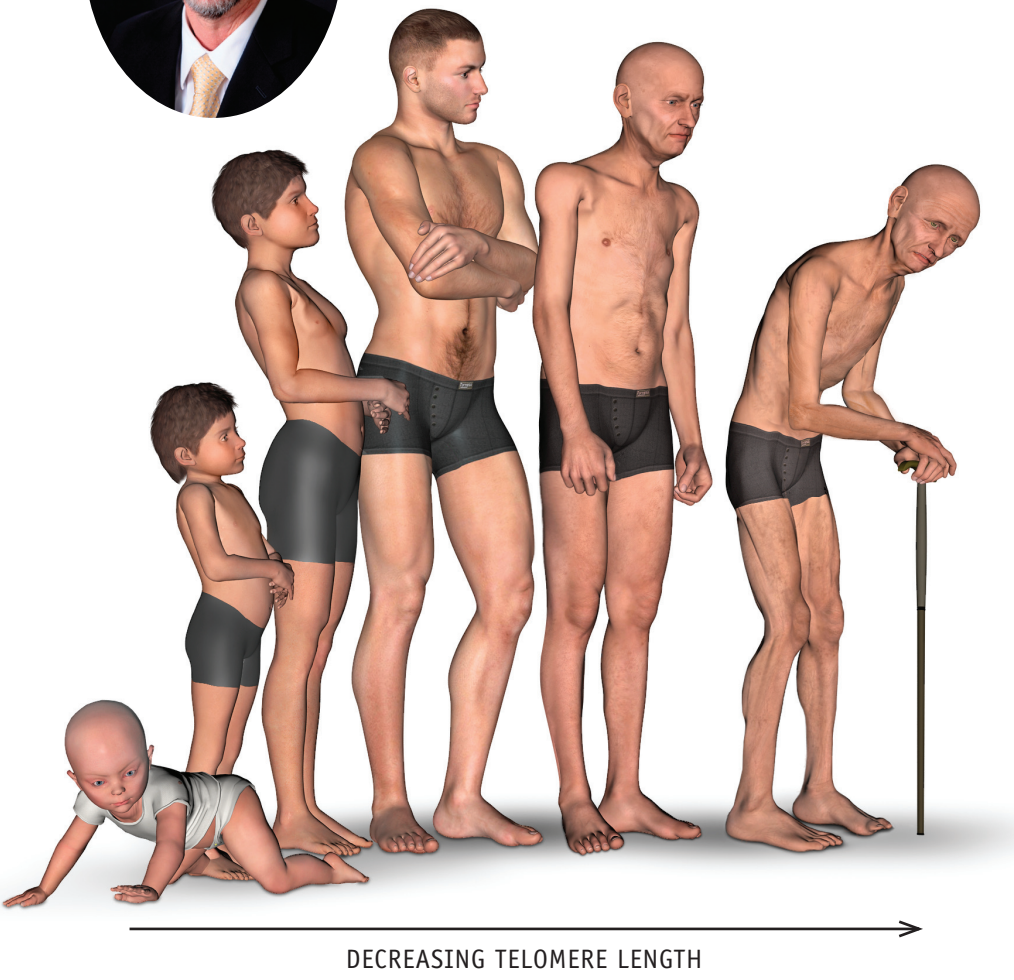
Bill Andrews on Telomere Basics:

CURING AGING

Bill Andrews, Ph.D. and Jon Cornell



Biography of William H. Andrews, Ph.D.

Dr. William H. Andrews has worked in the biotech industry for 28 years, focusing the last 16 years on finding ways to extend human lifespan through the intervention of telomere shortening in human cells.

Dr. Andrews earned his Ph.D. in Molecular and Population Genetics at the University of Georgia in 1981. He was a Senior Scientist at Armos Corporation and Codon Corporation, Director of Molecular Biology at Codon and at Geron Corporation, and Director of Technology Development at EOS Biosciences. He is presently the founder, President and CEO of Sierra Sciences, a biotech company in Reno, Nevada focused exclusively on finding drugs that will transiently induce the expression of endogenous telomerase in human cells.

While Director of Molecular Biology at Geron Corporation, Dr. Andrews was one of the principal discoverers of both the RNA and protein components of human telomerase and was awarded 2nd place as "National Inventor of the Year" in 1997 for this work. He is presently a named inventor on 35 US issued telomerase patents.

