Of Cells and Limits

Leonard Hayflick has been unafraid to speak his mind, whether it is to upend a well-entrenched dogma or to challenge the federal government. At 86, he's nowhere near retirement.

By Anna Azvolinsky | March 1, 2015

LEONARD HAYFLICK
Adjunct Professor of Anatomy
University of California, San Francisco

Leonard Hayflick learned to culture cells under the tutelage of a famous expert, Charles Pomerat. In 1958, after completing his fellowship in Pomerat's lab, Hayflick became a member of the Wistar Institute in Philadelphia, providing cell cultures for its researchers. "It was a golden opportunity because it would leave me with plenty of time and resources to do what I wanted to do without worrying about getting grants or being subject to supervision."

Hayflick was interested in detecting viruses that might cause human cancer. He would extract viruses from the culture fluid in which he grew cancer cells and attempt to transform normal cells into tumor cells. Human adult cells frequently contained unwanted viruses, so Hayflick obtained tissue from fetuses to use as a source of virus-free normal cells. He found that tissue from the lung, which is already a discrete organ by three months of embryonic development, was best for making fibroblast cell strains. "Like everyone else who did cell culture, I was persuaded to believe the 60-year-old dogma that when you culture cells, they have the capacity to divide forever. In practice, this never happened and people attributed their failure to ignorance of culturing methods, media formulation, and other things like the cleanliness of glassware."

50 population doublings, the cells were metabolically active but had stopped dividing. Together with cytogeneticist Paul Moorhead, he designed a simple but clever experiment to test whether the cells' limited capacity to replicate in culture was caused by microbial contamination or some unknown culture condition. It had recently been shown that female cells could be distinguished from male cells by the presence of Barr bodies formed from the inactive X chromosome in female cells. Hayflick mixed equal numbers of female cells that had divided 10 times with male cells that had doubled 40 times and found that after another 20 doublings of the mixed culture, only the female cells remained. "This showed that replication ceased not because of some extrinsic factor, but because of an intrinsic cell phenomenon—a revolutionary idea at the time."

> "It is possible that the etiology of all age-associated diseases is in the molecular state that exists in old cells."

Hayflick's study was published in *Experimental Cell Research* in 1961, after first being rejected by another prominent journal—*The Journal of Experimental Medicine*. The rejection letter came from Francis Peyton Rous who received the Nobel Prize a few years later for his discovery of chicken tumor viruses. "I can still quote from that letter: 'Anyone who has worked with tissue culture knows that if the cells are provided with the proper milieu in vitro they will replicate indefinitely.' He also called my suggestion that our observation suggests something about cellular senescence and aging 'notably rash.'" The theory that all cells are generally immortal in culture was first postulated at the birth of cell culture in the early 1900s and was well publicized by Alexis Carrel of Rockefeller University in New York City who had developed a cell strain from chicken heart cells that he claimed had been growing for more than 40 years.

After showing that normal cells are mortal, Hayflick also reported, for the first time, that cancer cells were uniquely immortal—a claim that could not be made without first establishing that normal cells are mortal. He also discovered that his normal human fetal cells had a memory. When fetal cells frozen at different population doublings were thawed, the cells remembered the doubling at which they had been frozen and only divided until they reached a total of 50 divisions.

Hayflick's work was criticized and he was ridiculed. It took about 10 years for a more general acceptance that normal cells have a limited life span in vitro, a phenomenon now known as the Hayflick limit. The Nobel Prize–winning discovery of telomere shortening and the expression of telomerase explained Hayflick's observations.

Here, Hayflick talks about creating one of the most widely used cell strains, how he sued the federal government, and his discontent with the field of aging research.

Hayflick Heads Out

An explosive interest. Hayflick grew up in southwest Philadelphia and became interested in chemistry in elementary school, sparked by the gift of a Gilbert chemistry set. Hayflick made fireworks and rockets and experimented with heat and cold production. His mother encouraged him, even signing a permission statement that allowed Hayflick to purchase metallic sodium. With string, tin cans, water, and the sodium, he built rockets that traveled three to four stories into the air.

College-bound. After completing high school in 1945, Hayflick was accepted by the University of Pennsylvania, but first enlisted and served 18 months in the US Army so he could help pay for college through the GI Bill. "That was a fortunate decision. Had I not enlisted, I would have been drafted for the Korean War."

Building confidence. After majoring in microbiology at Penn, Hayflick worked for the Philadelphia-based pharmaceutical company Merck, Sharp and Dohme and then returned to Penn as a graduate student. While earning a master's degree, Hayflick identified the first field isolate of a mycoplasma as the source of an ear infection in the rat colony at the adjoining Wistar Institute.

