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BIOLOGY OF AGING



# TELOMERES AND TELOMERASE

An introduction to aging science brought to you by the  
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## WHAT ARE TELOMERES?

Inside the nucleus of virtually all of our cells are 46 chromosomes, the thread-like packages that carry our genes. At the tips of these chromosomes, like the hard ends of shoelaces, are structures called telomeres. While they do not contain genes, telomeres are important for replication or duplication of the chromosomes during cell division. They are made up of approximately 1,000 to 2,500 copies of a repeated DNA sequence (the order of chemical building blocks in a stretch of DNA), TTAGGG.

Why do we need telomeres? When we are born, we don't have every cell our bodies will ever need. As we grow, we need new skin, bone, blood, and many other kinds of cells. Even as adults, we need to make new cells. For example, skin cells and those cells that line our intestines are constantly replaced. All of these reproducing cells need their telomeres for cell division. Without their telomeres,

our cells would be unable to reproduce at all.

Telomeres also play an important protective role in our cells. Their presence prevents important genetic material from being lost during cell division. They also serve as a "cap" on the ends of chromosomes, protecting chromosome ends from appearing broken. This is an important function, because broken chromosomes trigger unwanted biological responses.

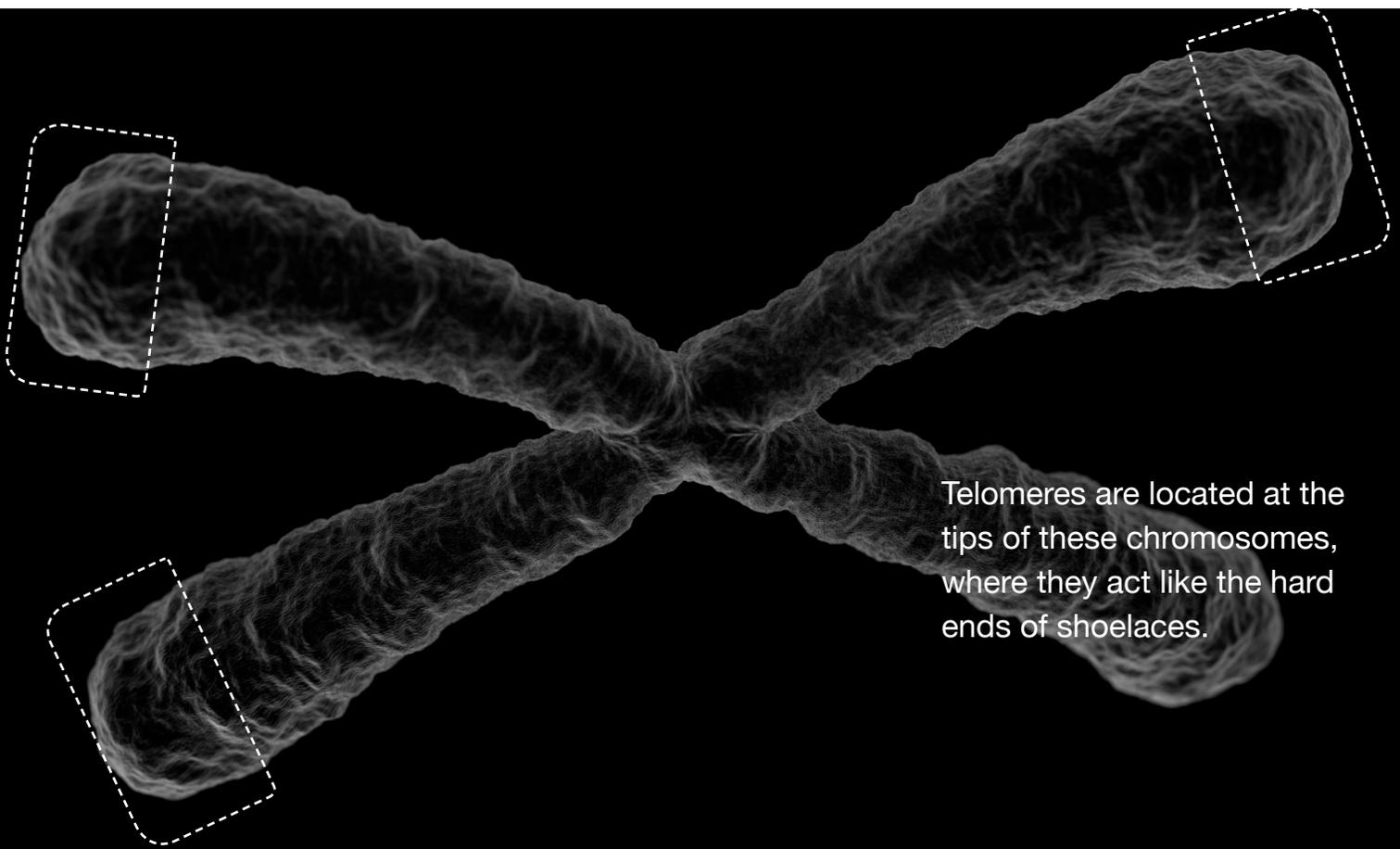
## HOW TELOMERES WORK

Telomeres are composed of double strands of deoxyribonucleic acid (DNA), except for the very ends, called telomere overhangs, which have single-strands. Many telomeres, including those from humans, appear to form t-loops—special folded structures where the single-stranded tail of the telomere is tucked into the more internal double-stranded part. T-loops are thought to be important for the protective-capping function of

telomeres. Research published in the January 21, 2003, issue of the *Proceedings of the National Academy of Sciences* suggests that the end of a cell's reproductive life may actually be triggered when this loop unravels, either due to DNA damage or to telomeres that have become excessively short.

Telomeres tend to get shorter over time. Two researchers, Alexei Olovnikov and James D. Watson, independently recognized that DNA replication machinery cannot copy chromosome ends completely. Watson named this the "end replication problem." Each time a normal cell divides, the ends don't get completely copied, and the telomeres become just a bit shorter. Eventually, telomeres are so short that the chromosome reaches a critical length, and no further cell division can occur.