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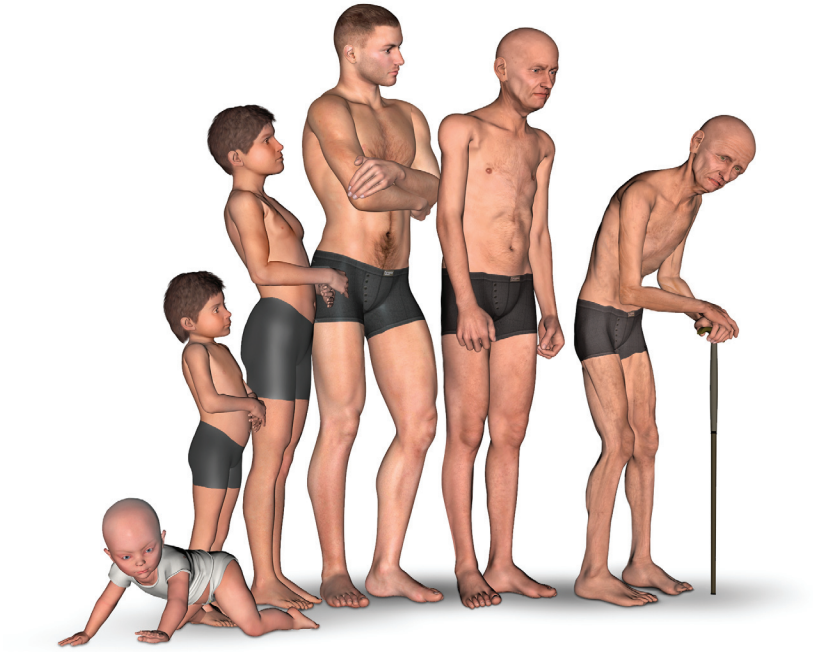

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Since before recorded history began, people have been searching for ways to live longer. We all know the story of Ponce de León's search for the elusive Fountain of Youth, but even two millennia earlier, emperor Qin Shi Huang of China was sending out ships full of hundreds of men and women in search of an Elixir of Life that would make him immortal. The desire to live forever is as old as humanity itself.

But it has only been in the last thirty years that science has made any real progress in understanding the fundamental question of why we age and what can be done about it. These discoveries have not been widely publicized – yet – and so most people are unaware of how close we are to curing the disease of aging once and for all.

Is Aging a Disease?

References to "the disease of aging" still make many people uncomfortable. After all, aging is a natural process that has existed forever – so how can it be a disease?

In fact, aging has not existed forever. Approximately 4.5 billion years ago, a cell came into existence on Earth that was the progenitor of every living organism that has since existed. This cell had the ability to divide indefinitely. It exhibited no aging process; it could produce a theoretically infinite number of copies of itself, and it would not die until some environmental factor killed it. When the ancestry of any given cell is traced back to this very first living cell, this lineage is called the cell's "germ line."

Much later – perhaps three billion years later – some cells of the germ line began to form multicellular organisms: worms, beetles, lobsters, humans. The germ line, however, was still passed on from one generation to the next, and remained immortal. Even with the inclusion of multicellular organisms, the germ line itself exhibited no aging process.

But, in some multicellular organisms, such as humans, certain cells strayed from the germ line and began to exhibit signs of aging. These cells aged because they became afflicted with a disease: their ability to reproduce themselves indefinitely became broken. The cause of this disease is still speculative, but many scientists are searching for cures.

The fact that a disease has existed in the genetic code of an animal for a very long time does not mean that it is not a disease. Thousands of diseases, from hemophilia to cystic fibrosis, have lurked in our genes for far longer than recorded history. These diseases should be cured, and aging is no exception.

The Cause of Aging

The root cause of aging is very straightforward: we age because our cells age.

In 1961, Leonard Hayflick, a researcher at the Wistar Institute in Philadelphia, discovered that there was a limit to the number of times a human cell could divide.[1] After about 70 divisions, a cell derived from embryonic tissue enters a stage where its ability to divide slows and eventually stops. This stage is called cellular senescence. Hayflick also observed that the number of times a cell could divide was governed by the age of the cells: cells from a twenty-year-old could divide more times than cells from a fifty-year-old, which in turn would divide more times than cells from a ninety-year-old.

Hayflick discovered that, in essence, there is a clock ticking inside every dividing cell of our body. Our aging process isn't simply a consequence of accumulated damage: there is a specific property of our cells that limits how long we can live.

The nature of this property was proposed independently in the early 1970s by both Soviet and American scientists.[2] When a cell divides, the genetic material inside that cell needs to be copied. This process is called DNA replication. These scientists suggested that the limitation on cell division is rooted in the very nature of DNA replication. The enzymes that replicate a strand of DNA are unable to continue replicating all the way to the end, which causes the loss of some DNA.