A new type of culture. Hayflick studied the behavior of mycoplasmas in cell culture before it was

known that these organisms are common, but often silent, contaminants of cell cultures. (See "Out, Damned Mycoplasma!," *The Scientist*, December 2013.) "This was the first time that anyone purposefully put mycoplasmas into cell culture."

Hayflick Hits His Stride

A popular cell strain. Hayflick cultured the famous WI-38 strain from fetal female lung tissue in 1962 and developed the technology to make poliovirus and other vaccines in this strain. By then, Hayflick was getting requests for his cells from all around the world. WI-38 cells have been used to produce most of the world's human virus vaccines, including those against adenoviruses, measles, rabies, chicken pox, mumps, rubella, polio, and hepatitis A. Hayflick gave WI-38 gratis to the world's human virus vaccine manufacturers because, at that time, living cells could not be patented. But while Hayflick promoted the use of his cells for vaccine production, there was resistance, mainly from the National Cancer Institute and the precursor of the Food and Drug Administration's vaccine-control authority, which made unfounded statements that using Hayflick's cells would be dangerous because of their alleged potential to mutate and become cancerous.

Mycoplasma expertise. As head of Wistar's tissue-culture facility and an expert on mycoplasmas, colleagues would send Hayflick cell cultures to be tested for their presence. A visit from an NIH colleague got Hayflick working on "walking pneumonia," known medically as primary atypical pneumonia, a common disease in young adults worldwide. Previously, researchers had been unsuccessful in isolating a virus as the etiological agent for this form of pneumonia. From an egg yolk sample thought to contain the causative virus, Hayflick isolated a mycoplasma that had not been described before and named it *Mycoplasma pneumoniae*. This was the first human disease shown to be caused by mycoplasmas, and it made the front page of *The New York Times*.

Aging clock. After moving to Stanford University in 1968, Hayflick's lab continued to investigate how human cells in culture maintain a memory of the number of divisions they have made. In 1975, he and his graduate student, Woodring Wright, showed that this counter was located in the nucleus.

A lawsuit. "My development of WI-38 was done without government or private support and because of the great demand for WI-38 cells, I was awarded an NIH contract only to 'store and distribute' the cells." When Hayflick moved to Stanford the contract ended, but the demand for WI-38 cultures continued. Hayflick began to charge $25—the same amount charged by the American Type Culture Collection at the time—to ship his cells, and put the funds into a holding account until a decision was made about disposition of those funds. "This seemed to me to be reasonable, but some people at the NIH and the FDA interpreted this to be theft of government property, and that I was selling WI-38 for personal gain. They entered my lab in my absence and confiscated all of the frozen WI-38 ampoules." Hayflick sued the federal government in 1975, claiming that the NIH had violated the Privacy Act of 1974 by releasing their findings before allowing him to reply and by illegally confiscating WI-38. "Who owns WI-38 or any other continuously dividing human cell culture is not easily settled. There are several stakeholders—the researchers who give the cells value, their institution, the individual from whom the cells are derived, and the organization that supports the research."

The suit was ultimately settled outside of court, at the request of the Justice Department, who found itself in an impossible position for a number of reasons. First, living cells were now patentable. Second, an executive order by President Reagan and the Bayh-Dole Act made it legal to use tax payer–supported research materials for commercial exploitation. Third, the emerging biotechnology industry was founded on materials developed in federally funded academic research labs. If the government prevailed in Hayflick's lawsuit, the biotech industry would be jeopardized, so industry lawyers prepared amicus briefs on Hayflick's behalf should his case go to trial. Finally, the NIH itself was by now offering products made in their own laboratories for commercial use.

As part of the settlement, Hayflick received some of the confiscated ampoules of WI-38 as well as the funds from mailing the WI-38 cultures. All of these funds went to pay his legal expenses.

And Hayflick had supporters. After the settlement, a letter published in *Science* signed by 85 scientists stated that they objected to the behavior of the NIH and the FDA (215:240-42, 1982). According to Hayflick, his position that biologists, like other scientists, have intellectual property rights helped start a revolution in thinking, resulting in many biologists later becoming successful entrepreneurs.

Due credit. Hayflick has been credited with starting the field of cellular biogerontology, the study of the aging of cells. His work set a new direction for aging research. "The focus had been on extracellular causes of aging such as stress or radiation. I suggested that what I had found was teaching us something about the biology of aging, and the term senescence became widely used. My work refocused

the field onto intracellular events as the etiology of aging," he says.