This cellular aging phenomenon is known as *replicative senescence* or the *Hayflick limit* (discovered by Leonard Hayflick in 1961). Telomere shortening is a major



Telomeres are located at the tips of these chromosomes, where they act like the hard ends of shoelaces.

factor limiting cell division. While some have likened this to a genetic biological clock, others have described the telomere as a fuse that becomes shorter and shorter, until it sets off a kind of cellular time bomb that wreaks havoc on the cell's internal workings. Today, researchers continue to probe the telomeric "timepiece," hoping to better understand the aging process and fight diseases.

## WHEN TELOMERES MALFUNCTION

As mentioned above, telomeres serve as protective caps on the ends of chromosomes. From time to time, defects in this capping function can occur, and may be related to the end loops unraveling.

The capping function can be lost if the telomere becomes too short or is deleted entirely, or if a telomere protein is missing or mutated. The body perceives uncapped chromosomes as broken DNA ends. In most cells, broken DNA ends spark one of two repair mechanisms.

The first is called *homologous recombination* (HR), in which a broken DNA end is fixed by copying the sequence from a similar, unbroken DNA molecule. In some situations, recombination between malfunctioning telomeres can be frequent enough to keep cells alive and keep telomeres very long.

In a second mechanism, the broken ends of two chromosomes with telomere failure simply fuse together. This is called *non-homologous end joining* (NHEJ). Under most circumstances, this end joining is an effective and useful DNA repair process. With malfunctioning human telomeres,

however, it can have devastating consequences.

If two different chromosomes are fused by way of telomere end joining, they cannot separate properly during replication. During cell division, a tug of war between the two daughter cells over the fused chromosome usually results in it being broken into two uneven pieces. Each daughter cell then inherits a chromosome with missing or extra DNA and has one (newly broken) end missing a telomere. That end is free to potentially cause another round of chromosome fusion and breakage. Through this mechanism, uncapped telomeres can wreak havoc, killing cells and rendering those that survive genetically abnormal.