As an analogy, think of a DNA as a long row of bricks, and of DNA replication as a bricklayer walking backwards on top of a brick wall laying a new layer on top of that row. When the end of the wall is reached, the bricklayer finds himself standing on top of the brick he's supposed to replicate. Since he can't put down a brick where his feet are, he steps back and falls off the wall - leaving the very end of the wall bare. As a result, the new copy of the wall is shorter.

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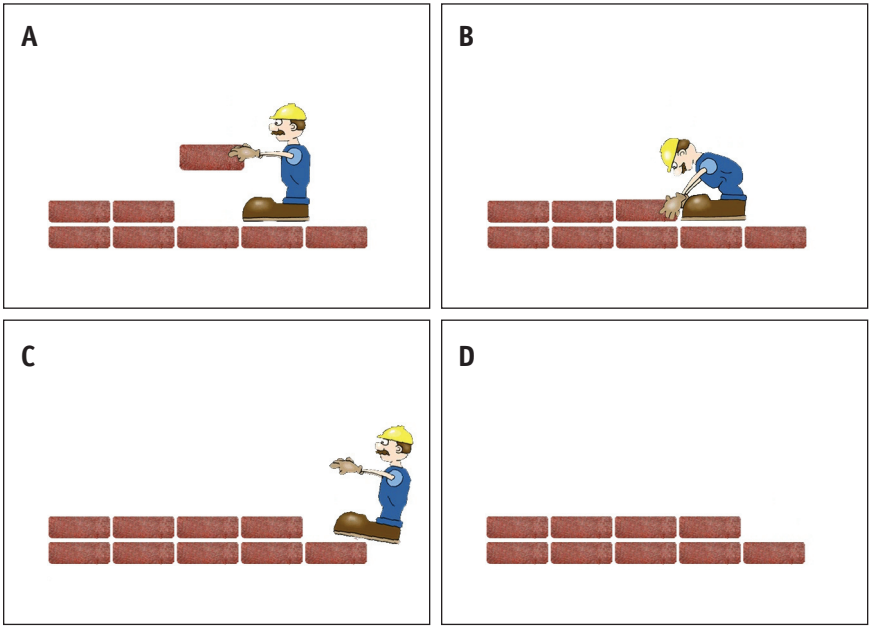
[1] Hayflick L. (1965). *The limited in vitro lifetime of human diploid cell strains*. Exp. Cell Res. 37 (3): 614–636.

[2] Olovnikov AM. *Principle of marginotomy in template synthesis of polynucleotides*. Doklady Akademii nauk SSSR. 1971; 201(6):1496-9. Watson, J. D. *Origin of concatemeric T7 DNA*. Nat New Biol. 1972; 239(94):197-201.



Just like this brick wall was copied imperfectly, our DNA is unable to perfectly copy itself; when a strand is replicated, the new strand is shorter than the old strand.

If we lost portions of the information encoded in our DNA every time it replicated, human life would be impossible. Our cells couldn't even divide enough times to allow us to be born. Fortunately, we are born with long, repetitive sequences of DNA at the end of each of our chromosomes, which later shorten during the normal DNA replication process.

These repetitive sequences are called "telomeres."

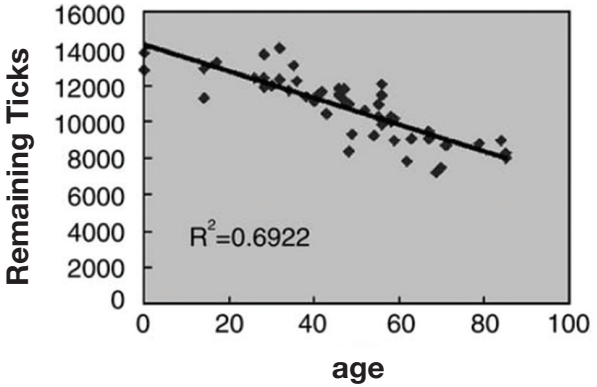
Telomeres, like all DNA, are made up of units called nucleotides, arranged like beads on a string. The nucleotides in human telomeres are arranged in the repeating sequence TTAGGG (two thymine nucleotides, one adenine nucleotide, and three guanine nucleotides). This sequence is repeated hundreds of times in tandem in every telomere.

Each time our cells divide and our chromosomes replicate, our telomeres become shorter. When we are first conceived, the telomeres in our single-cell embryos are approximately 15,000 nucleotides long. Our cells divide rapidly in the womb, and by the time we are born, our telomeres have decreased in length to approximately 10,000 nucleotides. They shorten

throughout our lifetime, and when they reach an average of about 5,000 nucleotides, our cells cannot divide any further, and we die of old age.

