. Compared with young NSG mice, aged mice exhibited impaired hippocampal-dependent learning and memory performance (Extended Data Fig. 2g–l) in modified Barnes maze²⁰ and contextual fear-conditioning tests; these differences were unrelated to pain, visual, and auditory sensory function (Extended Data Fig. 2m–p). Thus, NSG mice exhibit typical age-related hippocampal dysfunction observed in WT mice by Barnes maze (Extended Data Fig. 2q, r) and fear-conditioning¹⁵, seemingly at an earlier age.

To assess whether human cord plasma revitalizes aspects of brain function, plasma pools were created from cord, young adult, and elderly donors and injected intravenously into aged NSG mice every fourth day for 2 weeks before dissection of hippocampi for analyses (Fig. 1a). Whole-genome microarray analysis revealed transcriptional profiles from hippocampi that distinctly clustered by intravenous treatment (Fig. 1b); the groups separated across the first three principal components, explaining the most gene expression variance (Fig. 1c). Ontology-based comparison analysis of differentially expressed genes in human plasma- or vehicle-treated hippocampi suggested increased long-term potentiation (LTP) as a result of cord (Z-score = 2.12; $P = 2.68 \times 10^{-2}$) or young (Z-score = 0.96; $P = 2.59 \times 10^{-3}$) treatment, but not elderly ($P > 0.05$) plasma treatment. An additional predicted disease and functional pathway was memory (Z-score = 1.38; $P = 2.61 \times 10^{-2}$), although only as a result of cord plasma treatment. The immediate early gene (IEG) cFos (also known as Fos) was among the top three most significantly increased genes resulting from cord plasma treatment, suggesting a possible re-activation of genes linked to memory consolidation^{1,21,22}. To confirm the induction of IEGs and other genes linked to plasticity and memory, we measured gene expression by quantitative (q)PCR in dissected hippocampi from treated mice, finding that cord plasma treatment significantly increased expression of *Egr1*, *Junb*, *Camk2a*, *Bdnf*, *Ntr3*, *Ppp3ca*, *cFos*, and *Nptx2*, a late-response c-Fos/Junb target gene²³ (Fig. 1d). Notably, young adult human plasma treatment increased expression in a smaller subset of these genes, while elderly plasma did not lead to significant changes (Extended Data Fig. 3a, b). At the protein level, only cord plasma treatment significantly increased the number of c-Fos-expressing cells within aged dentate gyrus (Fig. 1e and Extended Data Fig. 3c). The activation seemed to depend on context, since the pattern was not observed in other regions, including amygdala and motor cortex (Extended Data Fig. 3d, e), nor was it observed in young hippocampi (Fig. 1f). Human plasma treatments did not alter phosphorylated cyclic AMP responsive element-binding protein (CREB)¹⁶ or hippocampal neurogenesis (Extended Data Fig. 3f–i), possibly because neural stem cells are depleted in the aged NSG brain. We next used the targeted recombination in active populations

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2019: Yousef and Wyss-Coray

Aged blood can impair young animal brain function. VCAM1 is a key culprit.

The 2019 paper by Hanadie Yousef et al., titled "Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1", published in *Nature Medicine*, investigates how aged blood negatively affects brain function, particularly in the hippocampus, a region critical for learning and memory. The study reveals that exposure to aged blood reduces the activity of neural precursor cells (NPCs) in the hippocampus, impairing neurogenesis—the process of generating new neurons. This effect is mediated by brain endothelial cells, which express higher levels of vascular cell adhesion molecule 1 (VCAM1) in response to aged blood. VCAM1 activates microglia, the brain's immune cells, leading to inflammation and further suppression of NPC activity. The researchers demonstrated that blocking VCAM1 or its signaling pathways restored NPC function and reduced microglial activation, highlighting VCAM1 as a key mediator of the detrimental effects of aged blood on the brain.

The study provides critical insights into how systemic factors in aged blood contribute to cognitive decline and reduced neurogenesis during aging. By identifying VCAM1 as a central player in this process, the research offers a potential therapeutic target for mitigating age-related brain dysfunction. The findings also underscore the importance of the brain vasculature and its interaction with systemic circulation in regulating brain health. This work advances our understanding of the mechanisms linking systemic aging to brain aging and suggests that targeting endothelial cell signaling could be a strategy to promote neurogenesis and cognitive resilience in older individuals.

Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1

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An aged circulatory environment can activate microglia, reduce neural precursor cell activity and impair cognition in mice. We hypothesized that brain endothelial cells (BECs) mediate at least some of these effects. We observe that BECs in the aged mouse hippocampus express an inflammatory transcriptional profile with focal upregulation of vascular cell adhesion molecule 1 (VCAM1), a protein that facilitates vascular-immune cell interactions. Concomitantly, levels of the shed, soluble form of VCAM1 are prominently increased in the plasma of aged humans and mice, and their plasma is sufficient to increase VCAM1 expression in cultured BECs and the hippocampi of young mice. Systemic administration of anti-VCAM1 antibody or genetic ablation of *Vcam1* in BECs counteracts the detrimental effects of plasma from aged individuals on young brains and reverses aging aspects, including microglial reactivity and cognitive deficits, in the brains of aged mice. Together, these findings establish brain endothelial VCAM1 at the blood-brain barrier as a possible target to treat age-related neurodegeneration.

Brain structure and function deteriorate with age, steadily driving cognitive impairments and susceptibility to neurodegenerative disorders in humans¹. How aging leads to these manifestations is poorly understood, but an increase in the activation of microglia, frequently referred to as 'neuroinflammation' (refs. 2–4), and a precipitous loss of stem cell numbers and activity in the dentate gyrus (DG) of the hippocampus, one of two neurogenic regions of the adult mammalian brain⁵, are commonly noted. The hippocampus is crucial for learning and memory and is particularly vulnerable to age-related neurodegeneration and diseases such as Alzheimer's disease (AD)⁶. Although many of these age-related changes in the brain may be the consequences of cell-intrinsic and brain-localized mechanisms of aging, we asked whether changes in secreted signaling proteins, dubbed the 'communicome'⁷, could be used to understand, characterize and quantify aspects of brain aging and cognitive impairment. Indeed, not only are such changes in plasma or cerebrospinal fluid proteomes abundant with aging and disease^{8,9}, but factors in blood or plasma from young mice or humans are also sufficient to increase brain function in the hippocampus^{10,11} and the subventricular zone (SVZ)¹². Conversely, young mice exposed to blood from aged individuals showed reduced neurogenesis and cognitive function in the hippocampus^{13,14}. Considering the tight regulation of transport of molecules across the blood-brain barrier (BBB) and its role as

a protective barrier with limited permeability to macromolecules¹⁵, it is currently unclear how factors promoting youth or aging may modulate brain function¹. Here we investigated the interaction between the circulating communicome and BECs in the context of brain aging.

Results

Aged BECs are transcriptionally activated. To determine the transcriptional changes associated with aging in BECs, we acutely isolated and pooled primary CD31⁺ BECs from the cortices and hippocampi of young (3-month-old) and aged (19-month-old) mice and analyzed their transcriptome using RNA-seq (Extended Data Fig. 1a,b). Unsupervised clustering analysis revealed prominent age-dependent differences in the transcriptome, with over 1,000 differentially expressed genes (Fig. 1a). Cell purity was confirmed on the basis of high gene expression values for genes with BEC-specific expression and very low or undetectable expression for other cell-type-specific markers of the central nervous system (CNS) (Fig. 1b and Extended Data Fig. 1c). GeneAnalytics pathway analysis of differentially expressed genes identified numerous pathways associated with aging (Supplementary Table 1), including cell adhesion, immune cell activation, stress response and vascular remodeling¹⁶. Analysis of the highly expressed and differentially expressed transcripts revealed an inflammatory and activated profile with age,

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Yousef H, Czupalla CJ, Lee D, Chen MB, Burke AN, Zera KA, Zandstra J, Berber E, Lehallier B, Mathur V, Nair RV, Bonanno LN, Yang AC, Peterson T, Hadeiba H, Merkel T, Körbelin J, Schwanninger M, Buckwalter MS, Quake SR, Butcher EC, Wyss-Coray T. Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1. *Nat Med.* June 2019; 25(6):988–1000.

2020: Conboy Lab

Exchanging out old animal plasma had the same effect as adding young blood.

This paper investigates the rejuvenating effects of blood exchange in old mice, focusing on a process called "neutral age blood exchange" (NBE), where half of the plasma is replaced with saline containing 5% albumin.

This approach dilutes plasma factors while maintaining albumin levels, mimicking the effects of young blood without using it. The study found that a single NBE significantly rejuvenated old mice, improving muscle repair, reducing liver fat and fibrosis, and increasing hippocampal neurogenesis—key benefits previously observed with young blood transfusions.

Proteomic analysis of serum from NBE and a similar human procedure, therapeutic plasma exchange (TPE), revealed a resetting of systemic signaling pathways, elevating proteins involved in tissue repair and immune responses.

Notably, albumin alone was not the primary driver of rejuvenation, as its levels remained stable with age and were unaffected by NBE/TPE.

Instead, the authors propose that diluting autoregulatory proteins, which interact with multiple signaling pathways, leads to long-lasting molecular and functional changes. This work advances our understanding of systemic rejuvenation and suggests that TPE, an FDA-approved procedure, could be repurposed to improve health and resilience in older individuals.

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Priority Research Paper

Rejuvenation of three germ layers tissues by exchanging old blood plasma with saline-albumin

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ABSTRACT

Heterochronic blood sharing rejuvenates old tissues, and most of the studies on how this works focus on young plasma, its fractions, and a few youthful systemic candidates. However, it was not formally established that young blood is necessary for this multi-tissue rejuvenation. Here, using our recently developed small animal blood exchange process, we replaced half of the plasma in mice with saline containing 5% albumin (terming it a "neutral" age blood exchange, NBE) thus diluting the plasma factors and replenishing the albumin that would be diminished if only saline was used. Our data demonstrate that a single NBE suffices to meet or exceed the rejuvenative effects of enhancing muscle repair, reducing liver adiposity and fibrosis, and increasing hippocampal neurogenesis in old mice, all the key outcomes seen after blood heterochronicity. Comparative proteomic analysis on serum from NBE, and from a similar human clinical procedure of therapeutic plasma exchange (TPE), revealed a molecular re-setting of the systemic signaling milieu, interestingly, elevating the levels of some proteins, which broadly coordinate tissue maintenance and repair and promote immune responses. Moreover, a single TPE yielded functional blood rejuvenation, abrogating the typical old serum inhibition of progenitor cell proliferation. Ectopically added albumin does not seem to be the sole determinant of such rejuvenation, and levels of albumin do not decrease with age nor are increased by NBE/TPE. A model of action (supported by a large body of published data) is that significant dilution of autoregulatory proteins that crosstalk to multiple signaling pathways (with their own feedback loops) would, through changes in gene expression, have long-lasting molecular and functional effects that are consistent with our observations. This work improves our understanding of the systemic paradigms of multi-tissue rejuvenation and suggest a novel and immediate use of the FDA approved TPE for improving the health and resilience of older people.

Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*, Feb 17. 2005; 433(7027):760-4.

2022: Tabula Muris Consortium

Single cell atlas shows multiple rejuvenation pathways from young blood.

The 2022 paper by Pálóvics et al., titled "Molecular hallmarks of heterochronic parabiosis at single-cell resolution", published in *Nature*, provides a detailed single-cell analysis of the effects of heterochronic parabiosis—a technique where the circulatory systems of young and old mice are connected—on aging and tissue rejuvenation. Using single-cell RNA sequencing, the researchers examined changes in gene expression across multiple tissues and cell types in young and old mice following parabiosis. They found that exposure to youthful blood reversed many age-related molecular changes in old mice, particularly in stem and progenitor cells, immune cells, and endothelial cells. These rejuvenating effects included the restoration of youthful gene expression patterns, improved cellular function, and reduced inflammation. Conversely, young mice exposed to old blood exhibited accelerated aging signatures, highlighting the bidirectional influence of systemic factors on aging.

The study identified specific molecular pathways and cell types that are particularly responsive to heterochronic parabiosis, providing insights into the mechanisms underlying systemic rejuvenation. Key pathways involved in tissue repair, inflammation, and metabolism were modulated by youthful circulation, suggesting that systemic factors in young blood can reset cellular aging clocks.

The researchers also identified potential candidate factors, such as GDF11 and CCL11, that may mediate these effects. These findings underscore the importance of systemic communication in aging and highlight the potential for targeting circulating factors to promote tissue repair and counteract age-related decline.

Article

Molecular hallmarks of heterochronic parabiosis at single-cell resolution

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 Check for updates

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The ability to slow or reverse biological ageing would have major implications for mitigating disease risk and maintaining vitality¹. Although an increasing number of interventions show promise for rejuvenation², their effectiveness on disparate cell types across the body and the molecular pathways susceptible to rejuvenation remain largely unexplored. Here we performed single-cell RNA sequencing on 20 organs to reveal cell-type-specific responses to young and aged blood in heterochronic parabiosis. Adipose mesenchymal stromal cells, haematopoietic stem cells and hepatocytes are among those cell types that are especially responsive. On the pathway level, young blood invokes new gene sets in addition to reversing established ageing patterns, with the global rescue of genes encoding electron transport chain subunits pinpointing a prominent role of mitochondrial function in parabiosis-mediated rejuvenation. We observed an almost universal loss of gene expression with age that is largely mimicked by parabiosis: aged blood reduces global gene expression, and young blood restores it in select cell types. Together, these data lay the groundwork for a systemic understanding of the interplay between blood-borne factors and cellular integrity.

Recent transcriptomic studies of major organs and cell types across the lifespan of the mouse (*Tabula Muris Senis*) uncovered both global and tissue-specific ageing signatures throughout the body^{3–5} providing a first glimpse at how ageing differentially impacts interconnected organs. However, how, or whether, the growing number of rejuvenation paradigms affect these global ageing pathways at a cellular level remains unknown. Heterochronic parabiosis (in which a young and an aged mouse share a common circulation) and systemic infusions of young blood are methods of rejuvenation with broad beneficial effects including on cognition, muscle strength and bone repair in mice⁶; young blood also reversed the DNA methylation age in several organs in rats. Several circulatory proteins have been determined to mediate at least some of the observed effects^{7–10} but the consequences of parabiosis on distinct organs and cells are incompletely understood.

Here we attempt to address this question by performing Smart-seq2-based single-cell RNA sequencing (scRNA-seq) of C57BL/6JN male mice following 5 weeks of heterochronic parabiosis, when mice had reached 4 and 19 months of age (equivalent to humans aged around 25 and 65 years). Targeted cell populations were captured via flow cytometry into microtitre plates from 20 organs—bladder, brain, brown adipose tissue (interscapular depot), diaphragm, gonadal adipose tissue (GAT; inguinal depot), heart, kidney, large intestine, limb

muscle, liver, lung, marrow, mesenteric adipose tissue (MAT), pancreas, skin (epidermis), spleen, subcutaneous adipose tissue (posterior depot), thymus, tongue and trachea—as detailed for each tissue and cell type (Fig. 1a, b, Extended Data Figs. 1, 2, Supplementary Tables 1–3, Supplementary Fig. 1). By integrating single-cell ageing data from the simultaneously collected *Tabula Muris Senis* (Extended Data Fig. 1a), we were able to match cell type annotations per tissue and compare parabiosis-mediated rejuvenation (REJ) and accelerated ageing (ACC) to normal ageing (AGE). Although mice from this parabiosis experiment and *Tabula Muris Senis* originate from the same cohort and were mapped to the same reference with the same parameters, the frozen cells were processed at different times. We therefore performed batch correction to account for technical artefacts (Extended Data Fig. 1j, k). Raw data are available from the Gene Expression Omnibus and a public Amazon Web Service (AWS) S3 bucket; gene-cell counts and metadata are available on Figshare, and the entire annotated dataset is available and accessible online via an interactive browser (see 'Data availability').

Cell-type-specific responses

To determine which cell types are susceptible to ACC or REJ during parabiosis (Fig. 1a), we analysed differential gene expression (DGE) in

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Pálóvics R, Keller A, Schaum N, Tan W, Fehlmann T, Borja M, Kern F, Bonanno L, Calcuttawala K, Webber J, McGeever A; Tabula Muris Consortium; Luo J, Pisco AO, Karkanias J, Neff NF, Darmanis S, Quake SR, Wyss-Coray T. Molecular hallmarks of heterochronic parabiosis at single-cell resolution. *Nature*. 2022 Mar;603(7900):309–314.

2023: Gladyshev and White

Long-term parabiosis associated with a 10% increase in lifespan.

The 2023 paper by Zhang et al., titled "Multi-omic rejuvenation and life span extension on exposure to youthful circulation", published in *Nature Aging*, investigates the effects of exposing aged organisms to youthful blood circulation on aging and lifespan. The study used a heterochronic parabiosis model, where the circulatory systems of young and old mice were connected, to demonstrate that exposure to youthful blood can rejuvenate aged tissues at the molecular, cellular, and functional levels. Through multi-omic analyses (including transcriptomics, epigenomics, and proteomics), the researchers found that youthful circulation reversed many age-related changes, such as reduced gene expression, altered epigenetic markers, and impaired protein homeostasis.

These rejuvenating effects were associated with improved tissue function, including enhanced muscle repair, reduced inflammation, and better metabolic health. Importantly, the study also showed that exposure to youthful circulation extended the lifespan of aged mice, providing direct evidence that systemic factors in young blood can counteract aging processes.

The study highlights the potential of youthful systemic factors to promote rejuvenation and longevity, offering insights into the mechanisms by which aging can be reversed at the organismal level. The researchers identified specific circulating factors and signaling pathways, such as those involving GDF11 and oxytocin, that contribute to the rejuvenating effects. These findings suggest that targeting systemic factors or mimicking their effects could be a promising strategy for anti-aging interventions. The study also underscores the importance of understanding the systemic nature of aging and how inter-tissue communication influences aging processes.

Multi-omic rejuvenation and lifespan extension on exposure to youthful circulation

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Heterochronic parabiosis (HPB) is known for its functional rejuvenation effects across several mouse tissues. However, its impact on biological age and long-term health is unknown. Here we performed extended (3-month) HPB, followed by a 2-month detachment period of anastomosed pairs. Old detached mice exhibited improved physiological parameters and lived longer than control isochronic mice. HPB drastically reduced the epigenetic age of blood and liver based on several clock models using two independent platforms. Remarkably, this rejuvenation effect persisted even after 2 months of detachment. Transcriptomic and epigenomic profiles of anastomosed mice showed an intermediate phenotype between old and young, suggesting a global multi-omic rejuvenation effect. In addition, old HPB mice showed gene expression changes opposite to aging but akin to several lifespan-extending interventions. Altogether, we reveal that long-term HPB results in lasting epigenetic and transcriptome remodeling, culminating in the extension of lifespan and healthspan.

Aging is the primary risk factor for chronic diseases^{1–3}. It brings accumulation of damage at many levels of biological organization and a pervasive and destructive decline of organ function, resulting in inevitable mortality. Although many attempts have been made to extend the lifespan and ameliorate specific aging phenotypes through interventions, aging itself has generally been regarded as an irreversible process. However, the recent development and application of advanced

aging biomarkers based on DNA methylation (that is, methylation clocks)^{4–7} have challenged this concept. With their assessment of the attenuated aging effect of various longevity interventions, including caloric restriction (CR), genetic models and cellular reprogramming, methylation clocks have been generally recognized as a robust predictor of organismal biological age, a measure of how old an organism is biologically rather than chronologically. Notably, DNA methylation

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Section 8.G: Cell Reprogramming

If the role of autophagy in aging might have been hard for our ancestors to imagine, then cellular reprogramming would have been impossible.