In addition to causing DNA repair in the form of recombination or end joining, broken DNA ends or uncapped telomeres can trigger other cellular responses. Because broken chromosomes are a severe form of DNA damage, cells are often exquisitely sensitive to their presence. Unrepaired broken DNA ends will often trigger cellular growth arrest, thereby preventing any cell division as long as the broken ends persist. In some human cells, broken DNA ends can trigger cellular suicide, a process known as apoptosis.

Because short telomeres are more common in older cells, telomere capping problems may be related to the development of cancer and other age-related diseases.

## TELOMERES AND AGING

Once a cell's telomeres have reached a critically short length, that cell can no longer divide. Its structure and function begins to fail. Some cells even die. In the

laboratory, most human cells can only divide 30 to 80 times before they stop reproducing. Cells taken from older persons and persons with premature aging syndromes undergo even fewer divisions before reaching senescence. Scientists know senescence is related to telomere length because adding telomerase, an enzyme that lengthens telomeres, to cells allows them to reproduce indefinitely.

One group of researchers looked at the cells of people with progeria, a disease that ages young children so rapidly that they die in their teens with many of the symptoms of old age. Their cells' chromosomes have exceptionally short telomeres, suggesting that the disease is causing rapid cell turnover. The cells are using up their ability to reproduce, which in turn may contribute to their premature aging.

Researchers have also developed a type of laboratory mouse that has defective telomerase. Selective breeding of these mice produced successive generations with signs of premature aging and shortened life spans, providing further evidence of the role of telomeres and telomerase in aging.

The entire aging process cannot be explained solely by telomere shortening. To date, evidence supporting the relevance of replicative senescence and telomere biology to cancer is rather strong; however, the direct evidence linking replicative senescence and human aging remains controversial and is of continued interest and effort of study. Some point out that no relationship exists between initial telomere length and a species' life span. Mice, for example, have much longer telomeres than humans,

and live only two years or so. This leads to the question concerning the role of replicative aging among different organisms and whether mice or humans represent the more common mammalian paradigm.

## TELOMERES AND OTHER AGE-RELATED DISEASES AND CONDITIONS

The shortening of telomeres has been associated with a number of diseases, many of them age-related. Shortened telomeres have been identified in aging skin, blood, and cardiovascular cells. And the cells of people with a variety of diseases—from atherosclerosis to hepatitis to blood disorders—have been found to have shortened telomeres.

## THE RELATIONSHIP BETWEEN TELOMERES AND CANCER

If telomeres give our cells finite life spans, how is it that cancer cells seem to possess infinite life spans? How can they reproduce and spread infinitely? How do cancer cells get around the limits that telomeres impose on our healthy cells, thereby becoming immortal?

In many non-human organisms, telomerase is always active when cells are dividing. This makes up for gradual telomere shortening from replication and cell division. This activity, regulated by certain proteins, keeps telomere length more or less constant.

Human cells are different. In most of them, telomerase is turned off. This means telomeres shorten as cells continue to divide. However, almost 90 percent of all cancer cells possess telomerase, while the remaining employ a mechanism called alternative lengthening



**Telomere shortening may be an active contributor to the genetic abnormalities that trigger cancer because dysfunctional telomeres drive genome instability.**

of telomeres (ALT) for telomere maintenance. Telomerase is an enzyme that “rewinds” our cellular clocks. It lengthens our shortened telomeres, replacing bits of DNA lost in ordinary cell division. If telomerase stops telomere shortening, then in theory, those cells with telomerase can live forever. Since most cancer cells contain telomerase, researchers believe it is a critical factor in conferring immortality upon these cells.