Leonard Hayflick had discovered that there was a clock ticking in every dividing cell of our body; telomere shortening explains what makes that clock tick.

The time remaining on this "telomere clock" can be measured from our blood cells. When such measurements are taken, a significant correlation is found between a person's age and the number of "ticks" remaining on the person's clock.[3]



[4]

Telomerase

Obviously, there must be a way for our bodies to re-lengthen telomeres. Otherwise, our sperm and egg cells would contain telomeres the same length as the rest of our cells, which would yield embryos as old as we are. Because so much cell division takes place in the womb, our children would then be born much older than us. Humanity could not exist more than a generation or two if this were the case.

However, our reproductive cells do not exhibit telomere shortening, and show no signs of aging. They are essentially immortal. They are our germ line – the same one that has been dividing since the beginning of life on this planet.

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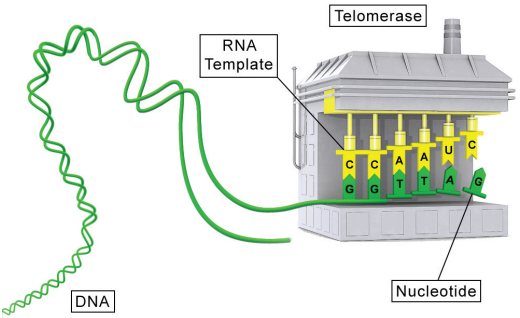
[3] Cawthon, R. M., K. R. Smith, et al. (2003). "Association between telomere length in blood and mortality in people aged 60 years or older." Lancet 361(9355): 393-5.

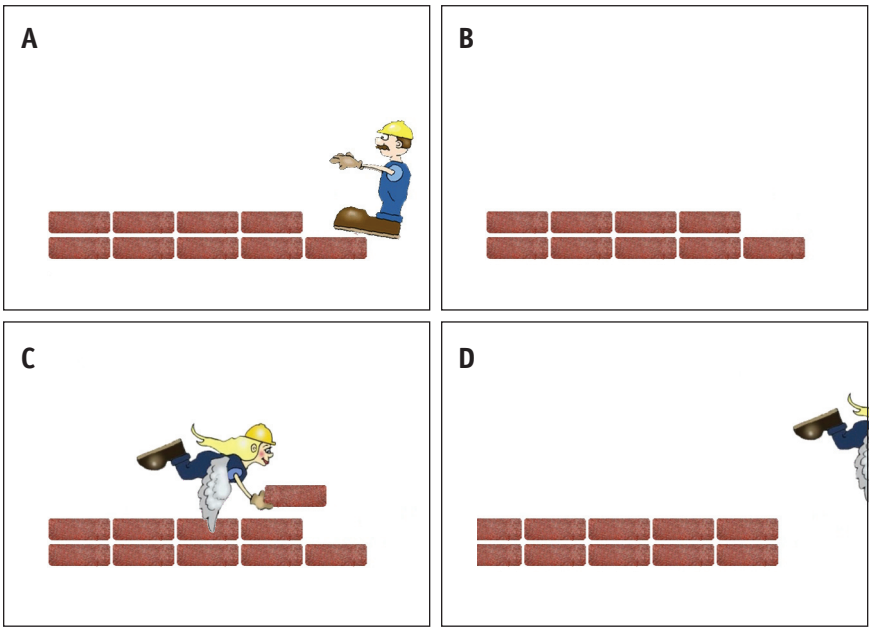
[4] Adapted from: Tsuji, A., A. Ishiko, et al. (2002). "Estimating age of humans based on telomere shortening." Forensic Sci Int 126(3): 197-9.

The reason these cells are immortal is that our reproductive cells produce an enzyme called telomerase. Telomerase acts like an assembly line inside our cells that adds nucleotides to the ends of our chromosomes, thus lengthening our telomeres.



In a cell that expresses telomerase, telomeres are lengthened as soon as they shorten; it's as though every time the "telomere clock" inside our cells ticks once, telomerase pushes the hands of the clock back one tick.

Telomerase works by filling the "gap" left by DNA replication. Returning to the analogy of the bricklayer that can't lay the last brick on the brick wall, telomerase would be like an angel that flies in and puts the last brick in place.

