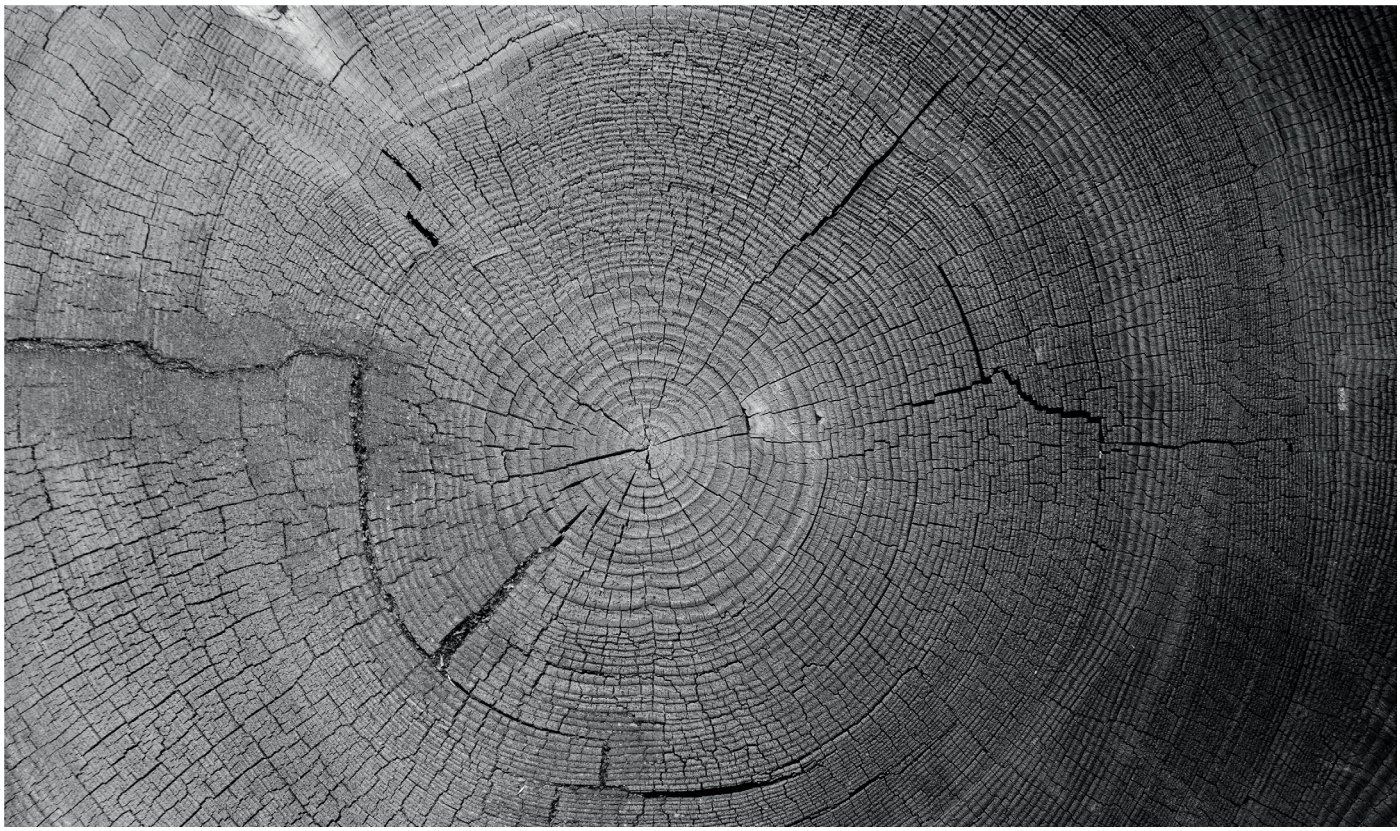
https://doi.org/10.1038/s41592-023-02140-2



Aging research comes of age

As money pours into aging research, the field can combine its many methods to home in on what underpins aging. Approaches differ, but researchers share the desire to not overpromise quick-fix anti-aging methods.

By Vivien Marx

"Finally, finally," says Vera Gorbunova, a biologist and geroscientist at the University of Rochester, where she co-directs the Rochester Aging Research Center and runs a joint lab with Andrei Seluanov. It's taken decades for aging "to be recognized as a legitimate area of research," she says.