Hayflick Has a Say

A matter of linguistics. "The term 'research on aging' has become muddled. Most scientists fail to understand that despite the fact that the 'aging' tag exists on their center, institute, or organization, most of the research is not on aging, but on age- associated diseases or longevity determinants. One problem that this linguistics issue creates is the diversion of more than 50 percent of the National Institute on Aging's budget to Alzheimer's disease research and less than 5 percent on the fundamental biology of aging. We should do Alzheimer's research, but not at the expense of research on the fundamental biology of aging. For more than 50 years, aging research has meant research on age-associated diseases and this will tell us nothing about the cause of aging. It is remarkable to observe that a major medical concept that 'the greatest risk factor for all age-associated diseases is aging' has resulted in so little research on aging. It is possible that the etiology of all age-associated diseases is in the molecular state that exists in old cells or cells near the end of their lineage. I've been vocal about this for 40 years. And as a consequence of that you don't make too many friends. But I am more interested in the truth than making friends."

Longevity vs. aging. "Many confuse aging with longevity determination, which is indirectly driven by genes. There are no genes for aging. There are genes to maintain the biological functionality of an organism up to and including reproductive success. There is no mechanism that immediately causes death after reproductive maturation; it's too costly. Longevity determination asks 'Why do we live as long as we do?' Aging research asks 'Why does everything ultimately fail?' and that is a huge difference."

Just the facts. Rebecca Skloot, author of *The Immortal Life of Henrietta Lacks,* had contacted Hayflick during her research for the book as he knew the personalities involved, including those who had originally cultured the HeLa cells. "Unfortunately, much of what I told her was hyped by her editors, as I'll give her the benefit of the doubt. There are a lot of inaccuracies in the book. One is the allegation that HeLa cells have saved lives and benefited the health of more people than any other cell population. That is false. WI-38 has benefited 2 billion people who have received vaccines made in these cells. Another is that HeLa cells were the first immortal cell population; that was L929 developed by Wilton Earle at the NIH nine years earlier. But this doesn't take away from importance of the book. It raises the property rights and ownership issue of self-duplicating biological entities, which I have been addressing for 30 years."

Retirement? Hayflick continues to mentor, consult, write, lecture, organize conferences on aging, and travel to meetings. "I have not stopped doing anything except running a wet lab. I've never retired because I don't know a definition of that word that applies. I have an enormous correspondence network worldwide, and I spend a lot of time keeping up with the scientific literature. I am also writing my autobiography. My mother lived to be 106, so I am hopeful that I may have inherited some of her longevity determinants."

Greatest Hits

- Developed the first normal human diploid fibroblast cell strains such as WI-38 and the technology for producing vaccines against polio and other human viral diseases
- Discovered that normal human cells are mortal, have a memory located in the nucleus, and eventually become senescent. This finding is now called "the Hayflick limit" and is explained by telomere attrition.
- Discovered that cancer cells are immortal, now explained by the expression of the enzyme telomerase
- Discovered that "walking pneumonia" in humans is caused by a previously unknown species of mycoplasma
- Invented powdered cell-culture media and the first inverted microscope for viewing cell cultures
- Changed the thinking of biologists about their intellectual property rights

Further Reading

"Medical Research: Cell Division" (*Nature,* 498:422-26, 2013)
"Hayflick, his limit, and cellular ageing" (*Nat Rev Mol Cell Biol,* 1:72-76, 2000)
"A Novel Technique for transforming the theft of mortal human cells into praiseworthy federal policy" (*Exp Gerontol,* 33: 191-207)
Web of Stories 2012 interview with Leonard Hayflick

Tags

telomerase, teleomeres, profile, mycoplasma, microbiology, longevity, intellectual property, Hayflick limit, cell senescence, cell biology and aging

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Comments

James V. Kohl
Posts: 453

March 4, 2015

> *The rejection letter came from Francis Peyton Rous who received the Nobel Prize a few years later for his discovery of chicken tumor viruses.*

My 2014 *invited* review of nutritional epigenetics detailed how differences in the microRNA/messenger RNA balance link viral microRNAs from ecological variation to ecological adaptations via the RNA-mediated differentiation of all cell types in all individuals of all species. It was returned **without review**.

The problem appears to be the clear link from viruses to RNA-mediated cell type differentiation via amino acid substitutions that stabilize DNA in the organized genomes of all species. That clear link led to the invitation to submit the review.

The invitation came after publication of Nutrient-dependent/pheromone-controlled adaptive evolution: a model and a series of other previously published works that detail the molecular epigenetics of biophysically constrained RNA-mediated protein folding.

Many of my "peers" still seem to think that mutations lead to the evolution of biodiversity. They won't consider the fact that viral microRNAS cause entropy or that nutrient-dependent microRNAs link entropic elasticity from DNA repair to the physiology of reproduction.

That anti-entropic fact links the metabolism of nutrients to species-specific pheromones that control the physiology of reproduction. The pheromones link RNA-mediated fixation of nutrient-dependent amino acid substitutions from metabolic networks to genetic networks in species from microbes to humans. See for examples in humans: Clinically Actionable Genotypes Among 10,000 Patients With Preemptive Pharmacogenomic Testing.