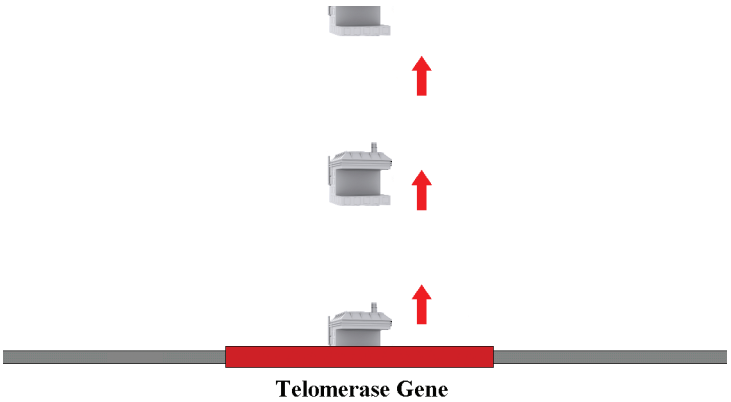


Telomere Length Therapy

So what about us? Can we insert the telomerase gene into all of our cells and extend our lifespan?

Inserting the gene directly into our DNA, through the use of viral vectors, is not a viable option. The main problem with this approach is that inserting genes into cells often causes cancer. That's because the gene gets inserted into our chromosomes at random sites, and if the wrong site is chosen, the gene can interrupt and disable cancer suppressor genes or turn on cancer-inducing genes. And you only need one out of the hundred trillion cells in your body to become cancerous in order to kill you.

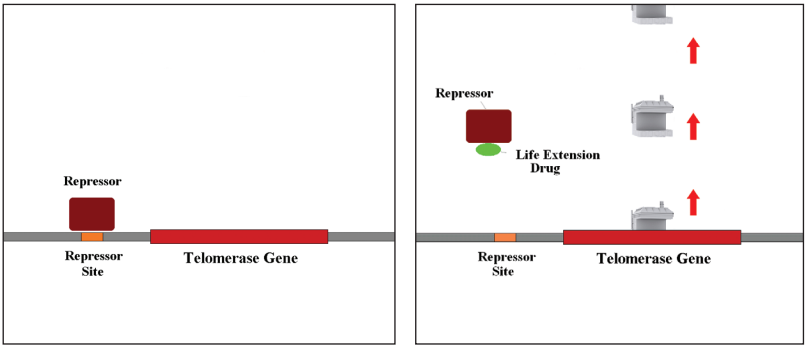
Fortunately, the telomerase gene already exists in all our cells. That's because the DNA in every one of our cells is identical: a skin cell, muscle cell, and liver cell all contain exactly the same genetic information. Thus, if the cells that create our sperm and egg cells contain the code for telomerase, every other cell must contain that code as well.



Telomerase being produced by the telomerase gene

The reason that most of our cells don't express telomerase is that the gene is repressed in them. There are one or more regions of DNA neighboring the telomerase gene that serve as binding sites for a protein, and, if that protein is bound to them, telomerase will not be created by the cell.

However, it is possible to coax that repressor protein off its binding site with the use of a small-molecule, drug-like compound that binds to the repressor and prevents it from attaching to the DNA. If we find the appropriate compound, we can turn telomerase on in every cell in the human body.



Compounds such as these have very recently been discovered. One such compound is TA-65, a nutraceutical discovered by Geron Corporation and licensed to TA Sciences. Additionally, Sierra Sciences, using a robotically-driven high-throughput drug screening effort, has discovered over two hundred compounds in twenty-nine distinct drug families that induce the expression of telomerase in normal cells.

However, the perfect drug hasn't been found yet. None of the compounds induce telomerase in large enough quantities that we can be confident in their ability to extend the lifespan of a cell; even the strongest known compound induces only 6% of the telomerase expression found in some immortal cell lines. Also, many of these compounds (with the notable exception of TA-65) are somewhat toxic to cell cultures and probably unsafe for human consumption.

Finding a more powerful drug will require more screening and more research, and the speed of that progress is dependent almost entirely on the level of funding that the project can achieve.

Proofs of Principle

There is a plan in place for inducing telomerase in all our cells. But will that plan work? Will it cure aging? That's the trillion-dollar question, and scientists have been trying to answer it for more than a decade. So far, all the signs point to yes: telomerase is a very likely cure for aging.

In 1997, scientists inserted the telomerase gene into normal human skin cells grown in a Petri dish.[5] When they observed that the telomerase enzyme was being produced in the cells, as hoped, they also observed that the skin cells became immortal: there was no limit to the number of times these cells could divide. When the lengths of the telomeres in these "telomerized" cells were examined, the scientists were surprised to see that the telomeres didn't just stop shortening: they got longer. The critical question, then, was whether the cells were becoming younger.

A few years later, scientists inserted the telomerase gene into human skin cells that already had very short telomeres. These cells were then grown into skin on the back of mice.[6] As one would expect, skin from cells that hadn't received the telomerase gene looked like old skin. It was wrinkled, blistered easily, and had gene expression patterns indicative of old skin.