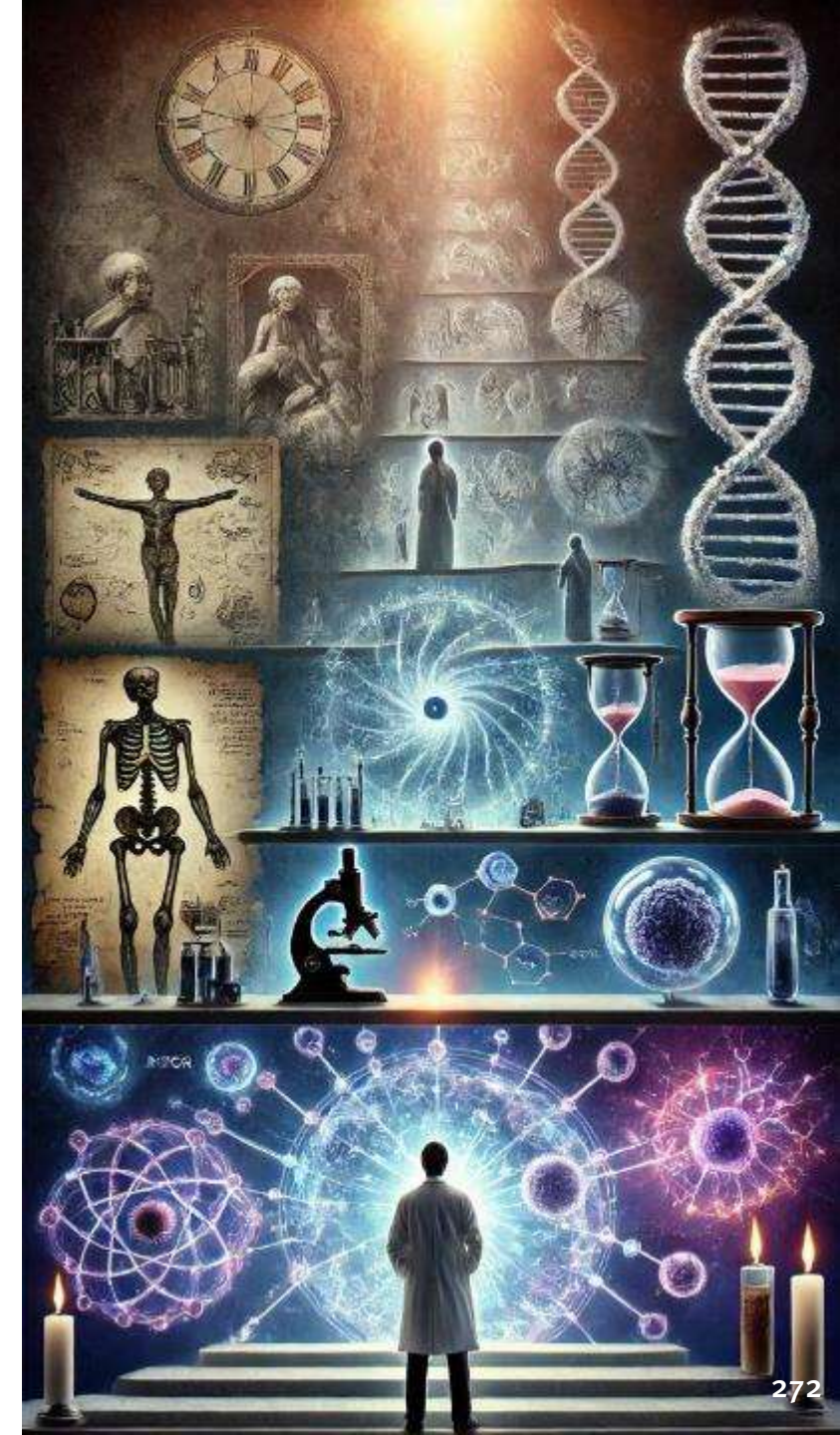
Even today, talk of programming cells back to a younger state furrows foreheads and arches eyebrows.

Of course, vague concepts of rejuvenation have been around forever. Drink this magic potion and you will be young again etc. But no one thought that there might be programmable transcription factors in the cell that could cause a cell to take on a younger phenotype.

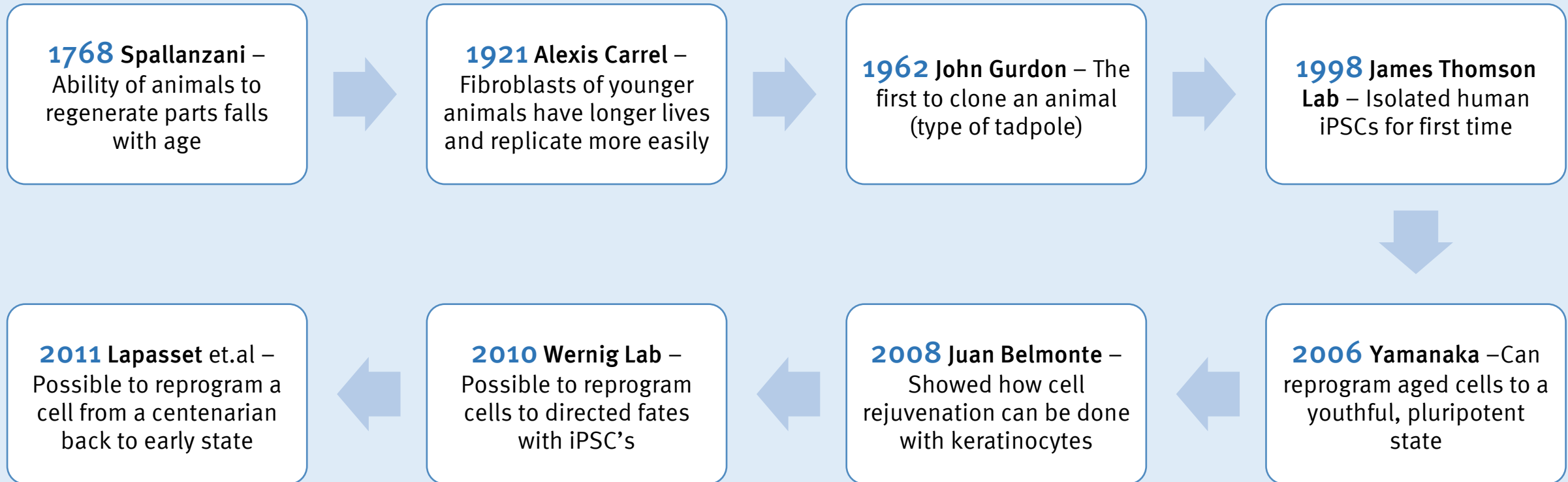
It has been no surprise that the time gap between Shinya Yamanaka's paper on programmable cell factors and the Nobel Prize was unusually short. Barbara McClintock had to wait nearly 40 years by contrast.

Since Yamanaka's stunning 2006 paper showing that four transcription factors (called OKSM) control a cell's conversion back to a pluripotent type there has been an outpouring of papers.

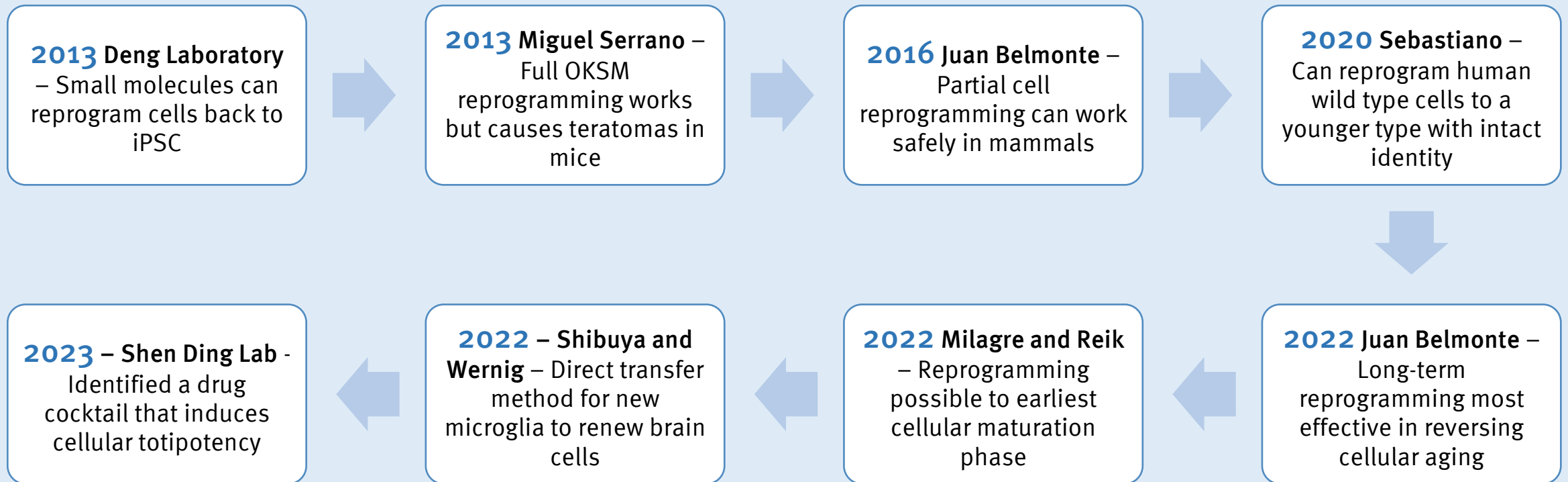
Perhaps the two most notable contributions since then have come from Juan Belmonte. In a 2008 paper, Belmonte and colleagues showed that they could reprogram cells to a younger state *in vitro*. Remarkably, in a 2016 paper Belmonte and colleagues showed by reprogramming of Yamanaka factors (OKSM) could reduce aging in a mouse. The answer was yes. This stunning result was one of the main factors behind the largest venture fundraiser in history. In 2019 Altos Labs hired Belmonte and raised \$3 billion in order develop anti-aging medicines based upon his partial reprogramming approach.



History: Cellular Reprogramming (1768 to 2012)



History: Cellular Reprogramming (2013 to 2023)



2006: Shinya Yamanaka

Yamanaka's 2006 paper demonstrated that aging-associated cellular states could be reversed, as the reprogramming process effectively reset the cells to a youthful, pluripotent state. This is the most cited paper in the history of aging research by far.

In their seminal 2006 paper, Shinya Yamanaka and Kazutoshi Takahashi demonstrated that somatic cells could be reprogrammed into induced pluripotent stem cells (iPSCs) using a defined set of transcription factors. The team identified four key factors — Oct4, Sox2, Klf4, and c-Myc (later termed the "Yamanaka factors") — that could revert differentiated cells to a pluripotent state.

Pluripotent stem cells have the remarkable ability to differentiate into any cell type in the body, a property previously thought to be exclusive to embryonic stem cells (ESCs). Yamanaka and Takahashi showed that the resulting iPSCs exhibited characteristics similar to ESCs, including the expression of pluripotency markers and the ability to self-renew.

Through a process of elimination, the researchers narrowed down the essential factors from twenty-four to the four mentioned above (often called OSKF). The reprogrammed iPSCs not only resembled ESCs in their morphology and gene expression profiles but also demonstrated functional pluripotency by contributing to tissues in chimeric mice and generating live offspring through tetraploid complementation. This work provided a powerful tool for regenerative medicine, disease modeling, and drug discovery, as iPSCs could be derived from patients' own cells, reducing the risk of immune rejection and enabling personalized therapies. For this groundbreaking contribution, Yamanaka was awarded the Nobel Prize in Physiology or Medicine in 2012.

References: GSK p. 354; Ramakrishnan p. 91.

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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SUMMARY

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions. Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development. These data demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors.

INTRODUCTION

Embryonic stem (ES) cells, which are derived from the inner cell mass of mammalian blastocysts, have the ability to grow indefinitely while maintaining pluripotency and the ability to differentiate into cells of all three germ layers (Evans and Kaufman, 1981; Martin, 1981). Human ES cells might be used to treat a host of diseases, such as Parkinson's disease, spinal cord injury, and diabetes (Thomson et al., 1998). However, there are ethical difficulties regarding the use of human embryos, as well as the problem of tissue rejection following transplantation in patients. One way to circumvent these issues is the generation of pluripotent cells directly from the patients' own cells.

Somatic cells can be reprogrammed by transferring their nuclear contents into oocytes (Wilmut et al., 1997)

or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.

Several transcription factors, including Oct3/4 (Nichols et al., 1998; Niwa et al., 2000), Sox2 (Avilion et al., 2003), and Nanog (Chambers et al., 2003; Mitsui et al., 2003), function in the maintenance of pluripotency in both early embryos and ES cells. Several genes that are frequently upregulated in tumors, such as Stat3 (Matsuda et al., 1999; Niwa et al., 1998), *E-Ras* (Takahashi et al., 2003), *c-myc* (Cartwright et al., 2005), *Klf4* (Li et al., 2005), and β -catenin (Kielman et al., 2002; Sato et al., 2004), have been shown to contribute to the long-term maintenance of the ES cell phenotype and the rapid proliferation of ES cells in culture. In addition, we have identified several other genes that are specifically expressed in ES cells (Maruyama et al., 2005; Mitsui et al., 2003).

In this study, we examined whether these factors could induce pluripotency in somatic cells. By combining four selected factors, we were able to generate pluripotent cells, which we call induced pluripotent stem (iPS) cells, directly from mouse embryonic or adult fibroblast cultures.

RESULTS

We selected 24 genes as candidates for factors that induce pluripotency in somatic cells, based on our hypothesis that such factors also play pivotal roles in the maintenance of ES cell identity (see Table S1 in the Supplemental Data available with this article online). For β -catenin, c-Myc, and Stat3, we used active forms, S33Y- β -catenin (Sadot et al., 2002), T58A-c-Myc (Chang et al., 2000), and Stat3-C (Bromberg et al., 1999), respectively. Because of the reported negative effect of Grb2 on pluripotency (Burdon et al., 1999; Cheng et al., 1998), we included its dominant-negative mutant Grb2 Δ SH2 (Miyamoto et al., 2004) as 1 of the 24 candidates.

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Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006 Aug 25;126(4):663-76.

2008: Juan Belmonte

If Yamanaka identified factors that could be modulated to restore differentiated cells back to iPSC's, then Belmonte showed how this might be practically done via retroviral programming of keratinocytes.

Juan Belmonte's 2008 paper, "Efficient and Rapid Generation of Induced Pluripotent Stem Cells from Human Keratinocytes," introduces a novel and efficient method for generating induced pluripotent stem cells (iPSCs) from human keratinocytes, the primary cells found in the outer layer of skin. The study demonstrates that keratinocytes can be reprogrammed into iPSCs more efficiently and rapidly than fibroblasts, the previously preferred cell type for this process.

Keratinocytes showed significantly higher reprogramming efficiency and required fewer reprogramming factors than was required by Yamanaka with his experiments using fibroblast cells, simplifying the overall procedure. Additionally, the reprogramming process was faster, making keratinocytes a more practical and effective cell source for iPSC generation.

The accessibility of keratinocytes, combined with their superior reprogramming efficiency, positions keratinocytes as an ideal cell type for producing patient-specific iPSCs. These iPSCs have significant potential for applications in disease modeling, drug screening, and regenerative medicine. Belmonte's work represents a major advancement in stem cell research, providing a more efficient and accessible approach to generating iPSCs and opening new possibilities for personalized medicine.

References: Ramakrishnan p. 189.

Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes

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The utility of induced pluripotent stem (iPS) cells for investigating the molecular logic of pluripotency and for eventual clinical application is limited by the low efficiency of current methods for reprogramming. Here we show that reprogramming of juvenile human primary keratinocytes by retroviral transduction with *OCT4*, *SOX2*, *KLF4* and *c-MYC* is at least 100-fold more efficient and twofold faster compared with reprogramming of human fibroblasts. Keratinocyte-derived iPS (KiPS) cells appear indistinguishable from human embryonic stem cells in colony morphology, growth properties, expression of pluripotency-associated transcription factors and surface markers, global gene expression profiles and differentiation potential *in vitro* and *in vivo*. To underscore the efficiency and practicability of this technology, we generated KiPS cells from single adult human hairs. Our findings provide an experimental model for investigating the bases of cellular reprogramming and highlight potential advantages of using keratinocytes to generate patient-specific iPS cells.

Induced pluripotent stem (iPS) cells have been derived from mouse¹ and human^{2–5} somatic cells by forced expression of a small number of defined factors. The reprogramming process appears highly inefficient and likely depends on many factors, including the age, type and origin of the cells used. In the mouse, iPS cells have been generated from derivatives of the three embryonic germ layers, including mesodermal fibroblasts¹, epithelial cells of endoderm origin⁶ and ectodermal keratinocytes⁷, whereas human iPS cells have been produced from fibroblasts. Here we investigate iPS cell generation from human primary keratinocytes to determine whether human epithelial cells are amenable to directed reprogramming and whether the efficiency of iPS generation can be increased by using keratinocytes. Keratinocytes, keratin-dense epithelial cells, generate the outer protective epidermal barrier of the skin surface as well as appendages such as hair and nails⁸. This process continues throughout life owing to the presence of self-renewing keratinocyte stem cells, which produce transit-amplifying cells that subsequently exit the cell cycle as they terminally differentiate⁹. Here we show reprogramming of human primary keratinocytes to pluripotency by retroviral transduction of four (*OCT4*, *SOX2*, *KLF4*, *c-MYC*) or three (*OCT4*, *SOX2*, *KLF4*) factors. The high efficiency of reprogramming with four factors allowed us to generate iPS cells from minute samples using single plucked hairs. This accessible source of more easily reprogrammable cells from either plucked hair or epidermal biopsies should facilitate progress toward the goal of safe, efficient iPS generation by transient delivery of reprogramming factors.

RESULTS

Generation of keratinocyte-derived iPS cells

We cultured keratinocytes from a normal human foreskin (age 4 years) in serum-free and low-calcium medium, which promotes a highly proliferative, undifferentiated state. Under these conditions, only keratin-positive keratinocytes grow (see below), and, upon addition of physiological levels of calcium, they readily differentiate and can form a fully differentiated stratified epidermis within a week (data not shown; see also ref. 9). N-terminal FLAG-tagged versions of human *OCT4*, *SOX2*, *KLF4*, *c-MYC* as well as *GFP* were cloned into a murine stem cell virus (MSCV)-derived retroviral vector, which provides higher transcriptional activation than the commonly used Mooney murine leukemia virus-based vectors¹⁰. We optimized the retroviral transduction of keratinocytes with *GFP* and found that two 45-min spinfections (centrifugation of cell culture plates in the presence of virus) 24 h apart resulted in nearly 100% infection of undifferentiated cells (Fig. 1a,b and Supplementary Fig. 1 online). We then used this infection protocol to transduce keratinocytes with retroviruses encoding the four factors. Fifty-thousand passage-1 to passage-5 keratinocytes were seeded (day 0) and infected on day 1 and 2 with a 1:1:1:1 mixture of retroviruses. Cells were trypsinized on day 4 and seeded onto a layer of irradiated mouse embryonic fibroblasts (MEFs) in embryonic stem (ES) cell medium.

Within 2–5 d (6–7 d post-infection), we detected several hundred small, tight cell colonies that grew rapidly and, by day 10 post-infection, displayed typical human (h)ES cell-like morphology (tight colonies of cells with a large nuclear to cytoplasmic ratio and prominent nucleoli;

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Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilić J, Pekarik V, Tiscornia G, Edel M, Boué S, Izpisua Belmonte JC. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol.* 2008 Nov;26(11):1276–84.

