LE Magazine July 1998

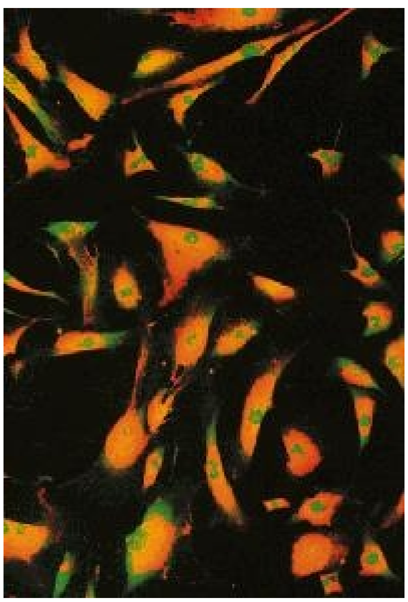
REPORT

Seeking the Key to Immortality

By Mary Nucci

Some of the most exciting science news in recent months has come from a California company that has been exploring cellular aging. The work of Geron Corp. and its university partners may herald a new phase in the quest for longer, healthier lives.

No matter how individual we like to consider ourselves, physically humans go through their lives in much the same programmed way. We are born, we grow up, we age, we die. There is, in short, a consistency in the programmed deterioration and breakdown of body systems.



At left, differential staining of replicatively young cells and senescent or "old" cells. Courtesy of Geron Corp.

But what if we could control these programmed events? Cell death is believed to be one of the factors, or even the prime factor, which results in the aging of our bodies. What if within our cells we could alter the eventual progression toward unwanted side effects of growing older?

In fact, some of the most exciting and far-reaching recent research offers the promise to manipulate the very life span of our cells.

That research has emanated from a Menlo Park, Calif., company named Geron Corporation, in conjunction with other research institutions. Geron's research demonstrates the aging effects of something called telomeres (TEE-low-meers)-the tiny end portions of DNA strands in certain cells in the body. These telomeres shorten as cells divide, and when they become short enough, cells die.

Further, the work of Geron and its research colleagues have demonstrated that an enzyme called telomerase (tuh-LAH-mer-ace) serves as a key to turning off the shortening of telomeres. The implications are potentially astounding: By activating the dormant telomerase in mortal cells, telomere shortening would stop, and the cells could live much longer.

Conversely, there might be a way to eliminate telomerase activity in immortal but dangerous cells, such as cancer cells. By inducing anti-telomerase activity in these immortal cells, their telomeres would begin to shorten with each cell division, and ultimately die.

It could conceivably result in a cure for cancer (Please see accompanying article).

Let's take a look at the premises behind telomere theory, and what led the Geron team of scientists to these findings.

Most cells in our bodies divide throughout their life spans. Some cells divide more often than others. But regardless of whether the cells divide frequently or not, before every cell divides it must make a copy of the genetic information carried in its DNA, the recipe book that each cell requires to function. During copying, small bits-anywhere from 50 to 200 nucleotides-are clipped from the ends of the DNA strands. These end portions of DNA, the telomeres, become shorter and shorter with each cell division. Often compared to the plastic ends of shoelaces, telomeres function to maintain the integrity of the DNA. When the integrity of DNA is compromised, the cell is not able to work correctly, eventually resulting in cell death.

Though telomeres do not appear to contain any vital genetic information, researchers long have believed they were extremely important in controlling the life span of the cell. Studies of cells grown in culture showed that certain cells seem to live indefinitely while others have a finite life span. The "immortalized" cells, which include cancer and male reproductive cells, differ from the "mortal" cells in one significant way: The telomeres at the ends of the DNA strands of the immortalized cells do not grow shorter with each cell division.

By contrast, in the somatic cells-all body cells other than reproductive cells-when the telomeres do get shorter, and when they reach a certain length, the cells no longer divide. Eventually the cells becomes senescent and die. These cells seem to have a programmed limit of about 50 cell divisions before they die, and this limit appears to be related to the length of the telomere end unit.

Telomerase researcher Woodring Wright, left, shown with colleague Jerry W. Shay, both of the University of Texas Southwestern Medical Center, believes that the ability to maintain telomerase-positive cells indefinitely in culture may lead to new therapies for cancer or genetic diseases. In 20 to 30 years, Wright believes, we may be able to modify telomere shortening in all cells of body to alter the process of aging.

Yet, for various reasons, many researchers have been unconvinced of the relationship between telomere length and cell senescence. For example, there does not appear to be a consistent pattern in the relationship between telomere shortening and cell life when comparing different species. The common mouse has telomeres that are three times the length of the human telomere, yet mouse cells do not live three times longer than human cells.