The skin grown from cells that had received the telomerase gene, on the other hand, looked young! It acted like young skin, and, most importantly, its gene expression patterns, as analyzed by DNA Array Chip analysis, were almost identical to the gene expression patterns of young skin. For the first time ever, scientists had demonstrably reversed aging in human cells.

Would the concept apply to living organisms? In November 2008, scientists published a paper describing how they had created cloned mice from mouse cells containing the inserted telomerase gene, which continually produced the telomerase enzyme.[7] These mice were shown to live 50% longer than cloned mice created from cells that didn't contain the inserted telomerase gene.

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[5] Bodnar, et al. *Extension of life-span by introduction of telomerase into normal human cells*. Science, 1998.

[6] Funk, et al. *Telomerase Expression Restores Dermal Integrity to in Vitro-Aged Fibroblasts in a Reconstituted Skin Model*. Experimental Cell Research, 2000.

[7] Tomas, et al. *Telomerase Reverse Transcriptase Delays Aging in Cancer-Resistant Mice*. Cell, 2008.

It's becoming increasingly clear that prevention of telomere shortening might be the best way to extend human lifespan beyond the theoretical 125-year maximum lifespan. How long this can extend the human lifespan is anyone's guess, but living a healthy, youthful life to 250, 500, or even 1,000 years is not outside the realm of possibility. More research needs to be done to answer that question.

The Cancer Question

The ability to divide forever and never age describes our ancestral germ line, but it also describes a much less pleasant type of cell line: cancer.

A cancer begins when something goes wrong in a cell, causing it to lose control over its growth. It begins to divide repeatedly, ignoring chemical signals that tell it to stop. However, the telomeres continue to shorten in these cells, and eventually, the cells reach a stage where they can no longer divide, at which point they enter a "crisis mode."

In the vast majority of cases, when this crisis is reached, the cells will enter senescence and stop dividing. However, very occasionally, they will find ways to re-lengthen their telomeres. When this happens, a cancer begins to divide not only uncontrollably but indefinitely, and this is when cancer becomes truly dangerous.

In most cases (85–95%), cancers accomplish this indefinite cell division by turning on telomerase. For this reason, forcing telomerase to turn off throughout the body has been suggested as a cure for cancer, and there are several telomerase inhibitor drugs presently being tested in clinical trials.

So, anti-aging scientists must be out of their minds to want to turn the telomerase gene on, right?

No! Although telomerase is necessary for cancers to extend their lifespan, telomerase does not cause cancer. This has been repeatedly demonstrated: at least seven assays for cancer have been performed on telomerase-positive human cells: the soft agar assay, the contact inhibition assay, the mouse xenograft assay, the karyotype assay, the serum inhibition assay, the gene expression assay, and the checkpoint analysis assay. All reported negative results.[8]

As a general rule, bad things happen when telomeres get short. As cells approach senescence, the short telomeres may stimulate chromosome instability.[9] This chromosome instability can cause the mutations normally associated with cancer: tumor suppressor genes can be shut off and cancer-causing genes can be turned on. If a mutation that causes telomerase to be turned on also occurs, the result is a very dangerous cancer.

Paradoxically, even though cells require telomerase to become dangerous cancers, turning on telomerase may actually prevent cancer. This is not just because the risk of chromosome rearrangements is reduced, but also because telomerase can extend the lifespan of our immune cells, improving their ability to seek out and destroy cancer cells.

It's fairly obvious that long telomeres in human beings are not correlated with cancer. If that were true, young people would get cancer more often than the elderly. Instead, we usually see cancers occurring in people at the same time they begin to show signs of cellular senescence – that is, at the same time their immune system begins to age and lose its ability to respond to threats. Extending the lifespan of our immune cells could help our bodies fight cancer for much longer than they presently can.

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[8] Jiang, X.-R. et al. *Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype*. Nature Genet., 21, 111–114 (1999); Morales, C.P., et. al. *Absence of cancer-associated changes in human fibroblasts immortalized with telomerase*. Nature Genet., 21, 115–118 (1999); Harley, C. B. *Telomerase is not an oncogene*. Oncogene 21(4): 494-502 (2002).

[9] Benn, P. A. *Specific chromosome aberrations in senescent fibroblast cell lines derived from human embryos*. Am J Hum Genet 28(5): 465-473 (1976); Meza-Zepeda, L. A., A. Noer, et al. *High-resolution analysis of genetic stability of human adipose tissue stem cells cultured to senescence*. J Cell Mol Med 12(2): 553-263 (2008); Boukamp, P., S. Popp, et al. (2005). *Telomere-dependent chromosomal instability*. J Investig Dermatol Symp Proc 10(2): 89-94 (2005).