2010: Marius Wernig

Marius Wernig and colleagues demonstrated that somatic cells could be directly reprogrammed into functional neurons without passing through a pluripotent state—a major advancement in the field of cellular reprogramming. This is one of the most heavily cited papers in the history of aging research.

In their groundbreaking 2010 *Nature* paper, Wernig Lab members and collaborators identified a combination of three transcription factors (Ascl1, Brn2, and Myt1l) that could convert mouse embryonic and postnatal fibroblasts into induced neuronal (iN) cells. These iN cells exhibited key neuronal characteristics, including the expression of neuron-specific markers, the ability to generate action potentials, and the formation of functional synapses. This discovery challenged the prevailing belief that cellular identity could only be reprogrammed by first reverting to a pluripotent state, offering a faster and potentially safer alternative to induced pluripotent stem cell (iPSC)-based approaches.

The study had significant implications for regenerative medicine and disease modeling, as it suggested that functional neurons could be generated directly from a patient's own fibroblasts, bypassing the risks of tumor formation associated with iPSCs. The researchers also found that Ascl1 was the critical initiator of neuronal reprogramming, while Brn2 and Myt1l enhanced maturation and functionality. Though the initial efficiency was low, this work laid the foundation for subsequent refinements in direct neuronal conversion, including human cell applications and in vivo reprogramming strategies. Wernig's paper thus opened a new avenue for studying neurological diseases and developing potential cell-based therapies for conditions like Parkinson's or spinal cord injury.

Direct conversion of fibroblasts to functional neurons by defined factors

Thomas Vierbuchen^{1,2}, Austin Ostermeier^{1,2}, Zhiping P. Pang³, Yuko Kokubu¹, Thomas C. Südhof^{3,4} & Marius Wernig^{1,2}

Cellular differentiation and lineage commitment are considered to be robust and irreversible processes during development. Recent work has shown that mouse and human fibroblasts can be reprogrammed to a pluripotent state with a combination of four transcription factors. This raised the question of whether transcription factors could directly induce other defined somatic cell fates, and not only an undifferentiated state. We hypothesized that combinatorial expression of neural-lineage-specific transcription factors could directly convert fibroblasts into neurons. Starting from a pool of nineteen candidate genes, we identified a combination of only three factors, *Ascl1*, *Brn2* (also called *Pou3f2*) and *Myt1l*, that suffice to rapidly and efficiently convert mouse embryonic and postnatal fibroblasts into functional neurons *in vitro*. These induced neuronal (iN) cells express multiple neuron-specific proteins, generate action potentials and form functional synapses. Generation of iN cells from non-neural lineages could have important implications for studies of neural development, neurological disease modelling and regenerative medicine.

The diverse cell types present in the adult organism are produced during development by lineage-specific transcription factors that define and reinforce cell-type-specific gene expression patterns. Cellular phenotypes are further stabilized by epigenetic modifications that allow faithful transmission of cell-type-specific gene expression patterns over the lifetime of an organism^{1,2}. Recent work showing that four transcription factors are sufficient to induce pluripotency in primary fibroblasts demonstrated that fully differentiated cells can be induced to undergo dramatic cell fate changes³. Similarly, the transfer of somatic cell nuclei into oocytes, as well as cell fusion of pluripotent cells with differentiated cells, have proven to be capable of inducing pluripotency^{4–6}. This remarkable transformation has been interpreted as a reversion of mature into more primitive developmental states, with a concomitant erasure of developmentally relevant epigenetic information¹⁰. Therefore, direct reprogramming between divergent lineages could be unique to reprogramming into an embryonic state, and might not be possible between different somatic cell states. However, cell fusion or forced expression of lineage-specific genes in somatic cells can induce traits of other cell types^{11,12}. For example, the basic helix-loop-helix (bHLH) transcription factor *MyoD* (also called *Myod1*) can induce muscle-specific properties in fibroblasts but not hepatocytes^{13,14}; ectopic expression of interleukin (IL)-2 and granulocyte-macrophage colony-stimulating factor receptors can lead to myeloid conversion in committed lymphoid progenitor cells¹⁵; expression of *Cebpa* in B cells or *PU.1* (also called *Spi1*) and *Cebpa* in fibroblasts induces characteristics of macrophages^{16–18}; deletion of *Ptc5* can induce B cells to de-differentiate towards a common lymphoid progenitor¹⁹; and the bHLH transcription factor neurogenin 3, in combination with *Pdx1* and *Mafk*, can efficiently convert pancreatic exocrine cells into functional β -cells *in vivo*²⁰. Here, we set out to determine whether specific transcription factors could directly convert fibroblasts into functional neurons.

A screen for neuronal-fate-inducing factors

Reasoning that multiple transcription factors would probably be required to reprogram fibroblasts to a neuronal fate, we cloned a

total of 19 genes that are specifically expressed in neural tissues, have important roles in neural development, or have been implicated in epigenetic reprogramming (Supplementary Table 1). A pool of lentiviruses containing all 19 genes (19F pool) was prepared to infect mouse embryonic fibroblasts (MEFs) from TauEGFP knock-in mice, which express EGFP specifically in neurons^{21–23} (see Fig. 1a for experimental outline). Great care was taken to exclude neural tissue from the MEF preparation, and we were unable to detect evidence for the presence of neurons or neural progenitor cells in these cultures using immunofluorescence, fluorescence activated cell sorting (FACS) and polymerase chain reaction with reverse transcription (RT-PCR) analyses (Supplementary Fig. 1). However, uninfected MEFs did contain rare Tuj1-positive, TauEGFP-negative cells with fibroblast-like morphology, indicative of weak Tuj1 (that is, β -III-tubulin) expression in non-neuronal cells (Fig. 1b, c and Supplementary Fig. 1a). In contrast, 32 days after infection with the 19F pool, we detected Tuj1-positive cells with typical neuronal morphologies and bright TauEGFP fluorescence (Fig. 1d,e). Thus, some combination(s) of the genes in the 19F pool was capable of converting MEFs into iN cells.

We next set out to narrow down the number of transcription factors required for generation of iN cells. Given their important roles in neuronal cell fate determination^{24–26} we first tested the bHLH transcription factors *Ascl1* (also known as *Mash1*) and *Neurog1* individually. Notably, we observed occasional Tuj1-positive, TauEGFP-positive cells exhibiting a simple mono- or bipolar morphology after infection with only *Ascl1* (Supplementary Fig. 2b). However, 19F iN cells showed more complex morphologies, which indicated that the activity of *Ascl1* alone was not sufficient to recapitulate the full activity of the 19F pool (compare to Fig. 1d, e). We therefore tested the neuron-inducing activity of *Ascl1* in combination with each of the remaining 18 candidate genes (Supplementary Fig. 2a). Five genes (*Brn2*, *Brn4* (*Pou3f4*), *Myt1l*, *Zic1* and *Olig2*) substantially potentiated the neuron-inducing activity of *Ascl1* (Supplementary Fig. 2a, b). Importantly, none of these five genes generated iN cells when tested individually (data not shown). Next, we tested whether combinatorial expression of these factors with *Ascl1* could further increase the induction of neuron-like cells by infecting

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Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*, Feb 25, 2010; 463(7284):1035–41.

Wernig Lab Making Excellent Progress in Cell Reprogramming for Age-Related Neurologic Diseases

Tyler Brown, *Stanford Daily*, May 27, 2011 (excerpt)

With the addition of four proteins, adult human skin cells can be transformed into neurons over a month-long period. The findings, reported yesterday in *Nature*, suggest a process that doesn't require the reprogrammed adult somatic cells called induced pluripotent stem (iPS) cells. The method may make it easier to produce patient- or disease-specific neurons for study.

"We are now much closer to being able to mimic brain or neurological diseases in the laboratory," said School of Medicine assistant professor and lead researcher Marius Wernig in a press release. He expressed hope that one day the cells could be used for human therapies.

The researchers showed they could convert human embryonic stem cells to neurons by infecting them with a virus that expressed the same proteins used in the study. This treatment, nicknamed "BAM" after an acronym of the three proteins, converted the embryonic stem cells into functional neurons within six days. The same treatment worked on iPS cells, but the process is labor-intensive and relies on cell lines that likely aren't as diverse as a natural population.

The challenge was to do the same for skin cells without stem cells as an intermediary. The researchers found that BAM treatment to skin cells from fetuses and newborns didn't have the same effect as it did on the stem cells. The addition of a fourth protein, called "NeuroD," was the tipping point; it triggered the skin cells' transformation into functional neurons within about four to five weeks. The cells expressed electrical activity characteristic of neurons and even integrated and interacted with mouse neurons on a laboratory dish.



Marius Wernig

*Professor and Co-Director of the Institute for Stem Cell Biology and Regenerative Medicine
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2011: Lapasset and Lemaitre

Possible to reprogram a centenarian's cell back to an early state.

The study by Lapasset et al., titled "Rejuvenating Senescent and Centenarian Human Cells by Reprogramming through the Pluripotent State", explores the potential of cellular reprogramming to reverse aging in human cells. The researchers used induced pluripotent stem cell (iPSC) technology to reprogram senescent cells and cells from centenarians (individuals over 100 years old) back to a pluripotent state. They found that this reprogramming process effectively reset the aging clock, erasing markers of cellular senescence and restoring youthful characteristics, such as telomere elongation, improved mitochondrial function, and reduced oxidative stress.

The rejuvenated cells exhibited a gene expression profile similar to that of young cells, demonstrating that the aging process can be reversed at the molecular level. This work provides strong evidence that aging is not an irreversible process, and that cellular reprogramming can restore youthful functionality to aged cells.

The study also highlights the potential of reprogramming as a strategy to combat age-related decline and diseases. By resetting senescent and centenarian cells to a pluripotent state, the researchers demonstrated that the aging process can be halted and even reversed. However, the study also acknowledges the challenges of applying this approach in vivo, as reprogramming can lead to tumorigenesis if not carefully controlled. Despite these challenges, the findings open up new possibilities for regenerative medicine and anti-aging therapies. The research underscores the plasticity of cellular aging and provides a foundation for developing interventions that could rejuvenate aged tissues and improve healthspan.

Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state

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Direct reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) provides a unique opportunity to derive patient-specific stem cells with potential applications in tissue replacement therapies and without the ethical concerns of human embryonic stem cells (hESCs). However, cellular senescence, which contributes to aging and restricted longevity, has been described as a barrier to the derivation of iPSCs. Here we demonstrate, using an optimized protocol, that cellular senescence is not a limit to reprogramming and that age-related cellular physiology is reversible. Thus, we show that our iPSCs generated from senescent and centenarian cells have reset telomere size, gene expression profiles, oxidative stress, and mitochondrial metabolism, and are indistinguishable from hESCs. Finally, we show that senescent and centenarian-derived pluripotent stem cells are able to redifferentiate into fully rejuvenated cells. These results provide new insights into iPSC technology and pave the way for regenerative medicine for aged patients.

Lapasset L, Milhavy O, Prieur A, Besnard E, Babled A, Aït-Hamou N, Leschik J, Pellestor F, Ramirez JM, De Vos J, Lehmann S, Lemaitre JM. Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. *Genes Dev.* Nov 1, 2011;25(21):2248-53.

2013: Deng Laboratory

It is possible to reprogram cells back to their iPSC state with small molecules.

Hongkui Deng is a Chinese immunologist and stem cell researcher and Director of the Institute of Stem Cell Research at Peking University.

The groundbreaking 2013 paper from his lab entitled “Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds,” demonstrates that somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) using only chemical compounds, without the need for genetic manipulation. This approach, known as chemical reprogramming, represents a significant advancement in stem cell research, as it avoids the risks associated with integrating foreign genes into the genome, such as mutations or tumor formation. The researchers identified a specific cocktail of small molecules that could induce pluripotency in mouse somatic cells, effectively resetting them to a stem cell-like state. These chemically induced pluripotent stem cells (CiPSCs) exhibited key characteristics of pluripotency, including the ability to differentiate into all three germ layers and contribute to chimeric embryos. This study provides a safer and more controllable method for generating iPSCs, with potential applications in regenerative medicine and disease modeling.

The study highlighted the potential of small-molecule compounds to replace or complement existing reprogramming strategies, reducing risks associated with genetic integration and providing a more controlled approach to generating pluripotent stem cells. The researchers demonstrated that CiPSCs exhibited key characteristics of pluripotency, including the ability to differentiate into various cell types and contribute to embryonic development.

Pluripotent Stem Cells Induced from Mouse Somatic Cells by Small-Molecule Compounds

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Pluripotent stem cells can be induced from somatic cells, providing an unlimited cell resource, with potential for studying disease and use in regenerative medicine. However, genetic manipulation and technically challenging strategies such as nuclear transfer used in reprogramming limit their clinical applications. Here, we show that pluripotent stem cells can be generated from mouse somatic cells at a frequency up to 0.2% using a combination of seven small-molecule compounds. The chemically induced pluripotent stem cells resemble embryonic stem cells in terms of their gene expression profiles, epigenetic status, and potential for differentiation and germline transmission. By using small molecule exogenous “master genes” are dispensable for cell fate reprogramming. This chemical reprogramming strategy has potential use in generating functional desirable cell types for clinical applications.

Pluripotent stem cells, such as embryonic stem cells (ESCs), can self-renew and differentiate into all somatic cell types. Somatic cells can be reprogrammed to become pluripotent via nuclear transfer into oocytes or through the ectopic expression of defined factors (1–4). However, exogenous pluripotency-associated factors, especially *Oct4*, are indispensable for establishing pluripotency (5–7), and previous reprogramming strategies have raised concerns regarding the clinical applications (8, 9). Small molecules have advantages because they can be cell permeable, nonimmunogenic, more cost-effective, and more easily synthesized, preserved, and standard-

ized. Moreover, their effects on inhibiting an activating the function of specific proteins are often reversible and can be finely tuned by varying the concentrations. Here, we identified small molecule combinations that were able to drive the reprogramming of mouse somatic cells toward pluripotent cells.

To identify small molecules that facilitate reprogramming, we searched for small molecule that enable reprogramming in the absence of *Oct4* using *Oct4* promoter-driven green fluorescent protein (GFP) expression (OG) mouse embryonic fibroblasts (MEFs), with viral expression of *Sox2*, *Klf4*, and *c-Myc*. After screening up to 10,000 small molecules (table S1A), we identified Forskolin (FSK), 2-methyl-5-hydroxytryptamine (2-Me-5HT), and D4476 (table S1B) as chemical “substitutes” for *Oct4* (Fig. 1, A and B, and figs. S1 and S2). Previously, we had developed a small molecule combination “VC6T” [VPA, CHIR9902 (CHIR), 616452, Tranylcypromine], that enable reprogramming with a single gene, *Oct4* (6). We next treated OG-MEFs with VC6T plus the chem-

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Hou P, Li Y, Zhang X, Liu C, Guan J, Li H, Zhao T, Ye J, Yang W, Liu K, Ge J, Xu J, Zhang Q, Zhao Y, Deng H., “Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds,” *Science*. Aug 9, 2013; 341(6146):651-4.

2013: Manuel Serrano

Full OKSM reprogramming works but causes teratomas in mice.

The 2013 study by Manuel Serrano and colleagues, titled "Reprogramming in vivo produces teratomas and iPS cells with totipotency features", published in *Nature*, investigates the effects of in vivo reprogramming in mice by activating the Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc (OSKM)) directly within adult somatic cells.

The researchers found that in vivo reprogramming led to the formation of teratomas, tumors containing cells from all three germ layers, demonstrating the pluripotency of the reprogrammed cells.

Remarkably, the induced pluripotent stem cells (iPSCs) generated in vivo exhibited features of totipotency, including the ability to contribute to both embryonic and extraembryonic tissues, such as the placenta and yolk sac. These findings suggest that the in vivo environment provides unique cues that enhance reprogramming efficiency and confer totipotent-like characteristics to the resulting iPSCs, which are not typically observed in iPSCs generated in vitro.

The study highlights the potential of in vivo reprogramming as a tool for generating iPSCs with enhanced developmental potential. However, the formation of teratomas underscores the challenges of controlling reprogramming in a living organism, as uncontrolled pluripotency can lead to tumorigenesis.

The research provides new insights into the mechanisms of cellular reprogramming and the influence of the in vivo environment on cell fate, offering a platform to study totipotency and tissue regeneration.

Reprogramming in vivo produces teratomas and iPS cells with totipotency features

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Reprogramming of adult cells to generate induced pluripotent stem cells (iPS cells) has opened new therapeutic opportunities; however, little is known about the possibility of *in vivo* reprogramming within tissues. Here we show that transitory induction of the four factors Oct4, Sox2, Klf4 and c-Myc in mice results in teratomas emerging from multiple organs, implying that full reprogramming can occur *in vivo*. Analyses of the stomach, intestine, pancreas and kidney reveal groups of dedifferentiated cells that express the pluripotency marker NANOG, indicative of *in situ* reprogramming. By bone marrow transplantation, we demonstrate that haematopoietic cells can also be reprogrammed *in vivo*. Notably, reprogrammable mice present circulating iPS cells in the blood and, at the transcriptome level, these *in vivo* generated iPS cells are closer to embryonic stem cells (ES cells) than standard *in vitro* generated iPS cells. Moreover, *in vivo* iPS cells efficiently contribute to the trophectoderm lineage, suggesting that they achieve a more plastic or primitive state than ES cells. Finally, intraperitoneal injection of *in vivo* iPS cells generates embryo-like structures that express embryonic and extraembryonic markers. We conclude that reprogramming *in vivo* is feasible and confers totipotency features absent in standard iPS or ES cells. These discoveries could be relevant for future applications of reprogramming in regenerative medicine.

Reprogramming into pluripotency remains an intense field of investigation that is providing many insights about cellular plasticity^{1,2}. Cellular reprogramming has been achieved under carefully controlled *in vitro* culture conditions³, whereas the *in vivo* tissue microenvironment is, in principle, conducive to cellular differentiation and opposed to reprogramming. However, we took note of remarkable examples in mice in which the normally irreversible state of cellular differentiation has been altered, inducing direct conversions *in vivo* from one cell type into a different one^{4–10}. Encouraged by these precedents, we have attempted to achieve reprogramming *in vivo*.

Generation of reprogrammable mice

We have generated reprogrammable mice similar, but not identical, to others previously described^{11–13}. A total of four transgenic mouse lines were obtained, each one carrying the transcriptional activator (rtTA) within the ubiquitously-expressed Rosa26 locus¹⁴ and a single copy of a lentiviral doxycycline-inducible polycistronic cassette encoding the four murine factors Oct4 (also known as Pou5f1), Sox2, Klf4 and c-Myc¹⁵ (Fig. 1a; Extended Data Fig. 1a). In two of the four transgenic lines, the cassette was highly induced in most tissues (Extended Data Fig. 1b) and mouse embryonic fibroblasts (MEFs) from these lines were efficiently reprogrammed *in vitro* upon addition of doxycycline (Extended Data Fig. 1c, d). The other two transgenic lines did not express the cassette and their derived MEFs did not reprogram upon doxycycline addition. We have named the two functional transgenic lines as i4F-A and i4F-B, i4F standing for 'inducible four factors'. We determined the integration sites of the transgenes, which in the case of line i4F-A is within an intron of the *Neto2* gene, and in the case of line i4F-B is within an intron of the *Pparg* gene (Extended Data Fig. 2a). Transcription of these two genes remained unaltered in a number of tissues, either with or without doxycycline (Extended Data Fig. 2b). We

conclude that transgenic lines i4F-A and i4F-B contain a functional inducible reprogramming transgene that is expressed in most tissues without affecting the resident endogenous genes.