Even within the same species, the role of telomeres has been unclear. In humans, nerve and muscle cells do not divide after infancy. Telomere shortening obviously has no role in these cells, yet they demonstrate cellular aging and death. Moreover, at cell senescence in human cells, there are still some telomere units on the ends of the DNA. There is no explanation for how the cell "knows" when the telomeres have reached a shortened length critical for cell senescence.

What the immortal cells share in common is the presence of the enzyme telomerase. All the cancer cell lines that have been tested so far (as well as male reproductive cells, interestingly) are positive for telomerase. Telomerase maintains the length of the telomeres on the ends of the strands of DNA by adding back or preventing the clipping off of telomere units that are normally removed during cell division. While mortal cells also have the gene for telomerase, but the gene is turned off, so that during cell division the telomeres at the end of the DNA are clipped.

It has been speculated that telomeres evolved in humans as a means to control cancer. During the course of prehistoric life, just as today, DNA accumulates mutations from mistakes in copying during cell division, environmental mutagens or exposure to ultraviolet radiation. When a certain critical mass of mutations has been reached, cells became cancerous.

Telomere control of cell division evolved in order to institute programmed cell death at the time that the number of mutations had reached critical mass. If there were no relation between telomere length and cell death, then the cells would be immortal and keep dividing. Instead of the rare cell becoming cancerous, as occurs in individuals with telomere control, many cells would become cancerous. Therefore, by limiting cell life with telomere control of cell division, the cells die at that point in a human's life when the likelihood of developing cancer is very high.

But a problem has arisen. While we have found ways to increase the average life span through proper sanitation, and to control cancer and other formerly life-threatening diseases, our cells are stuck with programs that dictate that they get old and die while we are still able to live an active, healthy and productive life.

Observation alone was not sufficient for most scientists to accept the theory of telomere control of cell division. It required the advent of cloning technologies to prove the theory of telomere-controlled cell death. Cloning (the ability to insert new or novel genes in specific cells) has enabled researchers to develop many new therapeutics based on human genes. Perhaps more importantly, though, cloning has improved our insight and understanding of cellular processes by allowing researchers to manipulate the genes and the genetic process itself.

In an article in the August 15, 1997, issue of *Science*, Nobel laureate Thomas Cech (pronounced CHECK) of the University of Colorado, Boulder, and colleagues at Geron, announced they had identified and sequenced the activation component of the human telomerase gene. This particular portion of the telomerase gene is responsible for "turning on" the gene. If the telomerase theory of programmed cell death was correct, once the gene was turned on, the cell would produce the telomerase enzyme and an increase in the life of the cell could be expected.

Dr. Andrea Bodnar and colleagues from Geron, along with Dr. Woodring Wright and colleagues at University of Texas

Southwestern Medical Center, in Dallas, cloned the activating component of the telomerase gene into skin fibroblasts and retinal pigment epithelial cells...two different kinds of somatic cells, each of which shows age-related changes.

The cells then were grown in cell culture and examined for their ability to divide. When compared with control cells that did not have the activation component of the telomerase gene, the manipulated cells exceeded the normal cellular life span by at least 20 cell divisions-a 40-percent increase in life span (*Science*, January 15, 1998).

Control cells that did not have the inserted telomerase gene activation component survived for the usual allotment of cell divisions before they entered cell senescence. With each cell division, the telomeres of the control cells became shorter. They also tested strongly positive for beta-galactosidase, a cell component associated with cellular aging. However, the manipulated cells maintained telomere length and continued to divide.

Only low levels of beta-galactosidase were found in the manipulated cells. Additionally, there was no evidence of cell mutation associated with the development of cancer.

The authors stated, "Our results indicate that telomere loss in the absence of telomerase is the intrinsic timing mechanism that controls the number of cell divisions prior to senescence."

In a reverse experiment performed by Geron scientists and collaborators at the Cold Spring Harbor Laboratory in New York, immortalized cancer cells were exposed to a type of molecule (called an "antisense" molecule) to shut down the telomerase gene. The antisense molecule "mortalized" the cancer cells with the result that after dividing for a finite time in culture, the cells aged and died. According to Dr. Mike West, founder of Geron, taken together these results "firmly demonstrated that the theory of telomere-related cell senescence was fact."

Mike West, a founder of Geron, believes "primordial stem cells" will enable scientists to develop therapies for the disease of aging.

(Following the initial reporting for this article, Mike West left Geron to form another company called Origen, which will develop primordial stem cells for the development of therapeutics.)