A funding surge is transforming aging research, says bio-gerontologist Steve Horvath, who has moved from a UCLA faculty post to Altos Labs in Cambridge, UK. Aging research has become a mature, dynamic field with promising results, and it's attracting bright minds.

This is letting researchers be more expansive and delve deeper, with tools including 'omics approaches, to design and do more stringent experiments that put discoveries on solid ground. Expanded study sizes can provide more generalizable results. The financial boost enables, he says, "a more ambitious, more precise exploration of the mechanisms of aging."

Many hallmarks of aging[1,2] have been identified, such as deterioration of cells or organs over time due to increased inflammation; genome instability; damage to DNA, proteins and lipids due to the reactive oxidative species that metabolic processes generate; and changes to telomeres, the repetitive DNA sequences at the ends of chromosomes that get shorter as a cell divides.

It's human to fall in love with one's favorite theory, says Horvath. He was once enamored of Denham Harman's theory that aging is caused by accumulated oxidative damage. But this has been debunked, and it's now known that reactive oxygen species play positive roles, too, such as in cell signaling.

With telomeres, he says, it's become clear "you don't want very short telomeres and you don't want very long telomeres." As cells divide, telomeres shorten and replicative senescence halts the cell cycle.

CREDIT: S. ESCRIBANO / GETTY IMAGES

Some rockfish have a brief life; others can live for over 200 years. Off the California coast, such as near the Farallon Islands, University of California, Berkeley researcher Peter Sudmant studies what might power this diversity.

Says Johns Hopkins University researcher Mary Armanios, excessively short telomeres capture only one aspect of aging, the one tightly associated with replicative senescence[3].

People with excessively long telomeres, she says, have delayed hair-graying and other cosmetic features that make them look younger than their chronological age. They face a higher cancer risk, but "the thresholds defining 'long length' remain to be characterized." Mechanisms independent of telomeres capture other features associated with aging, such as cardiovascular and neurocognitive disease and metabolic aging. One needs to appreciate that multiple mechanisms play a role, an awareness that plays into the eventual translation of findings for clinical benefit, she says.

Such translation should not be rushed. Like other researchers interviewed for this story, Gordan Lauc of the University of Zagreb says that private investors approach him who "want to have a magic solution," such as an anti-aging pill. At scientific conferences he sees vendors offering consumer products that strike him as mainly 'snake oil' without scientific grounding. "There is so much hype in longevity research," he says. "There is no regulation because aging is not a disease." He and others stay cautious about hastily devised antidotes to aging.

What is needed now, says Peter Sudmant, a biologist at University of California, Berkeley, are ways to discern which of the discovered hallmarks of aging cause aging and which correlate with aging. "That's tricky," he says. Some are at least a bit causative; "however, it is unclear which are the most important."

In the past, says Sudmant, the aging research community was "too quick to say 'this thing is it! This is the cause.'" And, he says, some members of the community are still too quick in this respect. It's likely that all discovered aging factors matter. Inflammation, which is a nuanced concept, certainly influences how mammals age, but "I would strongly caution against this being the 'one thing'," he says. "Yeast don't have immune systems, yet they still age."

Clocks of aging

Inflammation increases with age, but why it does so is still elusive, says Lauc. Glycan analysis might shed light on this. He studies how these branched, structurally diverse sugar molecules differ between individuals and how glycans affect diseases and aging. Glycans coat mammalian cells and attach to proteins such as immunoglobulin G (IgG). His company does large-scale glycan analysis, and its revenues buttress his grants and fund his team's glycan research. It's slow going as he and his team work on glycan clocks for diagnostic and basic research use. Glycans are complex molecules, and "the key problem with glycans is we need more people in the field," he says.

One aging signal comes from changes to the structure of glycans covalently linked to IgG. In conditions associated with low-grade systemic inflammation, such as cardiovascular disease, levels of galactose and sialic acid in IgG glycans drop, and this change is associated with accelerated aging, says Lauc. Tallying such changes is a kind of clock, one of many clocks aging researchers can consult to address the many basic unanswered questions about aging.