Reprogrammable mice generate teratomas

To test the possibility of *in vivo* reprogramming, we first treated i4F-A and i4F-B mice continuously with a high dose of doxycycline (1 mg ml⁻¹) in the drinking water. This treatment resulted in weight loss and severe morbidity in both transgenic lines after 1 week. Histological examination of the mice revealed alterations in many tissues, particularly profound in the intestine and pancreas. The intestinal epithelium showed a generalized cytological and architectural dysplasia (Extended Data Fig. 3a), probably responsible for the weight loss. A similar phenotype has been reported for mice with transgenic expression of Oct4 or c-Myc^{16,17}. In the case of the pancreas, mice presented multifocal dysplasia (Extended Data Fig. 3b). Taking into account these observations, we tested two milder induction protocols that turned out to be compatible with the long-term survival of the mice. In particular, 2.5-week treatment with low doxycycline (0.2 mg ml⁻¹) or 1-week treatment with high doxycycline (1 mg ml⁻¹), both followed by doxycycline withdrawal. Remarkably, after a variable period of time, treated mice succumbed to the presence of tumoral masses (Fig. 1b; Extended Data Fig. 4a), most of which consisted of teratomas (Fig. 1c; Extended Data Fig. 4b). Teratomas are a particular class of tumours that originate from pluripotent cells after a process of expansion and disorganized differentiation. Most of the teratomas (32/45, 71%) were well differentiated and presented abundant examples of the three embryonic germ layers (Fig. 1d). Therefore, the presence of teratomas in our reprogrammable mice is indicative of reprogramming into full pluripotency. Mice treated with the long-induction/low-doxycycline protocol developed teratomas faster and at a higher incidence rate than those treated with the short-induction/

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Abad M, Mosteiro L, Pantoja C, Cañamero M, Rayon T, Ors I, Graña O, Megías D, Domínguez O, Martínez D, Manzanares M, Ortega S, Serrano M. Reprogramming in vivo produces teratomas and iPS cells with totipotency features. *Nature*. Oct 17, 2013;502(7471):340-5.

2016: Juan Belmonte

Unlike his 2008 paper which looked at cells in vitro, this work by Belmonte and colleagues examined whether reprogramming of Yamanaka factors (OKSM) would reduce aging in an actual animal. The answer was affirmative.

In vitro studies have demonstrated that cellular reprogramming to pluripotency reverses cellular age, but alteration of the aging process through reprogramming has not been directly demonstrated *in vivo*.

Here, the researchers report that partial reprogramming by short-term cyclic expression of Oct4, Sox2, Klf4, and c-Myc (OSKM) ameliorates cellular and physiological hallmarks of aging and prolongs lifespan in progeroid space.

Similarly, expression of OSKM in vivo improves recovery from metabolic disease and muscle injury in older wild-type mice. Importantly, while constitutive generation of OSKM factors in a genetically altered mouse can cause cancer, partial reprogramming, as carried out in this paper did not.

Specifically, the authors showed that reprogramming of Yamanaka factors was associated with reductions of progerin, a key marker of physiologic aging, reductions in activation of stress-response genes in the P53 pathway and reduction of mitochondrial reactive oxygen species.

This was a landmark paper in aging because it was the first in vivo proof of principle for partial reprogramming of Yamanaka factors.

References: GSK p. 354; Ramakrishnan p. 188.

Article

Cell

In Vivo Amelioration of Age-Associated Hallmarks by Partial Reprogramming

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SUMMARY

Aging is the major risk factor for many human diseases. In vitro studies have demonstrated that cellular reprogramming to pluripotency reverses cellular age, but alteration of the aging process through reprogramming has not been directly demonstrated in vivo. Here, we report that partial reprogramming by short-term cyclic expression of Oct4, Sox2, Klf4, and c-Myc (OSKM) ameliorates cellular and physiological hallmarks of aging and prolongs lifespan in a mouse model of premature aging. Similarly, expression of OSKM in vivo improves recovery from metabolic disease and muscle injury in older wild-type mice. The amelioration of age-associated phenotypes by epigenetic remodeling during cellular reprogramming highlights the role of epigenetic dysregulation as a driver of mammalian aging. Establishing in vivo platforms to modulate age-associated epigenetic marks may provide further insights into the biology of aging.

INTRODUCTION

Embryonic development occurs as a unidirectional progression from a single-cell zygote to an adult organism. During embryogenesis and early stages of life, cells undergo a spatiotemporally orchestrated differentiation process, leading to the generation of all of the cell types that comprise an adult organism. These events take place within a stable environment that minimizes molecular and cellular damage. As an organism ages, however, there is a continuous and progressive decline in the mechanisms responsible for minimizing cellular damage. This eventually results in an organism's inability to maintain homeostasis (López-Otin et al., 2013; 2016).

The last decade of scientific research has dramatically improved our understanding of the aging process (Johnson

et al., 2013; Kanyon, 2010; Riera et al., 2016). The notion that cells undergo a unidirectional differentiation process during development was proved wrong by the experimental demonstration that a terminally differentiated cell can be reprogrammed into a pluripotent embryonic-like state (Gurdon, 1962; Takahashi and Yamanaka, 2006). Cellular reprogramming to pluripotency by forced expression of the Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc [OSKM]) occurs through the global remodeling of epigenetic marks (Buganim et al., 2012, 2013; Hanson et al., 2012; Polo et al., 2012). Importantly, many of the epigenetic marks that are remodeled during reprogramming (e.g., DNA methylation, post-translational modification of histones, and chromatin remodeling) are dysregulated during aging (Berayrou et al., 2015; Liu et al., 2013b; Polina and Brunet, 2011). In fact, epigenetic dysregulation has emerged as a key hallmark of the aging process (Sen et al., 2016). Several groups, including ours, have observed an amelioration of age-associated cellular phenotypes during in vitro cellular reprogramming (Lapasset et al., 2011; Liu et al., 2011; Mahmoudi and Brunet, 2012; Rando and Chang, 2012). Reprogramming of cells from centenarians or patients with Hutchinson-Gilford progeria syndrome (HGPS) a disorder characterized by premature aging, resets telomere size, gene expression profiles, and levels of oxidative stress, resulting in the generation of rejuvenated cells (Lapasset et al., 2011; Liu et al., 2011; Zhang et al., 2011).

Although these in vitro studies have been informative, the physiological complexity of the aging process demands an in vivo approach to better understand how reprogramming may affect cellular and organismal aging. Breakthrough studies led by the Serrano and Yamada groups have shown that cellular reprogramming to pluripotency, although associated with tumor development (e.g., teratoma formation), can be achieved in vivo in mice by the forced expression of the Yamanaka factors (Abad et al., 2013; Ohnishi et al., 2014). In addition, we and other groups have demonstrated that partial reprogramming in vitro by transient expression of OSKM can induce a dedifferentiated progenitor-like state (Kurita et al., 2013; Thier et al., 2012). Together, these observations suggest that cellular reprogramming may be used to promote tissue regeneration and led us



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Ocampo A et.al., In Vivo Amelioration of Age-Associated Hallmarks by Partial Reprogramming. *Cell*. Dec 15, 2016; 167(7):1719-1733.

2020: Sebastiano Lab

Can partially reprogram human wild type cells to a younger type with intact identity using an mRNA rather than gene therapy approach.

This paper provides detailed insights into the molecular mechanisms behind cellular rejuvenation. The researchers used transient reprogramming, which involves temporarily expressing a set of reprogramming factors (Oct4, Sox2, Klf4, and c-Myc) in aged cells.

This approach allowed the cells to regain youthful characteristics without fully reverting to a stem cell state, thus avoiding the risk of tumor formation. The findings suggest that aging is not a one-way process and that targeted interventions can restore cellular function, paving the way for future therapies aimed at combating aging and its associated diseases.

Researchers in Sebastiano's laboratory make iPS cells from human adult cells, such as those that compose skin, by exposing them over a period of weeks to a panel of proteins important to early embryonic development. They do so by introducing daily, short-lived RNA messages into the adult cells. The RNA messages encode the instructions for making the Yamanaka proteins. Over time, these proteins rewind the cells' fate - pushing them backward along the developmental timeline until they resemble young, embryonic-like pluripotent cells.

This key paper advanced the literature over Belmonte's 2016 work by using a more tractable technology to address reprogramming factors (mRNA) and by doing so with human rather than mouse cells. Also see: <https://med.stanford.edu/news/all-news/2020/03/old-human-cells-rejuvenated-with-stem-cell-technology.html>



ARTICLE

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Transient non-integrative expression of nuclear reprogramming factors promotes multifaceted amelioration of aging in human cells

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Aging is characterized by a gradual loss of function occurring at the molecular, cellular, tissue and organismal levels. At the chromatin level, aging associates with progressive accumulation of epigenetic errors that eventually lead to aberrant gene regulation, stem cell exhaustion, senescence, and deregulated cell/tissue homeostasis. Nuclear reprogramming to pluripotency can revert both the age and the identity of any cell to that of an embryonic cell. Recent evidence shows that transient reprogramming can ameliorate age-associated hallmarks and extend lifespan in progeroid mice. However, it is unknown how this form of rejuvenation would apply to naturally aged human cells. Here we show that transient expression of nuclear reprogramming factors, mediated by expression of mRNAs, promotes a rapid and broad amelioration of cellular aging, including resetting of epigenetic clock, reduction of the inflammatory profile in chondrocytes, and restoration of youthful regenerative response to aged, human muscle stem cells, in each case without abolishing cellular identity.

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2022: Juan Belmonte

Long-term *in vivo* reprogramming most effective in reversing cellular aging.

Juan Carlos Izpisua Belmonte and team's 2022 article, "In Vivo Partial Reprogramming Alters Age-Associated Molecular Changes," in *Nature Aging* explores the effects of partial cellular reprogramming in living organisms as a potential strategy to combat aging. The study builds on earlier work demonstrating that transient expression of Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc) can rejuvenate cells without fully reverting them to a pluripotent stem cell state.

In this research, Belmonte and his team applied partial reprogramming to aged mice, observing that the treatment reduced many molecular hallmarks of aging, such as DNA methylation patterns and gene expression profiles associated with aging. Importantly, the approach did not erase cellular identity, suggesting a safe and targeted way to mitigate age-related decline.

The findings highlight the potential of partial reprogramming to reset epigenetic markers and restore youthful cellular function *in vivo*. The study also underscores the importance of balancing rejuvenation with the preservation of tissue integrity, as prolonged or excessive reprogramming could lead to unintended consequences like tumor formation. Belmonte's work provides a foundation for future research into therapeutic applications of partial reprogramming, offering hope for interventions that could delay or reverse age-related diseases and improve healthspan. This research represents a significant step forward in understanding how to harness cellular reprogramming to address the biological processes underlying aging.

In vivo partial reprogramming alters age-associated molecular changes during physiological aging in mice

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Partial reprogramming by expression of reprogramming factors (Oct4, Sox2, Klf4 and c-Myc) for short periods of time restores a youthful epigenetic signature to aging cells and extends the life span of a premature aging mouse model. However, the effects of longer-term partial reprogramming in physiologically aging wild-type mice are unknown. Here, we performed various long-term partial reprogramming regimens, including different onset timings, during physiological aging. Long-term partial reprogramming lead to rejuvenating effects in different tissues, such as the kidney and skin, and at the organismal level; duration of the treatment determined the extent of the beneficial effects. The rejuvenating effects were associated with a reversion of the epigenetic clock and metabolic and transcriptomic changes, including reduced expression of genes involved in the inflammation, senescence and stress response pathways. Overall, our observations indicate that partial reprogramming protocols can be designed to be safe and effective in preventing age-related physiological changes. We further conclude that longer-term partial reprogramming regimens are more effective in delaying aging phenotypes than short-term reprogramming.

Aging is associated with widespread changes in cells and tissues that can result in chronic inflammation and a decline in metabolic homeostasis^{1–3}. A range of intervention strategies have been identified that can counteract such changes, reduce inflammation, restore metabolic homeostasis and thus delay the onset of age-related diseases^{4–7}. In recent years, cellular reprogramming using transcription factors has emerged as a powerful alternative strategy to rejuvenate aging cells, restore epigenetic marks and erase markers of cell damage, thus reversing cellular phenotypes associated with aging⁸. *Ex vivo* cellular reprogramming using Oct4, Sox2, Klf4 and c-Myc (OSKM) can completely erase epigenetic states imposed during development, allowing for the conversion of terminally differentiated somatic cells into pluripotent stem cells⁹. This conversion includes erasing aging phenotypes, as demonstrated in reprogrammed cells from individuals of advanced age and patients with premature aging syndromes, such as Hutchinson–Gilford progeria syndrome (HGPS)^{10–12}. Contrary to these beneficial *in vitro* effects, *in vivo* expression of reprogramming factors led to the generation of teratomas and death in mice^{13–15}. Following these results, our group was able to demonstrate that *in vitro* expression of the OSKM factors for short and repeated intervals (thus avoiding

continuous expression) lead to the amelioration of aging hallmarks in fibroblasts obtained from premature aging and normal mice¹¹ and in human cells from individuals of advanced age *in vitro*¹⁶. *In vivo*, partial reprogramming not only prevented the loss of cell identity and the appearance of tumors but also led to an extended life span in an accelerated aging mouse model¹⁷. Subsequently, other groups have shown in different animal models that partial reprogramming reduces fibrosis and scar formation in the skin, ameliorates aging phenotypes in dentate gyrus cells and promotes axon regeneration after injury^{17–19}. We have further found that short-term expression of Yamanaka factors (OSKM) in myofibers can induce satellite cell proliferation and accelerate regeneration in the muscle of young mice²⁰.

Although these are important steps forward to the possibility of using this strategy to increase health and life span in vertebrates, our initial experiments were mainly performed in a mutant background displaying an accelerated aging syndrome. Thus, confounding effects of the mutation (a point mutation in the *LMNA* gene) on the OSKM-elicited phenotypes could not be ruled out. To allay such concerns and evaluate the feasibility of age reversion by partial OSKM reprogramming more generally, we set out to evaluate the

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2022: Milagre and Reik

Reprogramming possible to earliest cellular maturation phase.

The article “Multi-omic rejuvenation of human cells by maturation phase transient reprogramming” by Gill et al., published in *eLife*, investigates the potential of transient reprogramming to rejuvenate aged human cells. The study builds on previous research showing that partial reprogramming using Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc) can reverse some aging-associated cellular changes without fully converting cells into a pluripotent state. The authors focus on optimizing the timing and conditions of reprogramming to achieve rejuvenation while preserving cellular identity and function. They introduce a novel approach called “maturation phase transient reprogramming” (MPTR), which involves a carefully controlled, short-term induction of reprogramming factors followed by a recovery period. This method aims to reset aging-related molecular changes without erasing the cells' specialized characteristics.

The researchers applied MPTR to human fibroblasts and endothelial cells, analyzing the effects using multi-omics approaches, including transcriptomics, epigenomics, and metabolomics. They found that MPTR significantly reduced aging-associated markers, such as DNA methylation changes and senescence-associated gene expression, while restoring youthful patterns of gene activity. Importantly, the treated cells retained their original identity and functionality, demonstrating that transient reprogramming can rejuvenate cells without inducing pluripotency or compromising their specialized roles. The study also identified key molecular pathways involved in the rejuvenation process, including improvements in mitochondrial function and reductions in inflammatory signaling.

References: Ramakrishnan p. 188.

Multi-omic rejuvenation of human cells by maturation phase transient reprogramming

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Abstract Ageing is the gradual decline in organismal fitness that occurs over time leading to tissue dysfunction and disease. At the cellular level, ageing is associated with reduced function, altered gene expression and a perturbed epigenome. Recent work has demonstrated that the epigenome is already rejuvenated by the maturation phase of somatic cell reprogramming, which suggests full reprogramming is not required to reverse ageing of somatic cells. Here we have developed the first “maturation phase transient reprogramming” (MPTR) method, where reprogramming factors are selectively expressed until this rejuvenation point then withdrawn. Applying MPTR to dermal fibroblasts from middle-aged donors, we found that cells temporarily lose and then reacquire their fibroblast identity, possibly as a result of epigenetic memory at enhancers and/or persistent expression of some fibroblast genes. Excitingly, our method substantially rejuvenated multiple cellular attributes including the transcriptome, which was rejuvenated by around 30 years as measured by a novel transcriptome clock. The epigenome was rejuvenated to a similar extent, including H3K9me3 levels and the DNA methylation ageing clock. The magnitude of rejuvenation instigated by MPTR appears substantially greater than that achieved in previous transient reprogramming protocols. In addition, MPTR fibroblasts produced youthful levels of collagen proteins, and showed partial functional rejuvenation of their migration speed. Finally, our work suggests that optimal time windows exist for rejuvenating the transcriptome and the epigenome. Overall, we demonstrate that it is possible to separate rejuvenation from complete pluripotency reprogramming, which should facilitate the discovery of novel anti-ageing genes and therapies.

Editor's evaluation

This study describes a novel “maturation phase transient reprogramming” (MPTR) method to restore the epigenome of cells to a more youthful state. The authors demonstrate the effectiveness of the method to reverse several age-related changes including remodeling of the transcriptome. The method performs favorably compared to other transient reprogramming protocols, and the study will be of interest to developmental biologists as well as researchers who study ageing.

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Competing interest: See page 19

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2022: Shibuya and Wernig

Cell therapies have the potential of being an effective approach for treating neurodegenerative conditions. However, the need for local delivery and the poor distribution of the transplanted cells hinder the development of effective treatments. Here, Shibuya et al. developed an efficient microglia replacement approach.

In their 2022 paper in *Science Translational Medicine*, Shibuya and Marius Wernig demonstrated a novel therapeutic approach for treating genetic brain diseases by replacing diseased microglia with healthy ones across the central nervous system (CNS). The study focused on a mouse model of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), a fatal neurodegenerative disorder caused by mutations in the CSF1R gene. The researchers used a bone marrow transplant strategy to introduce wild-type microglial progenitors, which then migrated widely and repopulated the CNS, effectively replacing the dysfunctional host microglia. This led to significant improvements in pathology, including reduced neuroinflammation, preserved myelin integrity, and extended survival, suggesting that CNS-wide microglia replacement could be a viable treatment for ALSP and similar disorders.

The study highlighted the critical role of microglia in maintaining brain health and showed that even in adult brains, these cells can be efficiently replaced. A key finding was that the new microglia not only integrated seamlessly but also functionally compensated for the genetic defect, mitigating disease progression. The approach bypasses the need for gene therapy or direct neuronal intervention, instead targeting the supportive glial environment. Members of the Wernig Lab are now working to get this therapy working in humans for broad treatment of neurodegenerative disease.

NEURODEGENERATIVE DISEASES

Treatment of a genetic brain disease by CNS-wide microglia replacement

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Hematopoietic cell transplantation after myeloablative conditioning has been used to treat various genetic metabolic syndromes but is largely ineffective in diseases affecting the brain presumably due to poor and variable myeloid cell incorporation into the central nervous system. Here, we developed and characterized a near-complete and homogeneous replacement of microglia with bone marrow cells in mice without the need for genetic manipulation of donor or host. The high chimerism resulted from a competitive advantage of scarce donor cells during microglia repopulation rather than enhanced recruitment from the periphery. Hematopoietic stem cells, but not immediate myeloid or monocyte progenitor cells, contained full microglia replacement potency equivalent to whole bone marrow. To explore its therapeutic potential, we applied microglia replacement to a mouse model for Prosaposin deficiency, which is characterized by a progressive neurodegeneration phenotype. We found a reduction of cerebellar neurodegeneration and gliosis in treated brains, improvement of motor and balance impairment, and life span extension even with treatment started in young adulthood. This proof-of-concept study suggests that efficient microglia replacement may have therapeutic efficacy for a variety of neurological diseases.