Objections to Finding a Cure

There are some who claim that a cure for aging is not a good thing, and that this is a technology that should never be researched in the first place. Some of the most common concerns about extending human lifespan are listed below, along with responses to these objections.

- **"Won't the Earth become overpopulated?"**

It stands to reason that extending our lifespans would increase the world population; after all, we've seen it happen before. In just over a century, the average life expectancy of a person living in the United States has increased from 47.3 in 1900 to 78.0 in 2008. Technologies including vaccines, antibiotics, chemotherapy, and antioxidants, as well as social advances such as sanitation, environmentalism, and an anti-smoking crusade have all contributed to this. Most recently, we've made attempts to push our lifespans out even further with technologies such as hormone replacement, caloric restriction, and Resveratrol.

And, indeed, these technologies have increased the size of our population. But something interesting also happened: population growth rate began to slow. Birthrates fell rapidly, and in less than four decades, the average number of children in a family was more than cut in half, from 6 to 2.9. Today, most researchers think we are headed quickly towards a stable population. Evidence is mounting that humans will simply not reach populations larger than our ability to sustain them: economics preclude us from doing that. As resources become scarce, prices rise, and as prices rise, family sizes shrink.

Is it a bad thing that our medical advances have nearly doubled our life expectancy? Most would say it's a decidedly good thing. So it's probably a safe bet that if we can drastically increase that figure again, future generations will also look back on it as beneficial.

- **"Won't Social Security be bankrupted?"**

Social Security is quickly heading toward bankruptcy right now – and the reason lies in the very nature of aging.

A typical person today works for forty to fifty years before retiring at age 65 or shortly thereafter. Although retirement is often framed as a reward

earned by a lifetime of hard work, the truth is that, not too long after reaching age 65, people inevitably become too sick and weak to continue working even if they wanted to. That's not the most desirable of rewards.

The fundamental problem with Social Security is that many of our modern medical advances have extended our lifespan – but have not expanded our healthspan to match. In 1935, when Social Security began, only about 57% of the population survived to age 65, and those who did only lived an average of 13 more years. Today, nearly 80% of the population survives to 65, and those who do typically live 17 more years.[10]

But these aren't our highest-quality years of life. Extending lifespan without improving healthspan has given us a large number of people who remain sicker longer, putting a historically unprecedented burden on the healthy to care for the sick.

If we felt as healthy and energetic at age 65 as we do at 30, why would we want to permanently retire? It would be far cheaper for the government to pay for a worker to take a ten-year vacation after forty years of work than to pay for seventeen years of decline and the staggering health care costs that accompany it. Not only that, but ten years of vacation as a healthy, youthful individual sounds like a much better reward for decades of hard work than seventeen years of decline.

• "Isn't curing aging unnatural or sacrilegious?"

Certainly, it can be argued that a cure for aging is unnatural. But it can also be argued that a human being, in his or her most natural state, is cold and hungry, infested with parasites, vulnerable to predators, and generally lives a life that Hobbes famously described as "nasty, brutish, and short."

In our natural state, we are susceptible to the disease of aging, and, similarly, we are susceptible to the disease of smallpox. Yet few among us would look back and claim that we made a horrible mistake when we unnaturally eradicated smallpox.

Sometimes, objections to finding a cure for aging are made on religious or philosophical grounds: some see such a cure as a defiance of natural order or of God's will. However, there are also many people whose religions

[10] U.S. Social Security Administration: http://www.ssa.gov/history/lifeexpect.html

and philosophies are exactly what drives them to seek a cure for aging. For example, Christian writer Sylvie Van Hoek believes that the search for the cure is not only compatible with belief, but that belief compels us to seek a cure:

> The Book of Genesis speaks of God's love. The creation stories describe the perfect world He created for us. After each creation He confirmed that it was good. There was no death or suffering in the Garden of Eden because it was not part of His plan. It couldn't have been because all that God creates is good; everything that is not good is the result of the absence of God. It was original sin that corrupted our perfect world. In failing to resist temptation and wanting to be like God---by eating from the forbidden tree of knowledge---man and woman turned away from God. This transformed the beauty of our nakedness into something shameful. Shame was impossible before the sin because nakedness meant that we enjoyed an intimate relationship with God. It was the sin that marked the beginning of our struggle with physical and moral suffering. Suffering is always the death of something, so physical death is just the far extreme along that same continuum.
>
> Critics [of anti-aging science] should read A Theology of the Body by John Paul II (Pauline Books, Boston, 2006). The recent pope eloquently expands on every bit of scripture concerning the body.
>
> In fact, I view [anti-aging science] as very much comporting to God's plan. He never wanted this for us. He created a different world, one that we corrupted. He could have turned away from us as we did to Him, but instead He sent the Christ to save us. He continues to work in the world today because He wants us to be happy. You may think you're doing something coldly scientific by fighting aging, but you're already up to your eyeballs in the fight against evil.[11]