Like many aging researchers, he thinks highly of the widely used epigenetic clock from the Horvath lab. Lauc was part of a project[4] that Horvath led in which several methods including this epigenetic clock were used to measure the outcome of an experiment in which rats showed marked age reversal. "But I'm always very cautious," he says, about the findings, which still need to be confirmed by others.

The researchers administered to rats an exosome-containing fraction from the plasma of young adult pigs. The rats' livers, hearts and brain showed improved function. Experiments that mix the blood of old and young mice are not new, but what struck him as new,

CREDIT: CLOCKWISE FROM TOP LEFT, P.H. SUDMANT, K. LEE, M. CHAMBERLAIN, SWFSC ROV TEAM, P.H. SUDMANT

says Lauc, was the way rat rejuvenation could be assessed with clocks.

The team studied the animals' physiology, did cognitive testing, and ran histological and biochemical tests. They profiled IgG glycosylation changes, too. Blood, heart and liver in these rats had halved their previously measured epigenetic age.

Horvath doesn't mind being called 'the father of epigenetic clocks'. He developed the first one in 2011, a paper that was "largely ignored," he says. That changed when he followed up with a multi-tissue one[5] in 2013. The method was seen as delivering an important biomarker of aging. The concept that methylation reflects activity of the cell's epigenetic maintenance system had gained traction. An epigenetic clock is a multivariate age estimator based on methylation measurements such as those from arrays from the Mammalian Methylation Consortium. The clock draws on public datasets of the millions of methylated sites in the human genome. Using a subset of methylated sites, a clock is built with a regression model and machine learning. Horvath has developed different species-specific and pan-tissue ones since then, as well as a pan-mammalian clock[6]. Species and different tissues within species show patterns of epigenetic changes.

Cohort studies have shown this epigenetic clock can predict both lifespan and healthspan because it reveals the difference between methylation-based age and chronological age, says Horvath. His second-generation epigenetic clock is called GrimAge — named after the Grim Reaper. In his view, it's a potent molecular predictor of mortality risk.

When he arrived at UCLA, "I had every prejudice against methylation," he says, as many did at the time. His colleague Eric Vilain suggested they assess methylation differences between Horvath's gay twin brother and Horvath, who is straight. "We found zero; there was nothing," says Horvath: no connection between methylation levels and sexual orientation. "Then, of course, I looked at aging effects, and the rest is history," he says.

In 2023, he published a universal pan-mammalian clock, and, "I'm quite pleased that it was even possible," says Horvath. The next step could be building a clock for all vertebrates, which all have cytosine methylation. "To me, this task is so difficult that I won't tackle it," he says, but he hopes the next generation of aging researchers might take it on.

Together with Horvath, Gorbunova built an epigenetic clock for naked mole-rats, and she is familiar with the pan-mammalian one, which she also likes. In her experience, it's less accurate than a species-specific one. More generally, she says, an epigenetic clock won't tell you it's time to write your will. But the clocks help to predict healthspan in large cohort analysis.

Altos Labs researcher Steve Horvath, posing in front of a grandfather clock from 1750, has built species-specific epigenetic clocks of aging, including one for walruses, and a pan-mammalian one. He often uses the hourglass and its sand as a metaphor for the methylation clock. The growing sand pile represents the age-related gain of methylation and the diminishing sand pile represents the age-related loss of methylation.

"We are very interested in developing more accurate clocks," she says. With the tools Horvath has, he can build such clocks fairly easily. "The only limitation really is sample availability," she says. To train the clock for individual species, one needs at least 100 samples.

Scientists face a wide choice of clocks[7] beyond the ones from the Horvath lab. "Aging clocks are great, but each of them is different, and we need to know what is each one of them measuring to be able to make any real use of them," says Lauc. "The plurality is good and bad," says Horvath. Two papers on a similar question might use different clocks, which makes it hard to compare results.

In his papers, when Horvath explains his choice of his second-generation epigenetic clock GrimAge as the best mortality risk predictor for blood samples, he includes an evaluation of other clocks. He sees other study authors evaluate clocks, too. One danger, he says, is that labs may try several "but only report from the one where they get the expected result," says Horvath. Not only is that a dissatisfying practice, it's bad for the field and can damage the reputation of clocks more generally.