INTRODUCTION

Rectifying disease pathology in the nervous system remains one of the grand challenges in medicine. In principle, neural cell therapies are an attractive approach because a one-time treatment has the potential to have long-lasting effects. Although local grafts are highly promising, such as cell transplantation for Parkinson's disease (1, 2), the large size of the human brain and the need for stereotactic injection complicate the homogeneous distribution of grafted cells even when highly migratory cells like oligodendrocyte precursors are transplanted (3). Thus, alternative approaches are needed to achieve more homogeneous brain incorporation, preferably delivered via the circulation.

Hematopoietic cell transplantation (HCT) is a widely used therapeutic procedure to replace a patient's hematopoietic system (4). In addition to diseases of the bone marrow, allogeneic HCT has been used to treat systemic genetic syndromes (5, 6).

The widespread distribution of hematopoietic cells and their continued turnover and replacement from bone marrow stem cells allow body-wide delivery of a functional gene product. For instance,

decades of experience have shown that HCT can rescue many pathological aspects in children with severe forms of genetic metabolic syndromes (7). However, central nervous system (CNS) involvement and resulting pathology have limited response to HCT presumably due to the inefficient replacement of brain-resident macrophages, microglia are long-lived and regenerated from within the brain (9–13). Moreover, peripheral administration of therapeutic gene products has limited efficacy as large molecules are unable to efficiently traverse the blood-brain barrier (BBB) (14, 15). Thus, cellular or molecular access to the brain is urgently needed to efficiently treat such genetic CNS diseases.

Here, we and others recently developed a rapid and near-complete replacement of microglia in mice with circulation-derived myeloid cells (CDMCs) eliminating the large variability observed after conventional bone marrow transplantation (BMT) (16, 17). These findings beg the question whether such widespread microglia replacement may be a potential solution for the unmet clinical need to effectively treat genetic brain diseases and reliably deliver therapeutic proteins into the CNS.

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2023: Ding Lab

Found small molecule approach to convert somatic cells back to totipotent cells – equivalent to cells in a human zygote

The Deng Lab demonstrated that mouse totipotent stem cells (totiSCs) can be generated from pluripotent stem cells using a defined chemical cocktail (CC), bypassing genetic manipulation.

This cocktail, comprising small molecules targeting signaling pathways and epigenetic modifiers, induced a reprogramming trajectory resembling the *in vivo* transition from pluripotency to totipotency.

The resulting totiSCs exhibited molecular and functional hallmarks of early embryonic cells, including activation of zygotic genome activation (ZGA)-associated genes and the ability to contribute to both embryonic and extraembryonic lineages in chimeric embryos¹.

This breakthrough provides a scalable, non-transgenic method to study totipotency mechanisms and early embryogenesis. The chemically induced totiSCs offer a model for dissecting regulatory networks of lineage specification and epigenetic reprogramming, with implications for regenerative medicine and developmental biology research.

To further prove that the resulting cells have a true totipotent state, the team tested their differentiation potential *in vitro* and also injected them into a mouse early embryo to see the differentiation potential *in vivo*. They found that the *in vivo* cells were functional and capable of differentiating.

Article

Induction of mouse totipotent stem cells by a defined chemical cocktail

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 Check for updates

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In mice, only the zygotes and blastomeres from 2-cell embryos are authentic totipotent stem cells (TotiSCs) capable of producing all the differentiated cells in both embryonic and extraembryonic tissues and forming an entire organism¹. However, it remains unknown whether and how totipotent stem cells can be established *in vitro* in the absence of germline cells. Here we demonstrate the induction and long-term maintenance of TotiSCs from mouse pluripotent stem cells using a combination of three small molecules: the retinoic acid analogue TTPNB, 1-azakenpaulone and the kinase blocker WS6. The resulting chemically induced totipotent stem cells (ciTotiSCs), resembled mouse totipotent 2-cell embryo cells at the transcriptome, epigenome and metabolome levels. In addition, ciTotiSCs exhibited bidirectional developmental potentials and were able to produce both embryonic and extraembryonic cells *in vitro* and in teratoma. Furthermore, following injection into 8-cell embryos, ciTotiSCs contributed to both embryonic and extraembryonic lineages with high efficiency. Our chemical approach to totipotent stem cell induction and maintenance provides a defined *in vitro* system for manipulating and developing understanding of the totipotent state and the development of multicellular organisms from non-germline cells.

In mammals, intrinsic totipotency is the capacity by a cell to give rise to all the differentiated cells in both embryonic and extraembryonic tissues and form an organism¹. In mice, only the zygote and its immediate descendants, 2-cell-stage blastomeres, are totipotent^{2–4}. As an embryo develops, TotiSCs undergo irreversible differentiation into the first two different lineages: the trophoblast, which generates the extraembryonic placenta, and the inner cell mass (ICM) which gives rise to the pluripotent epiblast (EPI) and primitive endoderm (PE), which are precursors of embryonic fetus and extraembryonic yolk sac, respectively. So far, TotiSCs have been generated *in vitro* only transiently through *in vitro* fertilization or somatic cell nuclear transfer using germline cells. Therefore, inducing and maintaining authentic TotiSCs *in vitro* from non-germline represents a key target.

Recent studies have reported observations of cells expressing certain 2-cell-stage genes from mouse pluripotent stem cells⁵ (PSCs), particularly in the presence of specific small molecules, cytokines or transgenes^{6,7}. Around 0.1–1% of cells in mouse embryonic stem (ES) cell and mouse induced pluripotent stem cell cultures express murine endogenous retrovirus with leucine tRNA primer (MERVL) and a few other 2-cell-stage-specific genes (these cells are called 2-cell-like cells (2CLCs)), and possess the limited differentiation potential of 2-cell blastomeres⁸. Subsequent studies have identified several crucial factors determining the 2CLC state, such as DUX⁹, and showed that modulating the expression of these factors was able to induce more 2CLCs. In addition, mouse expanded potential stem cells (mEPSCs) have the potential

to develop into both embryonic and extraembryonic lineages^{9,10}. However, the contribution of mEPSCs to extraembryonic tissues *in vivo* was challenged by a recent study with stringent criteria³. Notably, the transcriptional profiles of 2CLCs and mEPSCs actually more closely resemble mouse ES cells and post-implantation embryos than zygotes or 2-cell-stage totipotent embryos^{3,10}, therefore explaining their limited real developmental potentials compared with totipotent cells.

It has remained challenging to induce, capture and long-term maintain authentic TotiSCs *in vitro* from non-germline cells. In this article, we identify a combination of three small molecules that enable the induction and maintenance of ciTotiSCs from mPSCs, which closely resemble mouse totipotent 2-cell-stage embryos in terms of transcriptome, epigenome and metabolome, and developmental potentials into both embryonic and extraembryonic lineages.

Chemical induction of TotiSCs from mouse ES cells

To develop the conditions to induce TotiSCs, we first screened more than 3,000 chemical compounds using mouse ES cells with the 2-cell-stage-specific MERVL-tdTomato reporter and identified 23 compounds that could increase the number of tdTomato⁺ cells by threefold over the control condition (Fig. 1a,b and Extended Data Fig. 1a,b). Of note, we found several RAR agonists among those top hits. Although RAR agonists could induce massive generation of tdTomato⁺ cells in mouse ES cells (Extended Data Fig. 1c), these cells gradually died or

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792 | Nature | Vol 617 | 25 May 2023

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Ding Lab's Approach to Totipotent Cells

Scientists take the first step to master an all-powerful cell type in the beginning of life

Tsinghua University News Release 2022

From cloning to regeneration, how to find alternative paths to create or rejuvenate life has been one of the big questions for biologists. It is this question that's behind the work of generations of scientists who went on to win Nobel Prizes. It is also this question that drives the recent research led by Sheng Ding at Tsinghua University, School of Pharmaceutical Sciences, now published in the top scientific journal *Nature* magazine.

In the current study, Ding and colleagues have identified a drug cocktail that induces an all-powerful stem cell type at will, a cell type that can turn into an entire organism on its own. The researchers are also able to maintain the resulting cells' differentiation potential in the lab, allowing a stable system for later researchers to demystify the creation of life. This alternative path – obtaining a clean slate of life's earliest raw materials from more mature cells, instead of new sperms and eggs -- can have a wide range of implications. “Such an alternate to nature’s way of creating the beginning of life is a holy grail of biology”, Ding says.

Scientists call these all-powerful cells in the one-cell and two-cell embryo stages totipotent stem cells. And there are pluripotent and multipotent stem cells further down the continuum. “Normally after totipotent cells, none of the other stem cells have the possibility to turn into a life on its own,” Ding says. To better study and control the totipotent stem cells, Ding and his team established a system that achieves the induction and maintenance of these cells and confirmed their identity with stringent criteria. In this sense, “this paper is the first step and opens up tremendous opportunities,” he says.



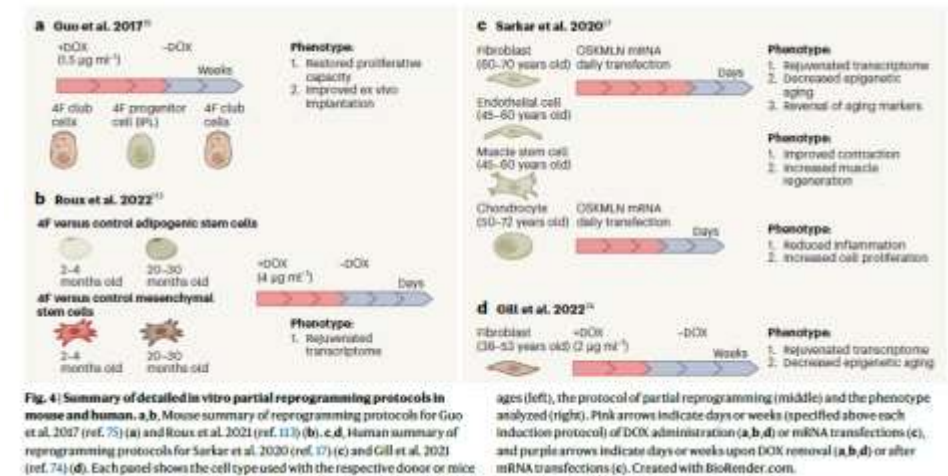
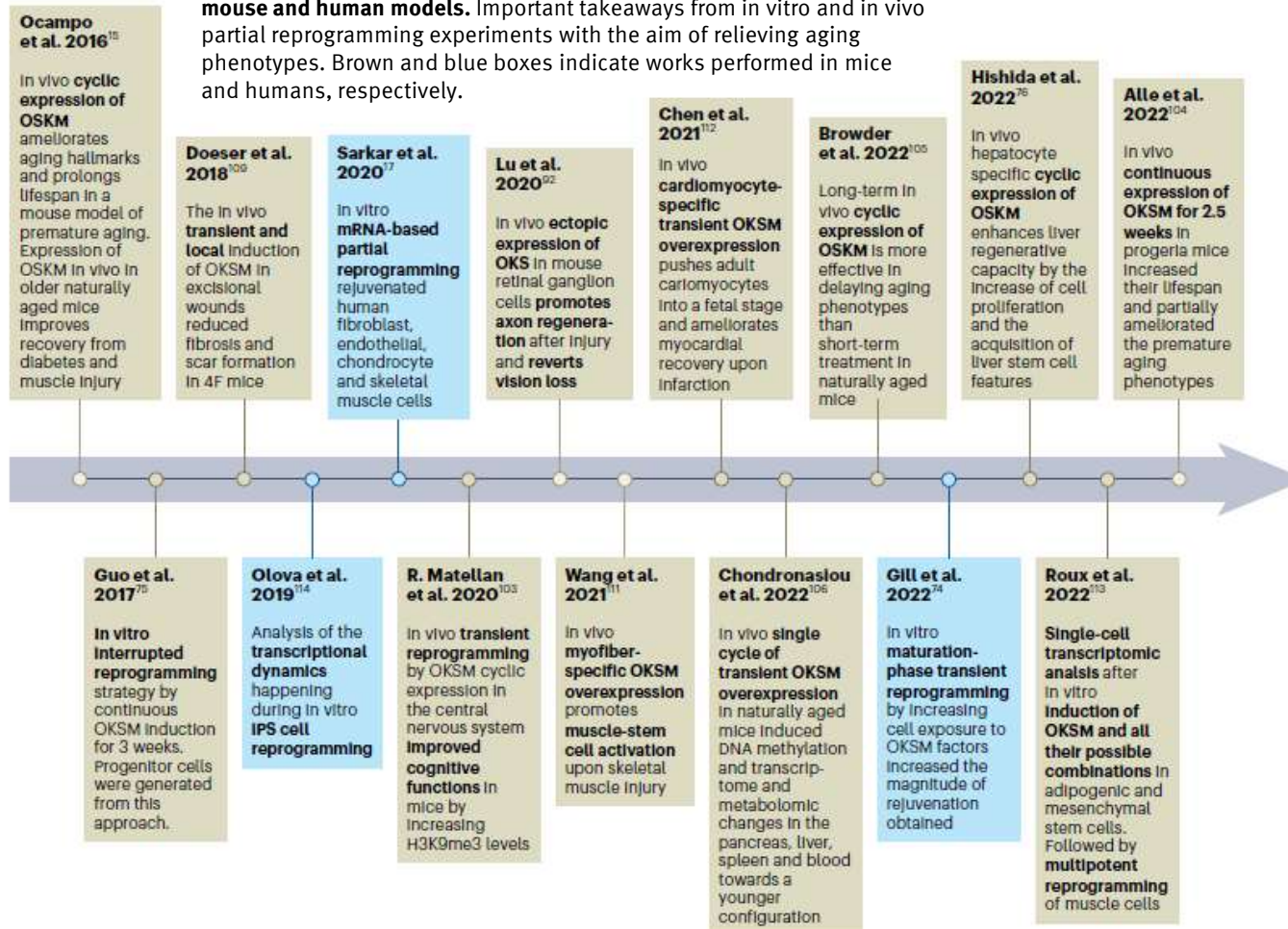
Shen Ding Lab Members on *Nature* paper, 2022

Tsinghua University, Beijing, China

2024: Sebastiano and Collaborators Summarize Rapidly Moving Reprogramming Methods and Literature

We found these charts to be quite helpful in putting together our review of the reprogramming literature.

Fig 2. Timeline highlighting publications on partial reprogramming in mouse and human models. Important takeaways from in vitro and in vivo partial reprogramming experiments with the aim of relieving aging phenotypes. Brown and blue boxes indicate works performed in mice and humans, respectively.



Source: Cipriano A, Moqri M, Maybury-Lewis SY, Rogers-Hammond R, de Jong TA, Parker A, Rasouli S, Schöler HR, Sinclair DA, Sebastiano V., “[Mechanisms, pathways and strategies for rejuvenation through epigenetic reprogramming](#),” *Nature Aging*, Jan 2024; 4(1):14-26.

Section 8.H: Epidemiology and Biomarkers of Aging (and Related Works)

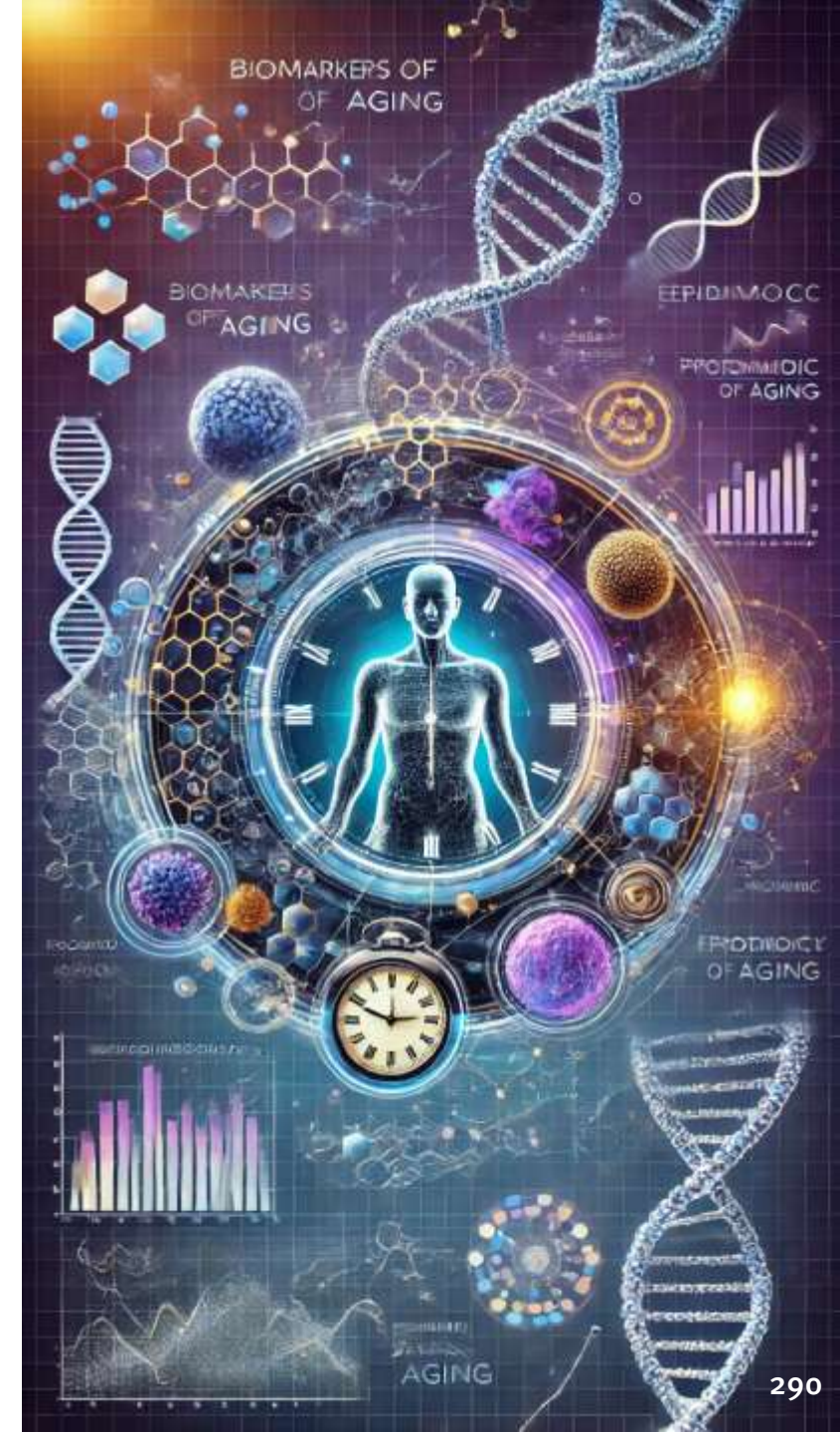
There are three different strands of the aging literature that are covered in this section:

1. A few popular books on aging that were published prior to 2005
2. Articles that discuss the epidemiology of lifespan and potential for increasing it
3. Articles on biomarkers of aging

To start on biomarkers, the idea is that if we could find surrogate markers for aging it would be a lot easier to measure whether anti-aging interventions are working. There has been some excellent work on proteomic determinants of mortality that are measurable with a blood sample. We also find Steve Horvath's "aging clock" concept to be alluring.

Jay Olshansky has written extensively on what it would take to increase human longevity. and his papers are full of interesting insights. We include a number of those here.

Finally, we have included Jimmy Carter's book *On the Virtues of Aging* and Ray Kurzweil's book *Fantastic Voyage*. They are both of historic interest and fascinating.



1987: Rowe and Kahn

First article to use the phrase “healthspan”. the authors distinguish between "usual aging," characterized by typical age-related declines, and "successful aging," where individuals maintain high physical and cognitive function, free from significant diseases and disabilities.