There may be some who will always have philosophical and religious concerns about anti-aging science. But aging can be a painful, torturous process: it seems difficult to argue that going through the final stages of

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[11] Van Hoek, Sylvie, Masters Theology Student at the College of Saint Elizabeth, Morristown, NJ. Personal communication, 2008.

decline is an inherently good thing, or that finding a way for all of us to remain fit and healthy is inherently evil.

• "Won't future generations face challenges, such as long-lived dictators, that could have been avoided?"

The short answer is yes. But the same can be said of any technology. When humans invented the car, we also created the problems of traffic safety and air pollution. When we invented factories and industrialized the manufacture of goods, we were forced to rebuild ancient economic and social structures. When we discovered fire, we also had to learn not to get burned.

But, looking back, we wouldn't have it any other way. Any progress comes with its own challenges, but rejecting progress because we don't trust future generations to deal with it is not the solution.

Other Cures for Aging

There are many theories on what causes aging[12], and they may all be true — different pieces of the puzzle of why we grow old. These theories can be looked at as multiple sticks of lit dynamite inside our cells, each stick of dynamite representing a different cause of aging. It's only the stick of dynamite with the shortest fuse that will kill us. Which theory of aging has the shortest fuse? No one knows for sure, but given the well-established correlation between telomere length and age, telomere shortening is a good bet.

Scientists around the world are looking for cures for aging, and control of telomere length is not the only one being discussed. In fact, there might even be better ways.

One approach that's receiving a lot of attention is stem cell therapy. Stem cell therapy actually works on a principle similar to telomerase activation; the idea is to periodically infuse the body with young cells to replace cells that have senesced.

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[12] For a review of theories of aging, see: Hayflick, Leonard (January 23, 1996). *How and Why We Age*. (Reprint ed.). Ballantine Books. ISBN 0345401557.

Some scientists feel that curing cellular senescence is only a single piece of the aging puzzle, and that aging must be addressed on other fronts. An example is Aubrey de Grey's "Strategies for Engineered Negligible Senescence"; De Grey believes that a cure for aging must include therapies that address not only cellular senescence but also cancer-causing mutations, mitochondrial mutations, intracellular junk, extracellular junk, cell loss, and extracellular crosslinks.

There are also theoretical approaches to curing aging which appear to be scientifically sound, but for which the technological groundwork has not fully been laid. These include nanotechnological methods of intelligently repairing cellular damage, where infinitesimally small robots could be programmed to maintain the body at an optimal state of health. Another exciting concept is "mind uploading" technology, in which the brain would be regularly scanned into a computer to safeguard it against damage to the body. Although it's unlikely that these technologies will come to fruition in the very short term, they do merit further research.

Ultimately, our goal is to extend our lifespans and healthspans and live a young, healthy life for as long as possible. Telomerase activation may or may not be the "magic bullet" needed to achieve that end, but it's a technology that's well within reach, and any extension of lifespan could allow us to live long enough to see the next technology developed.

To extend our lifespans indefinitely, all we need to do is enter a period of scientific progress where technologies that extend our lifespans more than one year are discovered each year. Authors Ray Kurzweil and Terry Grossman have coined a phrase to describe this strategy: "Live long enough to live forever."

In Conclusion

People often wonder why progress in finding a cure for aging isn't moving faster. A common impression is that aging cures are well-funded, but the science is out of our reach. That simply isn't true. The primary reason that aging isn't already cured is because of lack of funding.

What is most needed in order to find ways to extend our lifespan before that lifespan runs out on us is for the wealthy individuals that want to see aging cured in their lifetime to get together, review all the approaches that exist for curing aging, prioritize them, and then fund the ones on the top of the list. Besides lengthening telomeres, some of the candidates for funding were described in the previous section.

This kind of patron investment is the only plausible way to lay down a path to the cure for aging. The government doesn't support this kind of research, and venture capital is more focused on short-term profits than long-term cures.

If aging is cured in our lifetime, it will be because of these patrons, not because of brilliant leaps of intuition on the part of any scientist. When it comes to curing aging, the science is fairly straightforward; the funding is not.

For more information, visit our website:

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