After 30 years of debate whether aging is 'wear and tear' or something deterministic, says Horvath, "now we know, at least in mammals, there is something deterministic." Indeed, there is only a weak relationship between epigenetic changes and gene products, on the one hand, and changes in proteins or shifts in metabolomic data, on the other. Biology happens at the level of proteins, so it's unclear what precise effect methylation has. "And to this day, we struggle with that question."

More 'omics for aging research

Hypotheses and correlations about aging can now be deeply investigated experimentally with single-cell techniques, says Salvador Benitah, a molecular biologist, geneticist and aging researcher at Institute for Research in Biomedicine (IRB) Barcelona.

Previously, one might have had to focus a research project on, say, the relationship between macrophages and aging. Now researchers can combine molecular analyses of cells, tissues and organs and take a systems approach to whole organisms. "Now that we are doing the experiments, we realize that 'ooh, many of the things that we thought were important don't seem to be that important'," he says. To explore how a given perturbation affects healthspan and lifespan takes time and resources, says Benitah, which is why stronger funding stands to fortify the field.

Lauc views positively projects such as the Biomarkers of Aging Consortium, a collective

CREDIT: S. HORVATH

effort to characterize reliable biomarkers of aging.

What's still in its infancy in the aging field, says Benitah, is consensus. If another lab studies liver, muscle and skin as his does, when that team picks different ways to measure aging, "how do we compare that?" The community would benefit from established molecular signatures of tissue transcriptomes, metabolomes and proteomes as they relate to aging, says Benitah.

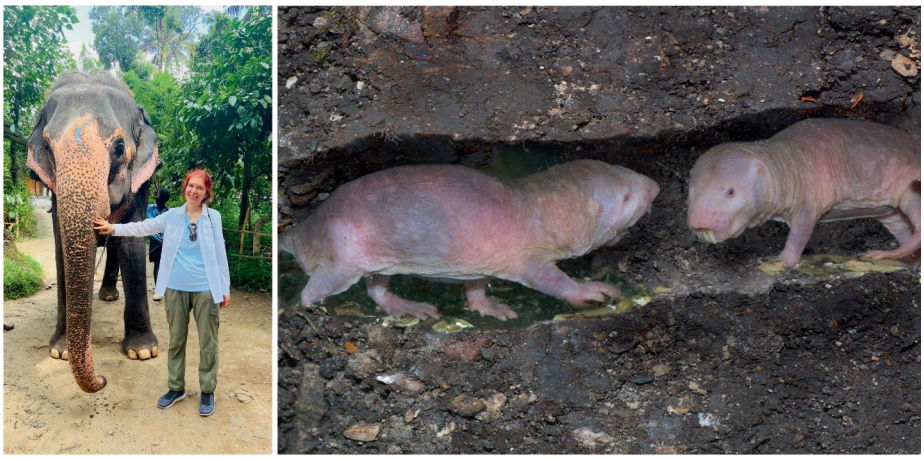
Among the resources he sees as empowering aging science is the single-cell atlas from the Tabula Muris consortium. The Tabula Muris Senis presents molecular and cell-type specific changes in over 350,000 cells from female and male aged mice. The atlas is, the authors note, "an essential companion to the genome" because it deeply characterizes phenotype and physiology and is intended as a reference to help with understanding cell biological changes over a lifespan.

Benitah also points to work from the Stanford University lab of Ann Brunet. She and colleagues have developed quantitative 'aging clocks'. Instead of using bulk tissue, they generated clocks with single-cell transcriptomic data. They used data from the neurogenic regions in the brains of mice of different ages and teased out cell-type-specific transcriptional signatures associated with aging. Other work he likes is from the Gorbunova lab. By transferring the trait of a longer healthspan and lifespan to a short-lived animal, the lab has done "amazing" work, says Benitah.

Whole-organism view

Oh, to be a naked mole-rat. Its life is spent mainly underground as a pinkish, more or less blind rodent about the size of a mouse and with much larger incisors. A mole-rat's lifespan is more than ten times a mouse's lifespan and they may reach age 40. That number might underwhelm, but, in people-years, the difference between these two rodent species translates to around ten times the average human lifespan, says Benitah. "It would be as if you would find a type of human that would live 800 years."