John W. Rowe and Robert L. Kahn’s 1987 article, *Human Aging: Usual and Successful*, published in *Science*, introduced a new framework for understanding aging. They challenged the traditional view that aging is solely a process of inevitable decline, instead distinguishing between “usual aging” and “successful aging.” Usual aging refers to the common trajectory marked by a gradual decline in physical and cognitive function, often influenced by lifestyle and environmental factors rather than aging itself. In contrast, successful aging is characterized by maintaining high cognitive and physical function, avoiding disease and disability and staying socially engaged. This perspective shifted the focus from merely treating age-related diseases to identifying factors that promote long-term health and functionality.

The authors emphasized that successful aging is not purely a matter of genetics but is largely shaped by behavior and environment. They highlighted the role of physical activity, social connections, and cognitive engagement in preserving health over time. Rowe and Kahn’s work laid the foundation for later research on aging interventions, influencing public health policies and gerontology. Their model suggested that individuals and societies could take proactive steps to enhance “healthspan”—the period of life spent in good health—rather than just extending lifespan. This article marked a turning point in aging research, fostering a more optimistic and proactive approach to aging and longevity.

Human Aging: Usual and Successful

JOHN W. ROWE AND ROBERT L. KAHN

Research in aging has emphasized average age-related losses and neglected the substantial heterogeneity of older persons. The effects of the aging process itself have been exaggerated, and the modifying effects of diet, exercise, personal habits, and psychosocial factors underestimated. Within the category of normal aging, a distinction can be made between usual aging, in which extrinsic factors heighten the effects of aging alone, and successful aging, in which extrinsic factors play a neutral or positive role. Research on the risks associated with usual aging and strategies to modify them should help elucidate how a transition from usual to successful aging can be facilitated.

RESEARCH ON AGING HAS EMPHASIZED LOSSES. IN THE absence of identifiable pathology, gerontologists and geriatricians have tended to interpret age-associated cognitive and physiologic deficits as age-determined. We believe that the role of aging per se in these losses has often been overstated and that a major component of many age-associated declines can be explained in terms of life style, habits, diet, and an array of psychosocial factors extrinsic to the aging process.

Research on aging has also emphasized differences between age groups. The substantial heterogeneity within age groups has been either ignored or attributed to differences in genetic endowment. That perspective neglects the important impact of extrinsic factors and the interaction between psychosocial and physiologic variables. In this article we offer our perspective on the place of extrinsic factors in the aging process, the long-term consequences of extrinsically initiated changes, and the implications of our views for gerontological research.

The Concept of Normality in Studies of Aging

In recent decades, increasing interest in the medical problems of older people and in the process of aging itself has stimulated substantial growth in physiologic, psychologic, and sociologic research on aging in human populations. Investigators involved in such studies have from the beginning recognized the importance of separating pathologic changes from those that could be attributed to aging per se. Thus, for physiologic studies careful guidelines have been developed to exclude individuals whose age-determined responses and behaviors might be contaminated by specific disease processes (1, 2). Results on the population remaining after such exclusions have then been interpreted as representing “normal” aging, especially when the data came from longitudinal studies

rather than cross-sectional comparisons of different age groups and were thus less confounded with cohort effects.

The concept of normality, explicit or implied, has served well in such research. During the past 30 years, numerous cross-sectional and longitudinal studies on populations carefully screened for disease have demonstrated major effects of age on such clinically relevant variables as hearing, vision, renal function, glucose tolerance, systolic blood pressure, bone density, pulmonary function, immune function, and sympathetic nervous system activity. More recently, related research has revealed characteristic changes with respect to cognitive and behavioral functions. Such nonpathologic changes are important not only as reflections of the aging process but also, in many cases, as precursors of pathology. They constitute the physiologic substrate for the influence of age on the subsequent presentation of disease, and perhaps on probable response to treatment and likelihood of complications.

Nevertheless, the division of populations into diseased versus normal and the division of research findings into disease-related and age-determined have serious limitations. Chief among these is the neglect of heterogeneity among older people in the nondiseased group with respect to many physiologic and cognitive characteristics, a heterogeneity that is important both within cultures and between cultures. A second limitation of the emphasis on normality is the implication of harmlessness or lack of risk. And a third limitation is the related implication that, risky or not, what is normal is somehow natural and therefore is or should be beyond purposeful modification. In short, the emphasis on “normal” aging focuses attention on learning what most older people do and do not do, what physiologic and psychologic states are typical. It tends to create a gerontology of the usual.

Successful and Usual Aging

Each of the foregoing limitations urges the development of an additional conceptual distinction within the normal category, which can be approximated by the contrast between usual on the one hand, and successful on the other. For example, while many important physiologic variables show substantial losses with advancing age on the average, an important characteristic of such age-grouped data is the substantial variability within groups (1–3). In many data sets that show substantial average decline with age, one can find older persons with minimal physiologic loss, or none at all, when

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10 JULY 1987

ARTICLES 143

Rowe JW, Kahn RL. “Human aging: usual and successful,” *Science*, Jul 10 1987; 237(4811):143-9.

1988: Jimmy Carter

Like Seneca, the former President argues that old age is a time of growth and contribution. His own life exemplified the ideals in this book.

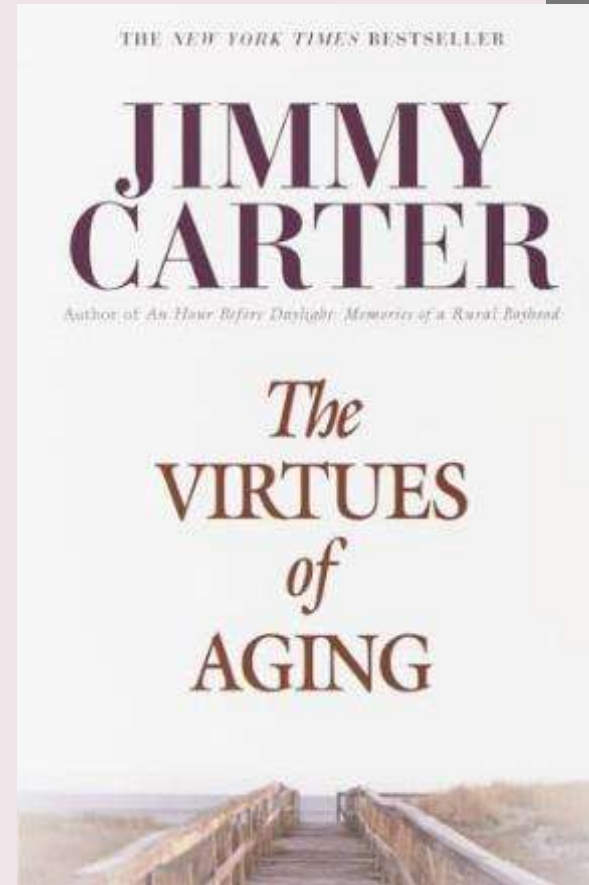
The *Virtues of Aging* by Jimmy Carter, published in 1998, is a thoughtful and inspiring exploration of the opportunities and challenges that come with growing older.

Drawing from his own experiences as a former U.S. President and his life after leaving the White House, Carter reflects on the positive aspects of aging, emphasizing that it can be a time of growth, fulfillment, and continued contribution to society.

He discusses the importance of maintaining physical health, mental sharpness, and emotional well-being, while also highlighting the value of strong relationships, lifelong learning, and finding purpose in later life. Carter's optimistic perspective challenges stereotypes about aging, portraying it as a phase rich with potential rather than one of decline.

This book is very much in the spirit of Seneca's book *De Senectute* (On Old Age). Cicero, through the character of Cato the Elder, argues that old age is not a burden but a time of great value. He highlights the virtues of aging, such as wisdom, freedom from youthful passions, and the opportunity to reflect on life.

Impressively, Mr. Carter made the most of his old age, living to 100 years. This book was very much a fitting tribute to what aging can be and to those who came before him such as Gabriele Paleotti.



Jimmy Carter, *The Virtues of Aging*, Ballantine Books, 1988. Inscribed by President Carter. From the medical library of Tim C. Opler.

1990: Olshansky & Cassel

Argued that getting to a human life expectancy over 85 is extremely unlikely. Interestingly, this prediction has already been disproven.

The authors of this 1990 paper in *Science* argue that while advances in medicine and public health have significantly increased average life expectancy, there is a natural upper limit to how long humans can live.

They challenge the optimistic claims of some scientists and demographers who suggested that human lifespan could be extended indefinitely through medical breakthroughs.

By analyzing historical and biological data, the authors conclude that the maximum human lifespan is unlikely to exceed 120 years, a limit set by the inherent biological constraints of human physiology, such as cellular aging, organ system decline, and the accumulation of damage at the molecular level. They further argued that getting to a human life expectancy of 85 years or more is highly unlikely anytime soon.

The authors note that while improvements in healthcare, nutrition, and lifestyle can reduce mortality rates and extend average life expectancy, they cannot eliminate the fundamental processes of aging.

Olshansky, Carnes, and Cassel caution against unrealistic expectations of radical life extension and advocate for a focus on improving the quality of life in old age rather than pursuing extreme longevity. The paper remains a foundational work in the field of aging research, providing a sobering perspective on the biological limits of human lifespan.

In Search of Methuselah: Estimating the Upper Limits to Human Longevity

S. JAY OLSHANSKY, BRUCE A. CARNES, CHRISTINE CASSEL

Estimates of the upper limits to human longevity have important policy implications that directly affect forecasts of life expectancy, active life expectancy, population aging, and social and medical programs tied to the size and health status of the elderly population. In the past, investigators have based speculations about the upper limits of human longevity on observations of past trends in mortality. Here the estimate of the upper bound is based on hypothesized reductions in current mortality rates necessary to achieve a life expectancy at birth from 80 to 120 years and an expectation of life at age 50 from 30 to 70 years. With the use of conditional probabilities of death from complete life tables for the United States, reductions in mortality required to achieve extreme longevity (that is, 80 to 120 years) were compared with those resulting from hypothetical cures for all cardiovascular diseases, ischemic heart disease, diabetes, and cancer. Results indicate that in order for life expectancy at birth to increase from present levels to what has been referred to as the average biological limit to life (age 85), mortality rates from all causes of death would need to decline at all ages by 55%, and at ages 50 and over by 60%. Given that hypothetical cures for major degenerative diseases would reduce overall mortality by 75%, it seems highly unlikely that life expectancy at birth will exceed the age of 85.

SINCE THE MID-19TH CENTURY THE HUMAN POPULATION HAS experienced one of the most important and dramatic changes in the history of the species—a near doubling of the expectation of life at birth from 40 to near 80 years (1). Most of the mortality declines and increases in life expectancy that occurred early in this century were a result of rapidly declining neonatal, infant, and maternal mortality. Today, mortality rates in younger and middle age groups are so low in the United States that the complete elimination of mortality before the age of 50 (about 12.4% of all deaths) would increase life expectancy at birth by only 3.5 years. Thus, the potential for additional increases in longevity relies on progress in dealing with the diseases of the elderly.

While there has always been a segment of the human population that survived to older ages, from ancient times until this century

only marginal improvements seem to have been made in extending life for the population aged 50 and older. Interestingly, in the last 25 years in the United States, age-adjusted death rates from the major cardiovascular diseases declined by more than 34% (2). Most of the declines in mortality and gains in life expectancy during this recent mortality transition were achieved in the elderly population—a phenomenon so unexpected and unexplained that it has been referred to as a new stage in the epidemiologic history of developed nations (3-5).

Having made such rapid advances in the extension of life during the last 100 years, researchers are now beginning to ask why this recent transition in mortality took place, how long it can continue, and what factors might contribute to future gains in life expectancy (6). These are crucial policy questions because they address estimates of the size, proportional distribution, and health status of the elderly population in the future.

A related issue of critical importance is whether further declines in mortality would lead to an increased active life expectancy or an expanded period of frailty and dependency. Unless active life expectancy is improved from present levels, the combination of population aging, a larger than predicted elderly population, and possible shifts in the distribution of frailty conditions among the oldest-old, would have an enormous adverse impact on government-funded programs such as Social Security and Medicare. Many interdisciplinary studies are currently addressed to this issue (7-12).

In this article we examine the methods that have been used to estimate the upper limits to human longevity and determine precisely what age-specific mortality declines would be required to achieve specified life expectancy targets. Insights gained from pursuing the second line of inquiry should help clarify issues concerning active life expectancy.

Terminology

Since research on human aging and longevity is an interdisciplinary effort, the terms used to describe these phenomena (for example, life expectancy, active life expectancy, life-span, average life-span, maximum life-span potential) are often used interchangeably (6). To avoid problems of definition, the terms we use are defined as follows: (i) "life expectancy" is the average number of years of life remaining for a population of individuals, all of age x , and all subject for the remainder of their lives to the observed age-specific death rates corresponding to a current life table. This is referred to in demography as "period life expectancy" because it is based on the risks of mortality that are present during a single time period (13). Although life expectancy may be calculated for any age, it is most often presented as life expectancy at birth. However, when the concern is mainly with older ages, the expectation of life at some

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634

SCIENCE, VOL. 250

Olshansky SJ, Carnes BA, Cassel C., "In search of Methuselah: estimating the upper limits to human longevity," *Science*. Nov 2 1990. 250(4981):634-40.

2001: Jay Olshansky

This popular book argues for compression of morbidity rather than outright efforts to change human lifespan.

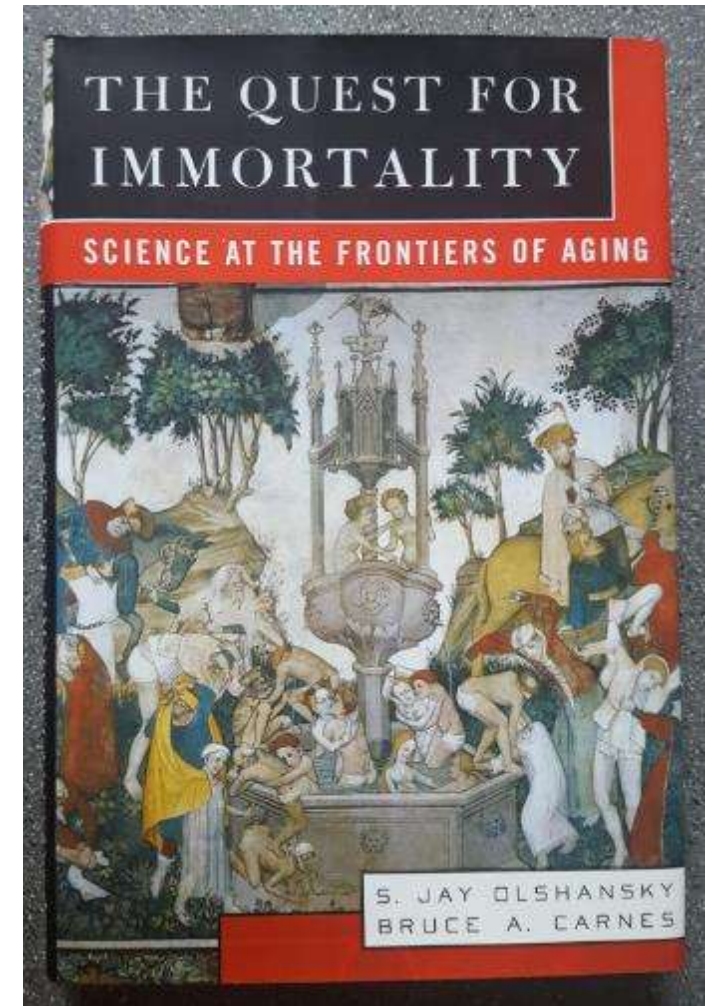
S. Jay Olshansky's *The Quest for Immortality: Science at the Frontiers of Aging* (2001) explores the scientific, social, and ethical dimensions of aging and the human pursuit of extended lifespan. Olshansky, a leading gerontologist, critically examines the history of efforts to combat aging, from ancient myths and alchemy to modern scientific breakthroughs. He discusses the biological mechanisms of aging, such as cellular damage, genetic factors, and the limits of human longevity, while debunking popular myths about anti-aging remedies and immortality.

Olshansky emphasizes that while science has made significant strides in understanding aging, the idea of "curing" aging or achieving immortality remains speculative and fraught with challenges.

The book also addresses the societal implications of significantly extending human lifespan, including the potential strain on resources, healthcare systems, and intergenerational equity.

Olshansky argues that the focus should shift from pursuing immortality to improving the quality of life in old age, a concept he terms "compression of morbidity." He argues that reduction of multi-morbidity is an important way to increase human life spans.

He advocates for research into healthy aging and preventive medicine, rather than unrealistic quests for eternal life. *The Quest for Immortality* serves as a sobering yet hopeful critique of humanity's fascination with defeating aging, urging a balanced approach that prioritizes scientific rigor, ethical considerations, and the well-being of future generations.



S. Jay Olshansky and Bruce A. Carnes, *The Quest for Immortality*, 2001. W.W. Norton: New York. From the medical library of Tim C. Opler. Book inscribed by Dr. Olshansky.

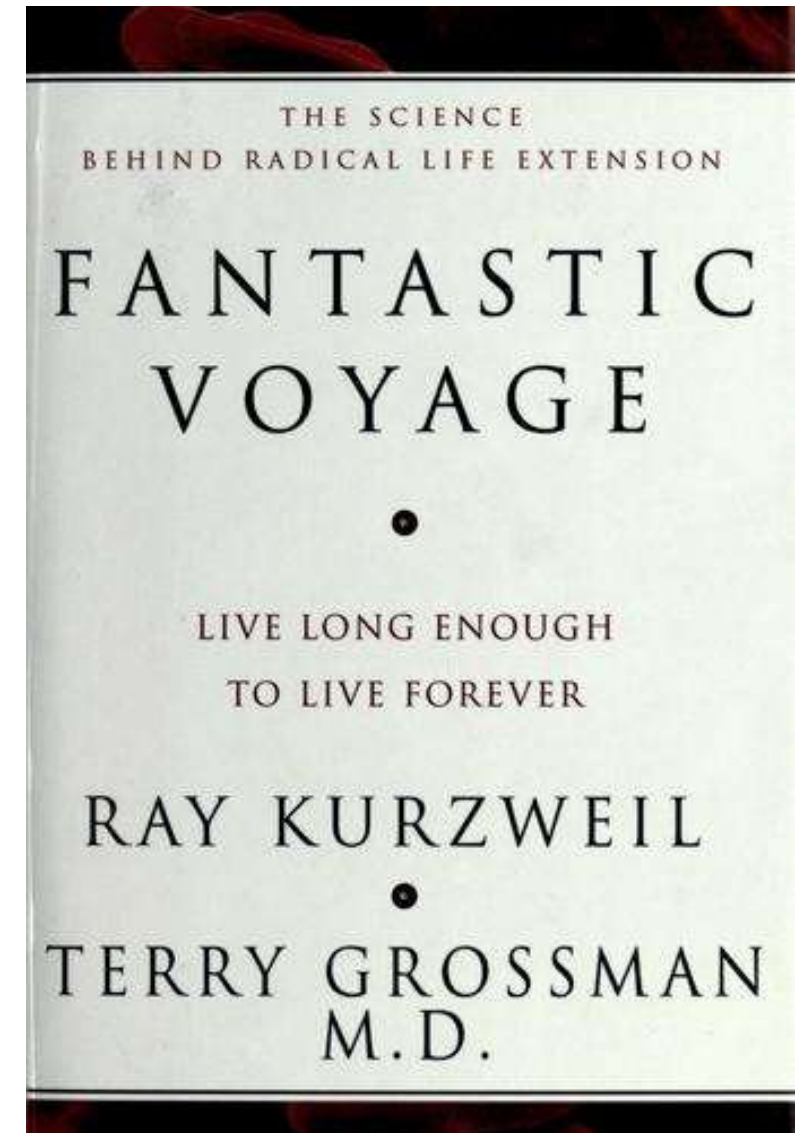
2004: Ray Kurzweil

In an exceedingly hopeful book, Kurzweil argued that if middle aged people can live long enough, until approximately 120 years, they will be able to live forever—as humanity overcomes all diseases and old age itself.