Inactivation of telomerase and the resulting telomere shortening likely evolved in humans to reduce the incidence of cancer. By causing replicative senescence, telomere shortening acts as a road block to the abnormally high amount of proliferation associated with the development of cancer. It takes many divisions for cells to accumulate enough mutations to become malignant. Cells that exhaust their replicative life span become senescent with only a few

mutations accumulated. Therefore, they remain pre-malignant and do not develop cancer.

However, telomere shortening itself may be an active contributor to the genetic abnormalities that trigger cancer because dysfunctional telomeres drive genome instability. In fact, there is growing evidence of an association between shortened telomeres and a greater risk of cancer development in humans. In addition, laboratory evidence has shown that shorter telomeres may contribute directly to the progression of the earliest stages of certain cancers. In an experiment involving cells taken from prostate cancer patients, researchers from the Johns Hopkins University School of Medicine found that telomeres in cells from precancerous lesions were four times shorter than telomeres in cells taken from surrounding normal tissue.

## THE ROLE OF TELOMERASE IN CANCER

Telomerase is the enzyme that replenishes shortened telomeres and allows cells to reproduce indefinitely. Found in only a few normal human cell types (germline cells, proliferating stem cells, and some immune cells), telomerase is present in as many as 90 percent of human cancers. This makes telomerase an attractive candidate for highly selective cancer drugs.

The evidence that activation of telomerase is necessary for most cancers to thrive is strong. Indeed, some scientists believe that telomerase activation is the main pathway by which cancer cells become immortal, that is, able to reproduce forever without limits. Cancer cells generally need to acquire four-to-six mutations to become malignant. On average,

a cell with a mutation would need to expand to at least a million cells (20 doublings) before it had a chance for another rare mutation to occur. If a cell can divide 30 to 80 times, pre-malignant cells can only acquire one-to-three mutations before they stop dividing. Replicative aging is thus a barrier against the formation of malignant cancer cells. Thus, say researchers, telomerase activation is necessary for most, but not all, cancers to grow.

A number of researchers have suggested that if telomerase is required for so many cancers to flourish, perhaps anti-telomerase drugs could be developed as cancer-fighting agents. Some have suggested that such a drug would have minimal side effects, since so few normal cells have active telomerase. Others caution that some side effects are possible because some normal cells, among them dividing germ-line lineages, stem cells, and some cells in the skin, blood, and gastrointestinal track, do have telomerase activity. Potential side effects include:

- *Blood toxicity.* Some populations of stem cells, which are the parents of mature blood cells, do use telomerase. Anti-telomerase drugs could, therefore, suppress the production of vital blood cells.
- *Immune toxicity.* Some infection-fighting cells use telomerase normally, so anti-telomerase drugs could, theoretically, weaken our ability to fight infection.
- *Skin toxicity.* While most of our skin cells have little telomerase activity, the skin stem cells that repair wounds do have some. Anti-telomerase drugs might cause delayed wound healing.

- *Gonadal toxicity.* Some normal telomerase activity is seen in the cells of the ovary and testes. Thus, some researchers speculate that anti-telomerase drugs could potentially interfere with fertility.

One drawback to the use of anti-telomerase drugs in treating cancer is the length of time needed for such drugs to have any effects. If tumor telomeres are long enough, it might take many cell divisions of telomerase inhibition before they're short enough to kill the tumor cell. Even if anti-telomerase drugs were developed in the near future, they would need to be used in conjunction with faster-acting anti-cancer drugs. The hope is that very rare surviving cancer

cells would require so many divisions to cause a relapse that their telomeres would become too short in the absence of telomerase.

### **Diagnostics**

The fact that telomerase is active in up to 90 percent of all cancer cells suggests that telomerase activity may help to diagnose early cancers so they can be treated quickly, before the cancer spreads. Theoretically, biopsies, tissue scrapings, and biological fluids such as blood may provide adequate cell samples for detecting telomerase activity. At a practical level, however, today's tests do not yet compare favorably with other tests, so their clinical application has been limited.