Examples from more than 14,000 patients now show what serious scientists have learned during the past two decades. What they have learned is exemplified in the honeybee model organism and many other model organisms.

In 2013, I wrote: "The honeybee already serves as a model organism for studying human immunity, disease resistance, allergic reaction, circadian

rhythms, antibiotic resistance, the development of the brain and behavior, mental health, longevity, diseases of the X chromosome, learning and memory, as well as conditioned responses to sensory stimuli (Kohl, 2012)."

In his 2003 presentation to the American Philosophical Society, Greg Bear told others about ancient viruses in the human genome that link sexual recombination and pheromonal interaction in multicellular organisms. The organisms communicate with each other, which links what is currently known about physics, chemistry, and molecular epigenetics to species-wide epigenesis and to the obvious anti-entropic examples of epistasis via metabolic and genetic networks.

Unfortunately, more than a decade after Greg Bear presented the facts about biodiversity in two of his science fiction novels, the accuracy of his claims about viruses goes largely unnoticed. Honeybee colony collapse is noticed. But, despite the fact that facts are facts and the fact that facts about viral microRNAs and nutrient-dependent microRNAs have replaced theories, theorists prefer their ridiculous theories.

Perhaps honeybee colony collapse has nothing to do with their nutrient-dependent pheromone-controlled reproduction. Perhaps Greg Bear was wrong when he claimed that "Networks from beehives to brains solve problems through the exchange and the selective cancellation and modification of signals. Species and organisms in ecosystems live and die like signals in a network."

Perhaps evolutionary theorists like Masatoshi Nei are correct and "...genomic conservation and constraint-breaking mutation is the ultimate source of all biological innovations and the enormous amount of biodiversity in this world." Mutation-Driven Evolution (p. 199).

Is there a model for that?

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mlerman
Posts: 71

March 4, 2015

When I came to The USA in 1980 I was in California and contacted Dr. Hayflick asking for a job in his lab. He was very receptive and answered I have no monies, no lab and am looking for support.. I gave him a copy of my paper just published in J. of Theoretical Biokogy, titled "The biological essense of resting cells in cell populations" Several weeks latter he replied saying that my theory of aging is correct.. Michael Lerman, Ph.D., M.D.

Sign in to Report

James V. Kohl
Posts: 453

Replied to a comment from mlerman made on March 4, 2015

March 7, 2015

Thanks Michael Lerman.

When an expert molecular biologist first examined my model in the early 90's, after several hours he advised me that I still needed to show gene activation in GnRH neurons due to mammalian pheromones, which the late Robert L. Moss's group showed in the months that followed via c-fos

expression.

To what do you attribute the subsequent lack of progress towards linking metabolic networks to genetic networks via the biophysically constrained chemisty of protein folding and conserved molecular mechanisms of RNA-directed DNA methylation and amino acid substitutions that differentiate all cell types in all individuals of all species via the physiology of their reproduction and fixation of the amino acid substitutions?

For example, I just read Wrangling Retrotransposons and learned about their portrayal of what obviously are viral microRNAs as "genomic parasites." I think that ridiculous representation may come from a biologically uninformed science journalist.

I'm concerned that someone who understands nothing about how the nutrient-dependent microRNA / viral microRNA balance controls cell type differentiation and proliferation has led others to believe that genomic parasites somehow contribute to the evolution of increasing organismal complexity.

That makes me think serious scientists may not believe that increasing organismal complexity is RNA-mediated via amino acid substitutions that link the epigenetic landscape to the physical landscape of DNA in the organized genomes of species from microbes to man. Do you know who else might be aware of the findings that

> *"...replacing either of the two amino acid residues, N191 or S195, located in the ENTV SU with the corresponding JSRV residues, H191 or G195, markedly increased the Env-mediated membrane fusion activity and infection. Reciprocal amino acid substitutions also partly switched the sensitivities of ENTV and JSRV pseudovirions to sHyal2-mediated SU shedding and inactivation."*

Single residues in the surface subunits of oncogenic sheep retrovirus envelopes distinguish receptor-mediated triggering for fusion at low pH and infection

They appear to extend what you suspected about resting cell types to health and/or pathophysiology via nutrient-dependent microRNAs that protect against the damage caused by viral microRNAs during thermodynamic cycles of protein biosynthesis and degradation, which occur throughout life and begin to fail with increasing age-associated mutations. Unfortunately, if people are taught to believe that mutations are sometimes beneficial, they may miss the fact that nutrient-dependent microRNAs are always beneficial. For example, they link ecological variation to the nutrient-dependent pheromone-controlled physiology of reproduction in species from microbes to humans by controlling viral microRNAs.

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