"Fantastic Voyage: Live Long Enough to Live Forever" (2004) by Ray Kurzweil and Terry Grossman is an optimist's guide to achieving radical life extension through the integration of current health practices and emerging technologies. Kurzweil is the leader of the transhumanist movement.

The book is structured around the concept of "Three Bridges to Longevity." Bridge One focuses on leveraging existing knowledge and technologies, such as personalized nutrition, exercise, stress management and medical treatments, to maintain health and extend life. The authors provide practical advice on optimizing diet, supplementation and lifestyle to combat aging and disease. Bridge Two explores near-future advancements in biotechnology, including genetic engineering, stem cell therapy and advanced diagnostics, which promise to revolutionize medicine and further extend human lifespans. Bridge Three envisions a more distant future where nanotechnology and artificial intelligence will enable the repair and enhancement of the human body at the molecular level, potentially allowing humans to overcome aging entirely.

Kurzweil and Grossman also discuss the ethical and societal implications of radical life extension, such as resource allocation and socioeconomic disparities in access to life-extending technologies.



Ray Kurzweil and Terry Grossman, *Fantastic Voyage: Live Long Enough to Live Forever*, Rodale, 2004. Inscribed by Ray Kurzweil. From the medical library of Tim C. Opler.

2012: James Carey

Groundbreaking finding on mortality plateaus in aging. Questions a key assumption in the fields of gerontology and evolutionary biology, although this was just seen in medflies.

It is generally assumed for most species that mortality rates increase monotonically at advanced ages. Mortality rates were found to level off and decrease at older ages in a population of 1.2 million medflies maintained in cages of 7,200 and in a group of approximately 48,000 adults maintained in solitary confinement.

The study demonstrated that mortality rates stop increasing at very advanced ages, a finding that contradicted the widely accepted Gompertz-Makeham law of mortality, which posits that mortality risk rises exponentially with age.

Thus, life expectancy in older individuals increased rather than decreased with age. These results cast doubt on several central concepts in gerontology and the biology of aging: (i) that senescence can be characterized by an increase in age-specific mortality, (ii) that the basic pattern of mortality in nearly all species follows the same unitary pattern at older ages and (iii) that species have absolute life-span limits.

The first concept challenged by Carey et al.'s findings is the idea that "senescence can be operationally defined and measured by an ever-increasing rate of mortality." Traditionally, aging has been associated with a continuous decline in physiological function, leading to an exponential increase in the risk of death. However, Carey et al. observed that mortality rates plateaued in the oldest medflies, suggesting that senescence does not inevitably lead to an ever-accelerating risk of death

Slowing of Mortality Rates at Older Ages in Large Medfly Cohorts

James R. Carey,* Pablo Liedo, Dina Orozco, James W. Vaupel

It is generally assumed for most species that mortality rates increase monotonically at advanced ages. Mortality rates were found to level off and decrease at older ages in a population of 1.2 million medflies maintained in cages of 7,200 and in a group of approximately 48,000 adults maintained in solitary confinement. Thus, life expectancy in older individuals increased rather than decreased with age. These results cast doubt on several central concepts in gerontology and the biology of aging: (i) that senescence can be characterized by an increase in age-specific mortality, (ii) that the basic pattern of mortality in nearly all species follows the same unitary pattern at older ages, and (iii) that species have absolute life-span limits.

Age-specific mortality rates (1) are used by gerontologists, demographers, and biologists in a number of interrelated ways including quantifying senescence in populations (2), comparing species (3), and inferring species-specific life-span limits (4). Surprisingly, the pattern of age-specific mortality is well known only for *Homo sapiens*, and even for humans data are sparse after age 85. For 48 species scattered across

various phyla, Finch estimated the level of mortality at the age of sexual maturity and the increase in the mortality rate with age, but he warned that the estimates "should be considered first approximations within a twofold range" (5). The estimates depend on the untested assumption that mortality increases at the same rate from sexual maturity to advanced old age; as Finch noted, "there is no a priori reason why mortality rates should conform to functions" of this type (6). The number of observations of age at death for any nonhuman species is small. In a typical study of mortality, the life-spans of some 20 to 50 individuals are observed in laboratory or field settings (7); only rarely has mortality in several thousand individuals been monitored (8). When only a few hundred individuals are observed, the pattern of age-specific mortality at older ages,

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SCIENCE • VOL. 258 • 16 OCTOBER 1992

457

Carey JR, Liedo P, Orozco D, Vaupel JW. Slowing of mortality rates at older ages in large medfly cohorts. *Science*. 1992 Oct 16;258(5081):457-61.

2013: Steve Horvath

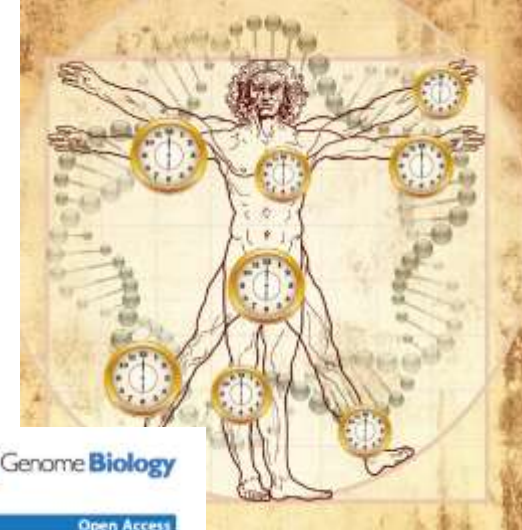
Showed that DNA methylation patterns are strongly related to age and could provide a prediction of biological age (a “clock”). This is the sixth most cited research paper on aging.

Steve Horvath's 2013 paper, "DNA Methylation Age of Human Tissues and Cell Types," introduced the first multi-tissue epigenetic clock, a novel tool for estimating biological age based on DNA methylation patterns.

Horvath analyzed DNA methylation data from a wide range of human tissues and cell types, identifying 353 specific CpG sites whose methylation levels strongly correlated with age. Using these sites, he developed a mathematical model that could accurately predict the chronological age of individuals based on their DNA methylation profile. Remarkably, this epigenetic clock was applicable across diverse tissue types, including blood, brain, muscle, and skin, making it a universal biomarker of aging. The study demonstrated that biological age, as measured by DNA methylation, could differ from chronological age, providing a new way to study the aging process and its variability among individuals.

The paper also revealed that certain tissues age faster or slower than others, highlighting the heterogeneity of aging across the body. Horvath's epigenetic clock provided a powerful tool for investigating the effects of genetics, lifestyle, and environmental factors on biological aging, as well as for evaluating interventions aimed at extending healthspan and lifespan.

References: Ramakrishnan p. 97.



Horvath Genome Biology · 14(R15)
<http://genomebiology.com/14/R0115>



RESEARCH

Open Access

DNA methylation age of human tissues and cell types

Steve Horvath^{1,2,3}

Abstract

Background: It is not yet known whether DNA methylation levels can be used to accurately predict age across a broad spectrum of human tissues and cell types, nor whether the resulting age prediction is a biologically meaningful measure. **Results:** I developed a multi-tissue predictor of age that allows one to estimate the DNA methylation age of most tissues and cell types. The predictor, which is freely available, was developed using 6,000 samples from 82 human DNA methylation array datasets, encompassing 51 healthy tissues and cell types. I found that DNA methylation age has the following properties: first, it is close to zero for embryonic and induced pluripotent stem cells; second, it correlates with cell passage number; third, it gives rise to a highly heritable measure of age acceleration; and, fourth, it is applicable to cancerous tissues. Analysis of 6,000 cancer samples from 12 datasets showed that all of the considered 20 cancer types exhibit significant age acceleration, with an average of 36 years. Low age acceleration of cancer tissue is associated with a high number of somatic mutations and TP53 mutations, while mutations in steroid receptors greatly accelerate DNA methylation age in breast cancer. Finally, I characterize the 353 CpG sites that together form an aging clock in terms of chromatin states and tissue variance.

Conclusions: I propose that DNA methylation age measures the cumulative effect of an epigenetic maintenance system. This novel epigenetic clock can be used to address a host of questions in developmental biology, cancer, and aging research.

Background

An increasing body of evidence suggests that many manifestations of aging are epigenetic [1,2]. This article focuses on one particular type of epigenetic control: cytosine-5 methylation within CpG dinucleotides (also known as DNA methylation). Age-related DNA hypomethylation has long been observed in a variety of species, including salmon [3,4], rats [5], and mice [6]. More recent studies have shown that many CpGs are subject to age-related hypermethylation. A vast literature characterizes genes or genomic regions that either get hypomethylated or hypermethylated with age [6–14]. Previous studies have shown that age-related hypermethylation occurs preferentially at CpG islands [9], at insulator chromatin domains [15], and at Polycomb target

protein targets [16]. The epigenetic landscape varies markedly across tissue types [16–18] and many age-related changes depend on tissue type [9,19]. But several recent studies have shown that age-dependent CpG signatures can be defined independently of sex, tissue type, disease state and array platform [10,13–15,20–22]. While several recent articles describe age predictions based on DNA methylation data sets for defining and evaluating an age predictor, the astonishing accuracy across most tissues and cell types justifies its designation as a multi-tissue age predictor. Its age prediction, referred to as DNAm age, can be used as a biomarker for addressing a host of questions arising in aging research and related fields. For example, I show that premature aging diseases (such as progeria) do not resemble healthy normal aging according to DNAm age and that interventions used for erasing induced pluripotent stem (iPS) cells reset the

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Steve Horvath, 2013, DNA methylation age of human tissues and cell types, *Genome Biology*, 14: R1-R15.

2013: Lopez-Ortin et.al. – Hallmarks of Aging

This widely followed review outlined nine key biological hallmarks of aging, providing a comprehensive framework for understanding aging mechanisms and guiding research.

The 2013 paper "The Hallmarks of Aging" by López-Otín et al., published in *Cell*, is a foundational review that outlines nine key biological processes driving aging. These hallmarks include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Each hallmark represents a distinct yet interconnected mechanism contributing to the progressive decline in cellular and organismal function.

For example, genomic instability and telomere attrition lead to DNA damage and cellular aging, while epigenetic alterations disrupt gene expression patterns. Loss of proteostasis results in the accumulation of damaged proteins, and deregulated nutrient sensing impairs metabolic balance. Mitochondrial dysfunction reduces energy production and increases oxidative stress, while cellular senescence and stem cell exhaustion limit tissue repair and regeneration. Finally, altered intercellular communication promotes chronic inflammation and tissue dysfunction. Together, these hallmarks create a vicious cycle of decline that characterizes aging.

The paper emphasizes that these processes are not isolated but interact in complex ways, amplifying each other's effects. For instance, mitochondrial dysfunction can exacerbate oxidative stress, leading to further genomic instability and epigenetic changes.

The Hallmarks of Aging

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Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death. This deterioration is the primary risk factor for major human pathologies, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. Aging research has experienced an unprecedented advance over recent years, particularly with the discovery that the rate of aging is controlled, at least to some extent, by genetic pathways and biochemical processes conserved in evolution. This Review enumerates nine tentative hallmarks that represent common denominators of aging in different organisms, with special emphasis on mammalian aging. These hallmarks are: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. A major challenge is to dissect the interconnectedness between the candidate hallmarks and their relative contributions to aging, with the final goal of identifying pharmaceutical targets to improve human health during aging, with minimal side effects.

Introduction

Aging, which we broadly define as the time-dependent functional decline that affects most living organisms, has attracted curiosity and excited imagination throughout the history of humankind. However, it has only been 30 years since a new era in aging research was inaugurated following the isolation of the first long-lived strains in *Caenorhabditis elegans* (*C. elegans*) (Klass, 1983). Nowadays, aging is subjected to scientific scrutiny based on the ever-expanding knowledge of the molecular and cellular bases of life and disease. The current situation of aging research exhibits many parallels with that of cancer research in previous decades. The cancer field gained major momentum in 2000, with the publication of a landmark paper that enumerated six hallmarks of cancer (Hanahan and Weinberg, 2000), recently expanded to ten (Hanahan and Weinberg, 2011). This categorization has helped to conceptualize the essence of cancer and its underlying mechanisms.

At first sight, cancer and aging may seem to be opposite processes: cancer is the consequence of an aberrant gain of cellular fitness, whereas aging is characterized by a loss of fitness. At a deeper level, however, cancer and aging share common origins. The time-dependent accumulation of cellular damage is widely considered to be the general cause of aging (Gems and Partridge, 2013; Kirkwood, 2005; Vijg and Campisi, 2008).

Concomitantly, cellular damage may occasionally provide aberrant advantages to certain cells, which can eventually produce cancer. Therefore, cancer and aging can be regarded as two different manifestations of the same underlying process—namely, the accumulation of cellular damage. In addition, several of the pathologies associated with aging, such as atherosclerosis and inflammation, involve uncontrolled cellular overgrowth or hyperactivity (Blagosklonny, 2008). Based on this conceptual framework, several critical questions have arisen in the field of aging regarding the physiological sources of aging-causing damage, the compensatory responses that try to re-establish homeostasis, the interconnection between the different types of damage and compensatory responses, and the possibilities to intervene exogenously to delay aging.

Here, we have attempted to identify and categorize the cellular and molecular hallmarks of aging. We propose nine candidate hallmarks that are generally considered to contribute to the aging process and together determine the aging phenotype (Figure 1). Given the complexity of the issue, we have emphasized current understanding of mammalian aging while recognizing pioneer insights from simpler model organisms (Gems and Partridge, 2013; Kenyon, 2010). Each hallmark should ideally fulfill the following criteria: (1) it should manifest during normal aging; (2) its experimental aggravation should accelerate aging; and (3)

2014: Brian Kennedy and Felipe Sierra



This consensus commentary was highly influential in U.S. gerontology policy. The key idea was that high burden of chronic disease costs are linked to lifespan and that efforts to improve lifespan can be used to manage disease.

In their 2014 *Cell* article, "Geroscience: Linking Aging to Chronic Disease," Kennedy et al. present a comprehensive overview of geroscience, an interdisciplinary field that explores the connections between aging and chronic diseases.

The authors argue that aging is the primary risk factor for many chronic conditions, such as cancer, cardiovascular disease, and neurodegenerative disorders, and that understanding the biological mechanisms of aging could lead to interventions that delay or prevent these diseases. They highlight key hallmarks of aging, including genomic instability, cellular senescence, and mitochondrial dysfunction, and emphasize how these processes contribute to age-related pathologies. The paper advocates for a shift in focus from treating individual diseases to targeting the underlying aging processes, proposing that such an approach could extend healthspan and reduce the burden of chronic illness.

The authors call for increased research into geroscience, stressing its potential to revolutionize medicine by developing therapies that simultaneously mitigate multiple age-related diseases, thereby improving quality of life and reducing healthcare costs.

Senior author Felipe Serra coined the phrase “geroscience” from his long-standing position as Director of the Division of Aging Biology at the National Institute of Aging.

Geroscience: Linking Aging to Chronic Disease

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Mammalian aging can be delayed with genetic, dietary, and pharmacologic approaches. Given that the elderly population is dramatically increasing and that aging is the greatest risk factor for a majority of chronic diseases driving both morbidity and mortality, it is critical to expand geroscience research directed at extending human healthspan.

The NIH mission is “to seek fundamental

knowledge about the nature and behavior

of living systems and the application

of that knowledge to enhance health,

lengthen life, and reduce illness and

disability.” NIH-funded research is struc-

tured to address the major diseases

driving morbidity and mortality. Interrog-

ating and developing therapeutics for

one disease at a time has often been

productive. Will, however, the success of

this approach be sustainable for the

chronic aging diseases, such as neurode-

generative and metabolic syndromes,

most cancers, and cardiovascular dis-

ease? Findings in the last few decades

have made it impossible to ignore the

integrative nature of human physiology.

Pathologies thought to be disparate are

now understood to be connected. This

awareness raises the possibility that the

stated goals in the NIH mission statement

are best achieved by novel integrated

approaches to health and disease, with

the understanding that biological systems

change with age.

Basic aging research has always been

intriguing but until recently seemed to

hold few concrete solutions to advance

health. Despite limited resources, the field

recently erupted and now offers routes

to achieve a new and ambitious goal—a

substantial extension in health life ex-

pectancy, or “healthspan.” The lynchpin

is that aging itself is the predominant

risk factor for most diseases and condi-

tions that limit healthspan. Moreover,

interventions that extend lifespan in

model organisms often delay or prevent

many chronic diseases. This knowledge

launched the era of geroscience, which

strives to understand how aging enables

chronic disease and seeks to develop

novel multi-disease preventative and

therapeutic approaches.



Cell 159, November 6, 2014 ©2014 Elsevier Inc. 709

Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, Franceschi C, Lithgow GJ, Morimoto RI, Pessin JE, Rando TA., “Geroscience: linking aging to chronic disease,” *Cell*, Nov 6, 2014; 159(4):709-13.

Felipe Sierra: A Giant in Driving Aging Research Forward

NIH Announcement, May 6, 2020

Felipe Sierra, Ph.D., director of NIA's Division of Aging Biology (DAB) and an international advocate for growing the field of geroscience, retired from federal service on April 30, 2020, after leading the division for 14 years.

A native of Chile, he came to the U.S. after studying biochemistry at the Universidad de Chile. He received his Ph.D. in Biochemistry and Molecular Biology at the University of Florida, and then did postdoctoral work at the University of Geneva, Switzerland. While working for Nestlé in Switzerland, he became interested in the biology of aging. This pursuit led him back to academia and the U.S., first as an assistant professor at the Medical College of Pennsylvania, and later as an Associate Professor at the Lankenau Institute for Medical Research.

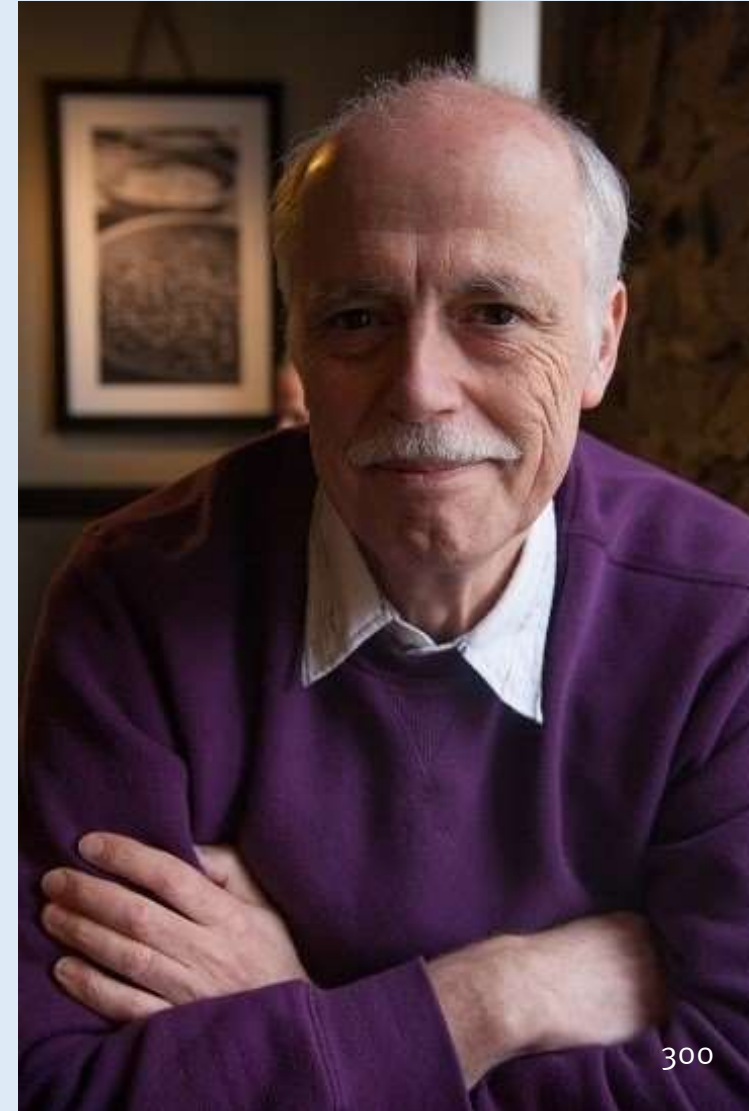
In 2002, Sierra joined the NIA DAB as a program officer, becoming the director in 2006. He soon founded the trans-NIH Geroscience Interest Group (GSIG), which pushes for better understanding of the ways in which the basic biology of aging is a major risk factor for age-related diseases like cardiovascular disease, cancer, diabetes and Alzheimer's. In 2013 and 2014 he received NIH Director's Awards for this effort.