Some scientists believe that telomerase activation is the main pathway by which cancer cells—such as this breast cancer cell— become immortal.



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### **Prognostics**

Telomerase biology may also help to identify cancer survivors who might benefit from supplemental therapies after surgery. In a recent study, the expression of telomerase was a strong predictor of long-term outcome. In breast cancer survivors without lymph node involvement, 98 percent of those with the lowest levels of telomerase expression survived more than 12 years. Among patients with the highest levels of telomerase, there were no survivors after 12 years.

### **THE FUTURE OF TELOMERE AND TELOMERASE RESEARCH**

At the leading edge of cancer research, scientists are currently looking at ways to help the immune system identify and target malignant cells by way of their telomerase expression, leaving normal cells unharmed. In one clinical trial, 12 patients with advanced prostate cancer were vaccinated with immune cells that could do just that. All patients with detectable levels of circulating tumor cells showed declines ranging from six to a thousand fold. Limited clinical trials are now underway to test this approach with other types of cancer.

Another approach uses oncolytic viruses to attack telomerase-expressing cells. These are genetically engineered viruses that specifically infect and destroy cancer cells by penetrating their membranes and replicating inside them. The replication is triggered by the presence of telomerase.

### **TISSUE REJUVENATION**

If low telomerase is related to the shortened telomeres that mark an aging cell, perhaps we can “turn on” the expression of this

gene to lengthen the lifespan of a cell or organ. One goal of tissue engineering is to overcome organ failure by infusing cells with telomerase. A patient donates cells that are modified in culture by the introduction of telomerase. The cells are then returned to the patient to correct a deficiency. Thus far, 17 different cell types have been used to engineer 22 different kinds of tissues. The limitations of this technology are considerable, however, emphasizing our limited understanding of the complexity of aging cells.

The use of a donor's own cells to build new tissue has its limitations because of the limited lifespan of most cells—especially cells from older patients. This inability to proliferate, however, can be overcome if the donor's cells are infused with a specific gene, called hTERT, that switches on telomerase expression. Telomerase then restores length to shortened telomeres.

In one proposed form of therapy, cells with shortened telomeres are donated by a patient. Telomere length is restored in culture with an infusion of hTERT. Cells are then reintroduced in the patient's body to restore tissue function. One application, for example, might be in arterial bypass grafting in aging cardiac patients.

While cell therapy offers tremendous promise in clinical medicine, the safety issue dominates the future acceptability and widespread use. Researchers are grappling with certain questions such as: What is the likelihood for long-term change that might trigger abnormal tissue growth? Specifically, what is the risk of cancer?

The results of a study at Duke University suggest that telomerase is not independently capable of inducing tumor formation. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1413782/>

The over expression of telomerase activity in cultured cells failed to result in malignant change in a number of cell types. The results of various analyses of this Duke study thus far, suggest no evidence of tumor formation of either the hTERT cells or the control cells. Another laboratory at the UT Southwestern Medical Center has also reported that long-term effect of telomerase immortalized human fibroblast does not show cancer-associated changes. <http://www.ncbi.nlm.nih.gov/pubmed/9916803>

While cell therapy appears to be an exciting field with tremendous promise, future research endeavors involving hTERT cell therapy need to carefully explore long-term risks. For

example, might hTERT-modified cells be more dangerous if mutational changes months or years after transplantation cause the activation of oncogenes? Also, is any potential risk of hTERT gene therapy acceptable—even when given to save lives?

Future research must also unravel the phenomenon of cellular aging even in newly engineered tissues. In the smooth muscle cells described above, for example, age-related changes to cells were not reversed by transplantation of cells modified with telomerase. In fact, collagen synthesis decreases and consequently reduces the strength of even robustly engineered vessels.

Research into telomeres and telomerase is still in its early phases, but scientists have learned much in these last few years. The knowledge they have amassed and continue to pursue offers enormous potential for understanding and perhaps controlling the diseases of old age.

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