Naked mole-rats do not appear to get cancer. In her lab, Gorbunova and team, along with colleagues from other universities, found that one anticancer mechanism in these rodents seems to be provided by the exceptional elasticity of the mole-rat's skin cells[8]. The cells secrete high-molecular-mass hyaluronan (HMM-HA), a polymer that is a component of the extracellular matrix. HMM-HA is likely an adaptation to subterranean tunnel life. It's anti-inflammatory, protects from oxidative stress and appears to offer cancer protection.



Among vertebrates, lifespan varies widely. Naked mole-rats (right) are long-lived rodents. Vera Gorbunova from the University of Rochester, here with a long-lived vertebrate, says that cross-species analysis in aging research will yield new discoveries.

More recently, she and her team, along with colleagues, generated transgenic mice with cells overexpressing the naked mole-rat hyaluronic acid synthase 2 gene (nmr*Has2*)[9]. Its product, HAS2, mainly produces HMM-HA. The nmr*Has2* mice show a transcriptional signature found in species that live longer lives, the mice also have lower inflammation levels, and both sexes have a longer healthspan and lifespan. Mice often develop lymphomas when they age, but these mice were more resistant to chemically induced skin cancer and spontaneous cancer.

In their paper, the team notes that their finding "opens new avenues for cancer prevention and life extension," but, says Gorbunova, it's still a long way from applying the findings about mole-rats to people. Like many aging researchers, she receives calls from investors and people keen on signing up for a clinical trial. She is involved in several biotech startups but runs no trials with people. At some point in the future one might happen, she says, likely in partnership with someone who seeks to drive applications. "Basic science is really my passion," she says.

To study Alzheimer's disease, Gorbunova also works on a different rodent species, the degu. Mice do not develop Alzheimer's disease, but, as they age, degus do. In her view, cross-species analysis in aging research will deliver new discoveries. With today's tools, scientists no longer need to limit themselves to, for example, working with yeast to address topics of interest.

More in the zoo

Vertebrate lifespan varies immensely, and aging researchers want to understand what underpins this variation: a pygmy goby's life ends after five weeks, a turquoise killifish lives for four to six months, a bowhead whale lives over 200 years, some rockfish can live as long as the bowhead, and Greenland sharks live around 400 years.

> "I think all those models are awesome!" says Peter Sudmant.

Aging mechanisms are likely conserved across vertebrate species, just tuned differently, says Itamar Harel of the Hebrew University in Jerusalem. In this sense, any vertebrate is suited for aging research. To explore longevity mechanisms, primarily on the cellular level, "the naked mole-rat is a fantastic model," he says. The rockfish is well-suited for studies in comparative evolution. "However, if you wish to test how specific paradigms experimentally affect longevity and healthspan, you have to use a naturally short-lived vertebrate." That would include the turquoise killifish, which he uses, as a molecular geneticist who studies the mechanisms underpinning aging. Some groups, he says, comprehensively describe correlations across many organs, species and ages while others, such as his group, focus on a specific mechanism.

For studies of extreme longevity, rockfish are ideal, says Gorbunova. Sudmant and his lab study rockfishes of the Pacific Ocean.

Some species live 11 years and the rougheye rockfish, *Sebastes aleutianus*, reaches over 200 years of age.

He and his colleagues sequenced and assembled over 100 genomes de novo of 88 rockfish species and found positive selection of DNA maintenance pathways[10]. The immune-related butyrophilin genes have seen copy-number expansion in rock fish. The team also found different mutation rates, particularly more CpG-to-TpG mutations in longer-lived fish.

CpG dinucleotide sites in the genome are particularly prone to DNA methylation, which is a form of real-time genome tuning. Methylated cytosine is just one reaction — a hydrolytic deamination — away from becoming a thymidine, so CpGs are essentially a mutation waiting to happen. CpGs "have a weird property of being very likely to turn into TpGs," says Sudmant.

The team found that rockfish with longer lifespans show more CpG to TpG mutations. The mutations do not themselves cause a long lifespan, but they are a shifted mutational signature. In populations with different generation times, this shift shapes genetic variation.