As NIA's DAB director, he attracted a new generation of young investigators to research in the biology of aging. Through the GSIG, he convened several successful Geroscience Summits, edited the first book on geroscience, and wrote numerous influential articles to advance the evolving field. A talented artist, Sierra painted the cover art for the inaugural issue of the journal GeroScience. **In addition to geroscience, he worked to spearhead understanding of concepts of healthspan as well as molecular and cellular resilience.**

"Felipe truly helped change the conversation on aging and geroscience at the NIH and internationally," said NIA Director Richard J. Hodes, M.D. "He leaves a legacy of curiosity and collaboration in the field that will continue to influence future generations of scientists."

"Felipe made geroscience an internationally recognized research field. He also enhanced the infrastructure for research in basic biology in a way that will help sustain the field long after his departure from NIA," said Ron Kohanski, Deputy Director, NIA DAB. "Personally, I value our 15 years together with Felipe as my friend and mentor."

Source: <https://www.nia.nih.gov/news/dr-felipe-sierra-nia-division-aging-biology-director-retires>



2021: The deCODE Team

Modern Proteomics: A simple blood draw can give a very good read of mortality risk. One protein, GDF-15, dominates the ability to predict mortality and is only surpassed in predictive power by age itself.

In a June 2021 research paper in *Communications Biology* scientists from deCODE, a subsidiary of Amgen, describe a predictor of human mortality. Using a dataset of ~5000 protein measurements in 22,913 Icelanders, the scientists developed a predictor of life expectancy that substantially outperformed predictors based on traditional risk factors.

The top predictor of mortality is a non-specific marker called GDF-15. A five-marker model does extraordinarily well in predicting mortality. The AUC in a logit model runs in the low 80's up to around 88% (in the best model).

This is without including traditional risk factors but with age and sex included. None of the top five predictors are associated with traditional phenotypic disease but all are related in some way to subclinical pathology.

A proteomic predictor gives a good estimate of health from a single blood draw (at least if you were born in Iceland). The difference in mortality from the 99th risk percentile to the 1st percentile was over 300 to 1. To compare, the best published finding ever for the life insurance industry today using conventional diagnostics is 11 to 1.

“The predictor gives a good estimate of general health from a single blood draw,” says Thjodbjorg Eiriksdottir scientist at deCODE genetics.



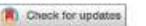
Thjodbjorg Eiriksdottir and Kari Stefansson, deCODE

communications biology

ARTICLE

<https://doi.org/10.1038/s42003-021-02289-6>

OPEN



Predicting the probability of death using proteomics

Thjodbjorg Eiriksdottir¹, Steinthor Ardal¹, Benedikt A. Jonsson¹, Sigrun H. Lund¹, Erna V. Ivarsdottir¹, Kristjan Norland¹, Egil Ferkingstad¹, Hreinn Stefansson¹, Ingileif Jonsdottir^{1,2,3}, Hilma Holm¹, Thorunn Rafnar¹, Jona Saemundsdottir¹, Gudmundur L. Norddahl¹, Gudmundur Thorgeirsson^{1,2,3}, Daniel F. Gudbjartsson^{1,2}, Patrick Sulem¹, Unnur Thorsteinsdottir^{1,2}, Kari Stefansson^{1,2} & Magnus O. Ulfarsson^{1,2}

Eiriksdottir T. et.al. Predicting the probability of death using proteomics. *Commun Biol.* 2021 Jun 18;4(1):758.

2023: Tony Wyss-Coray

One organ in a person's body can age faster than the rest — with implications for health and mortality.

In this 2023 *Nature* paper Wyss-Coray and colleagues studied 11 major organs and report that all of them can be subject to 'accelerated' ageing, as defined by the levels of certain proteins in the blood.

Around one-fifth of the more than 5,600 people who participated in the study met the authors' criteria for accelerated ageing for at least one organ. Such hyper-aged organs are linked to a higher prevalence of disease, and having one organ of unusually advanced age is linked to a higher risk of premature death, the study found.

The findings have significant implications for early disease detection and personalized medicine. By identifying organ-specific aging signatures in blood, the study provides a non-invasive method to monitor organ health and predict disease risk. For example, accelerated brain aging was linked to cognitive decline and Alzheimer's disease, while accelerated heart aging was associated with cardiovascular disease.

This work highlights the potential of plasma proteomics as a tool for tracking organ health and aging, offering new opportunities for early intervention and targeted therapies to mitigate age-related diseases. Wyss-Coray's research represents a real advance in understanding the systemic nature of aging and its impact on organ function and disease.

Article

Organ aging signatures in the plasma proteome track health and disease

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Animal studies show aging varies between individuals as well as between organs within an individual^{1–4}, but whether this is true in humans and its effect on age-related diseases is unknown. We utilized levels of human blood plasma proteins originating from specific organs to measure organ-specific aging differences in living individuals. Using machine learning models, we analysed aging in 11 major organs and estimated organ age reproducibly in five independent cohorts encompassing 5,676 adults across the human lifespan. We discovered nearly 20% of the population show strongly accelerated age in one organ and 1.7% are multi-organ agers. Accelerated organ aging confers 20–50% higher mortality risk, and organ-specific diseases relate to faster aging of those organs. We find individuals with accelerated heart aging have a 250% increased heart failure risk and accelerated brain and vascular aging predict Alzheimer's disease (AD) progression independently from and as strongly as plasma pTau-181 (ref. 5), the current best blood-based biomarker for AD. Our models link vascular calcification, extracellular matrix alterations and synaptic protein shedding to early cognitive decline. We introduce a simple and interpretable method to study organ aging using plasma proteomics data, predicting diseases and aging effects.

Aging results in organism-wide deterioration of tissue structure and function that drastically increases the risk of most chronic diseases. Comprehensive studies of the molecular changes that occur with aging across multiple organs in mice have identified unique molecular aging trajectories and timings^{1–4}, and susceptibility and resilience to diseases of aging in specific organs such as the brain, heart and kidney varies substantially across the population⁴. However, little is known about how human organs change molecularly with age. A molecular understanding of human organ aging is of critical importance to address the massive global disease burden of aging and could revolutionize patient care, preventative medicine and drug development⁵. In particular, preclinical studies have demonstrated that rejuvenating interventions affect organs differently^{3,6}. To translate these studies into transformative

medicines, we must be able to accurately measure aging across the body and understand the diversity of human aging not only across but also within individuals.

While many methods to measure molecular aging in humans have been developed^{7–9}, most of them provide just a single measure of aging for the whole body. This is difficult to interpret given the complexity of human aging trajectories. Some recent methods have used clinical chemistry markers which include some markers of organ function^{10–13}. However, many of these markers have low organ specificity, making them difficult to interpret for organ specific aging. Methods to measure brain aging have used MRI-based brain volume and functional connectivity measurements, which are costly and do not provide molecular insights¹⁴, or have required tissue samples, which prevents their

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2024: Austin Argentieri and Cornelia van Duijn

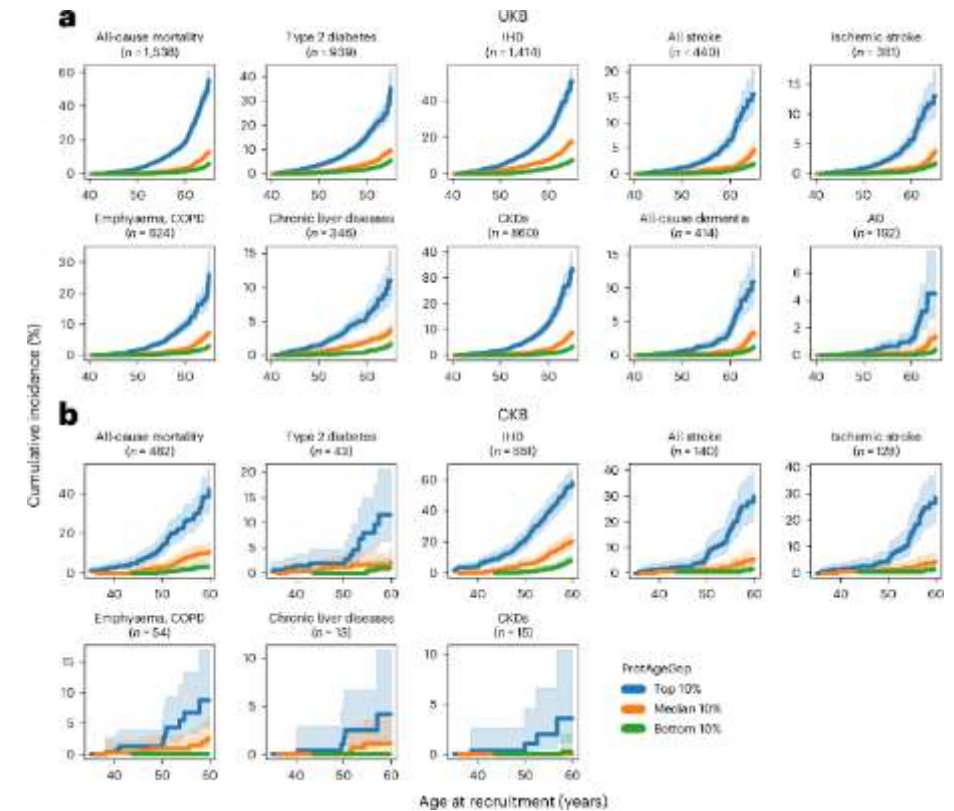
Proteomic Aging Signatures Predict Disease Risk and Mortality Across Diverse Populations.

Argentieri and van Duijn of Oxford developed a machine learning model that uses blood proteomics information to estimate a proteomics age clock for a large sample of participants from the UK Biobank (UKB) ($n = 45,441$; age range, 40–70 years). They further validated this model in two biobanks across the world: the China Kadoorie Biobank (CKB) ($n = 3,977$; age range, 30–80 years) and FinnGen ($n = 1,990$; age range, 20–80 years).

These biobanks represent geographically and genetically distinct populations that differ from the UKB in age range and morbidity profiles. They assessed the influence of the proteomic age gap (defined as the difference between protein-predicted age and chronological age) on 27 aging-related phenotypes related to biological, functional and cognitive function; all-cause mortality; and incidence of 26 common age-related diseases.

They identified 204 proteins that accurately predict chronological age, and further identified a set of 20 aging-related proteins that capture 91% of the age-prediction accuracy of the larger model. They demonstrated that their proteomics age clock showed age-prediction accuracy in independent participants from China and Finland similar to its performance for the UK Biobank.

This proteomic aging measure was associated with the incidence of 18 major chronic diseases (including diseases of the heart, liver, kidneys and lungs, diabetes, neurodegeneration and cancer), multimorbidity and all-cause mortality.



Cumulative incidence of various diseases and mortality (above plots) by decile of proteomic age gap (ProtAgeGap) (in years) in the UKB (a) and CKB (b). Incidence rates are shown for the subsequent 11–16 years (UKB) or 11–14 years (CKB) of follow-up after recruitment for each given age at recruitment. All plots show the cumulative density of events at a given timepoint on the basis of the Kaplan–Meier survival function, with 95% confidence intervals (lighter shading).

Argentieri, M.A., Xiao, S., Bennett, D. et al. Proteomic aging clock predicts mortality and risk of common age-related diseases in diverse populations. *Nature Medicine*, Aug 8, 2024: 30, 2450–2460

2025: HELO Framework

Andrea Maier and colleagues around the globe introduced the HEalthy LOngevity (HELO) conceptual framework in 2025. This framework provides a means for quantifying and managing healthspan in a population health setting.

The article "Awareness, knowledge, and motivations about lifespan, healthspan, and Healthy Longevity Medicine in the general population: the HEalthy LOngevity (HELO) conceptual framework" introduces the HELO framework, a model designed to assess public understanding and engagement with the concepts of lifespan, healthspan, and Healthy Longevity Medicine (HLM).

The framework is structured around three core components: awareness, which measures public recognition of these terms; knowledge, which evaluates the depth of understanding regarding their implications; and motivations, which explore personal drivers such as personality traits, behaviors, beliefs, and health perceptions that influence an individual's interest in pursuing healthy longevity. The authors argue that while life expectancy has increased, public understanding of the importance of maintaining health during extended years remains limited, necessitating better strategies for public education and engagement in longevity-focused healthcare.

The aging group Hevolution has been sponsoring annual healthspan [summits](#) in Saudi Arabia and, in the most recent event, healthspan concepts like HELO received substantial discussion.



Awareness, knowledge, and motivations about lifespan, healthspan, and Healthy Longevity Medicine in the general population: the HEalthy LOngevity (HELO) conceptual framework

Belinda Wang · Anna Szücs · Elena Sandalova · E. J. Horberg · Paul A. O'Keefe · Louis Island · Hans J. Meij · Sonny Rosenthal · Andrea B. Maier

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Abstract The global population is ageing and the gap between lifespan (total years lived) and healthspan (years lived free of diseases) is increasing. Healthy Longevity Medicine (HLM) is an approach to optimise health and healthspan, and it has substantial public health implications. Despite those implications, the understanding of public perspectives on this field is lacking. The HEalthy LOngevity (HELO) framework was developed through a literature review guided by expert discussions across disciplines to include evidence-based concepts of health-related decision-making, ageing, and HLM. The framework organises concepts into three components. The first two components, awareness and knowledge, explore

public perception and understanding of the healthy longevity field, respectively. The third component, motivations, reflects factors underlying motivations towards healthy longevity. These include personality, current behaviours, personal values and beliefs, and health-related perceptions. The framework outlines the theoretical foundation to explore public knowledge and interest in healthy longevity. The framework will be refined based on findings from qualitative focus groups in Singapore and then applied to quantitative population surveys globally. These HELO initiatives aim to inform strategies for integrating HLM into public healthcare, promoting health and healthspan.

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References

General References

Cushing	Yale University, School of Medicine Library, 1943, <i>The Harvey Cushing Collection of Books and Manuscripts</i> , New York: Schumans.	PMM	John Carter and Percy Horace Muir, 1967, <i>Printing and the Mind of Man</i> , London: Cassell and Company.
DSB	Dictionary of Scientific Biography, 2008	Waller	Hans Sallander, 1955, <i>Biblioteca Walleriana – The Books Illustrating the History of Medicine and Science</i> , Stockholm.
GMN	Fielding H. Garrison, Leslie T. Morton, and Jeremy M. Norman, 2024, <i>An Interactive Annotated World Bibliography of Printed and Digital Works in the History of Medicine and the Life Sciences</i> from Circa 2000 BCE to 2024.	Wellcome	Wellcome Library, 1962. <i>A Catalogue of Printed Books in the Wellcome Historical Medical Library</i> , 3 volumes, London.
Iowa	Richard Eimas, 1980, <i>Heirs of Hippocrates – The Development of Medicine in a Catalogue of Medical Books in the Health Science Library, the University of Iowa</i> , Iowa City: Friends of the University of Iowa Libraries.	Wing	Wing, D.G., 1945, <i>Short-title catalogue of books printed in England, Scotland, Ireland, Wales, and British America, and of English books printed in other countries, 1641-1700</i> , New York: Index Society, 1945-51.
Krivatsky	Peter Krivatsky, 1989, A catalogue of seventeenth century printed books in the National Library of Medicine, Bethesda MD: U.S. Department of Health and Human Services.		
Norman	Diana H. Hook and Jeremy M. Norman, 1991, <i>The Haskell F. Norman Library of Medicine and Science</i> , San Francisco: Jeremy Norman & Co.		
Osler	William Osler, 1929, <i>Biblioteca Osleriana</i> , Oxford: Oxford University Press.		

Gerontologic References

Achenbaum	W. Andrew Achenbaum, 1995, <i>Crossing Frontiers: Gerontology Emerges as a Science</i> , Cambridge: Cambridge University Press.	Hayflick	Leonard Hayflick, 1994, <i>How and Why We Age</i> , New York: Ballantine Books.
Attia	Peter Attia, 2023, <i>Outlive</i> , New York: Harmony Books.	JSG	F. Brad Johnson, David Sinclair and Leonard Guarente, "Molecular Biology of Aging," <i>Cell</i> , Jan 22, 1999.
Bailey	William G. Bailey, 1987, <i>Human Longevity from Antiquity to the Modern Lab: A Selected Annotated Bibliography</i> , Greenwood Press: New York.	Medawar	Peter Medawar, 1957, <i>The Uniqueness of the Individual</i> , London: Methuen., CA: University Press.
Campisi	Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E. From discoveries in ageing research to therapeutics for healthy ageing. <i>Nature</i> , July 10, 2019, pp.183-192.	Morley	John E. Morley, 2004, <i>A Brief History of Geriatrics, The Journals of Gerontology Series A Biological Sciences and Medical Sciences</i> .
Comfort	Alex Comfort, <i>Ageing: The Biology of Senescence</i> , New York: Rinehart, 1956	NYAM	A. Malloch and E.J. Rhodebeck, 1929, Catalogue of an exhibition of books etc., on old age, held in the Library of the New York Academy of Medicine in connection with the first annual Graduate Fortnightly, Bulletin of the New York Academy of Medicine 5:119
Freeman	Joseph Freeman, <i>Aging: Its History and Literature</i> , 1979, New York: Human Sciences Press.	Ramakrishnan	Venki Ramakrishnan, 2024, <i>Why We Die</i> , New York: William Morrow.
Grmek	M.D. Grmek, 1958, <i>On Ageing and Old Age</i>	Schafer	Daniel Schäfer, 2011, <i>Old Age and Disease in Early Modern Medicine</i> , London: Pickering & Chatto (trans. P. Baker).
Gruman	Gerald J. Gruman, 1966, <i>A History of Ideas About the Prolongation of Life: The Evolution of Prolongevity Hypotheses to 1800</i> , Transactions of the American Philosophical Society, (Volume 56, Part 9)	Shock	Nathan Wetherill Shock, 1951, <i>A classified bibliography of gerontology and geriatrics</i> . Stanford, CA: University Press.
GSK	Leonard Guarente, David Sinclair and Guido Kroemer, "Human trials exploring anti-aging medicines," <i>Cell Metabolism</i> , Feb 6, 2024.	Stambler	Ilia Stambler, 2020, <i>A History of Life-Extensionism, Encyclopedia of Biomedical Gerontology</i> , Volume 2, Elsevier, pp. 228-237
Haycock	David Boyd Haycock, 2008, <i>Mortal Coil: A Short History of Living Longer</i> , New Haven, Yale University Press.		

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