Beyond the many factors that can cause DNA mutations, such as sunlight or chemicals, mutations can occur by chance, especially during replication, says Sudmant. One important moment is when producing sperm and eggs. That's when changes can deliver new mutations to offspring, and this makes them an important source of evolutionary variation. Older parents pass on more mutations to their offspring, and many of those changes are due to CpG-to-TpG shifts, says Sudmant. And the long life affects the genetic diversity of these fish because they have more CpG to TpG mutations.

Aging mechanisms are likely conserved across vertebrate species, just tuned differently, says Itamar Harel.

Harel and his team focus on the turquoise killifish[11]. At three months of age, *Nothobranchius furzeri* is like a 40-year-old human in some ways. Harel and his team, along with colleagues at other institutions, used CRISPR-based gene editing to affect this fish's energy homeostasis.

The older fish with edited genomes had metabolisms more like those of younger fish and lived 20% longer than fish with unedited genomes. The fish with edited genomes had metabolic patterns that resembled those of intermittent fasting, even though their eating regimen had not changed. Particularly in male gene-edited fish, age-related loss of metabolic homeostasis was restored. The team is studying the outcome to learn more about the sex differences.

Benitah is intrigued by this metabolism-related result in killifish. Caloric restriction can extend lifespan, he says, but its cost is that it cuts an organism's energy budget. In his own work, he focuses on a different aspect of metabolism: the influence of circadian clocks on aging-related metabolism changes.

Many factors shape metabolism, including genetic factors, diet and exercise. Metabolism is on a clock, and each organ's metabolism has its own clock such that the one in muscle, for example, affects the one in liver, he says. When clocks fade, diseases can occur. Why circadian rhythms fade as we age, "we don't know yet."

Benitah and colleagues assessed how aging-related changes in circadian rhythms affect organ-wide processes such as the way senescent cells accumulate in tissue. When a muscle is injured, stem cells are involved in muscle repair. Some resident stem cells become senescent. The senescent cells are not just bystanders: they appear to signal to stem cells, and that hampers their ability to regenerate and repair muscle after injury[12]. As a molecular biologist, he says, "I would be fascinated to understand molecularly why some of the stem cells become senescent and the others don't."

One culprit is CD36, a membrane protein through which fats reach the cell. In senescent cells CD36 is upregulated and seems to help senescent cells secrete a pro-senescence signal. Among the next steps in his lab is to manipulate pathways in vivo to study how manipulating CD36 affects tissue and the overall organism.

The blossoming of aging research is a big shift from when he started to work on aging 12 years ago, says Salvador Benitah.

Across the tree of life, different selective forces have molded aging and lifespan phenotypes differently. This means that studying aging in different species sheds light on different, important things about the highly conserved pathways associated with aging, says Sudmant. "I think all those models are awesome!" he says, and they are useful in aging research. "Remarkably, studying things as diverse as rockfish and degus and mole-rats and killifish gives us insights into humans."

The blossoming of aging research is a big shift from when he started to work on aging 12 years ago, says Benitah. Gone are the days of small meetings and researchers working in disparate disciplines such as stem cell science or physiology. He says of aging research, "Now, it's huge."

Vivien Marx ✉
Nature Methods.
✉e-mail: v.marx@us.nature.com

Published online: 2 January 2024

References

1. Li, S., Vazquez, J. M. & Sudmant, P. H. *Trends Genet.* **39**, 830–843 (2023).
2. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. *Cell* **186**, 243–278 (2023).
3. Armanios, M. *Annu. Rev. Genomics Hum. Genet.* **23**, 363–381 (2022).
4. Horvath, S. et al. *Geroscience* https://doi.org/10.1007/s11357-023-00980-6 (2023).
5. Horvath, S. *Genome Biol.* **14**, 1–20 (2013).
6. Lu, A. T. et al. *Nat. Aging* **3**, 1144–1166 (2023).
7. Horvath, S. & Raj, K. *Nat. Rev. Genet.* **19**, 371–384 (2018).
8. Tian, X. et al. *Nature* **499**, 346–349 (2013).
9. Zhang, Z. et al. *Nature* **621**, 196–205 (2023).
10. Kolora, S. R. R. et al. *Science* **374**, 842–847 (2021).
11. Astre, G. et al. *Dev. Cell* **58**, 1350–1364.e10 (2023).
12. Moiseeva, V. et al. *Nature* **613**, 169–178 (2023).