Boehringer Mannheim, a world leader in the development and marketing of laboratory assays and kits, will be working with Geron to develop cancer diagnostics based on telomerase. In certain cancers, high levels of telomerase are predictive of a poor prognosis, while in others the presence of telomerase is a marker for a pre-malignant conditions.

Nobel laureate Thomas Cech, (right) one of the identifiers of the activation component of the human telomerase gene.

Telomerase screening will be useful for cancer diagnosis as well as to aid physicians on how best and how aggressively to treat patients. Pharmacia & Upjohn and Kyowa Hakko of Japan are collaborating with Geron on the development of telomerase-based therapeutics. The three-way restricted agreement between Geron, Pharmacia & Upjohn, and Kyowa Hakko will pay Geron more than $88 million for the single target program of telomerase-inhibition cancer products.

Telomerase researcher and Geron collaborator Woodring Wright believes that in the near term the ability to maintain telomerase-positive cells indefinitely in culture will provide valuable tools for researchers to study cell processes. Such cells can be used to replace or complement those cells already being used for laboratory studies on cancer.

After that, the manipulation of the telomerase gene may lead to new therapies for cancer or genetic diseases. Manipulation of the telomerase gene offers promise for cancer therapy by de-activating the gene in immortalized cancer cells. For genetic diseases, researchers may be able to remove cells from a patient, choose those that are free of defects, rejuvenate cells by activation of the telomerase gene, and give them back to the patient.

In 20 to 30 years, Wright believes, we may be able to modify telomere shortening in all cells of body to alter the process of aging.

"I disagree that it will mean we will live 150 to 200 years, as there are other factors affecting aging," Wright says. "But walking out on a long limb, I believe there will be a 10-percent difference in life span with telomerase activation. Replacing the transmission on a car with 90,000 miles will not affect the brakes from giving out, but on average you will increase the life span of the car."

Aside from the development of diagnostics to screen for cancer therapy and treatment, probably one of the first applications of telomerase research will be in the production of immortal cell lines for use in research laboratories or for drug development. Immortalized human cells will provide invaluable tools for scientists limited by the finite life span of human cells in culture.

These immortalized cells may also be used to produce specific gene products, such as proteins or enzymes, for human therapeutics. The use of proteins for therapeutics is limited by the fact that animal proteins are generally different from human proteins, and using animal therapeutic proteins often results in an allergic response that can be fatal. Human proteins are preferable for use, but so far human cells have not proven to be capable of creating these products in culture in sufficient quantities, partly because of limitations due to the life span of the cells in culture.

By genetically manipulating an immortalized cell prior to cell culture, it could be possible to produce a cell line that can divide indefinitely. This replicative immortality will enable researchers to grow vast quantities of cells. By specifically manipulating the genetic makeup of the cell prior to growing it in culture, it will be possible to induce the cell to make a specific gene product, which could then be isolated and purified, and used either as a research tool or as therapy for various diseases.

Patients with genetic deficiency diseases like hemophilia, or diseases where a gene product is lost due to gene inactivation, such as diabetes, would greatly benefit from the ready availability of the human protein they lack that causes their disease.

A very long-range goal involving telomerase activation of cells, according to West, would be the creation of "customized" primordial stem cells (PSC). PSCs are cells that have not yet differentiated into muscle or skin or any of the other kinds of cells that are found in the body. Before cells differentiate, they are totipotent-that is, they have the capability to become any kind of cell. Once differentiation has occurred, the cell is locked into a specific pattern. Differentiation also results in a loss of immortality as the gene for telomerase becomes inactive.

The use of PSCs may revolutionize medicine because researchers can generate any kind of cell they desire, while offering the "broad possibility to make a variety of cell types survive longer in culture, such as for grafts," according to Dr. Matthew Meyerson of the Whitehead Institute for Biomedical Research, in Cambridge, Mass. The PSC could be altered for the specific need of an individual...that is, genetically manipulated to create specific cells with specific characteristics.

Manipulating the gene information in the PSCs to create a universal cell that would not react with an individual's immune system would make it possible to transplant cells and not prompt the immune response that can lead to graft rejection or death. Currently, physicians control graft rejection by giving patients immune-suppressing drugs, but that leaves the patients vulnerable to the simplest of infections.

This new technology may also be used to address diseases associated with aging. Geron is evaluating PSC technology for the transplantation of retinal cells for macular degeneration, nerve cells for neurodegenerative disorders, and cardiomyocytes for heart disease.

West notes that this PSC research is a "very profound application for the future to generate new cells and tissues from new cells," but that this technology is "definitely a technology for the 21st century."

Now that the scientists have identified the gene and its product, it will be easier to define a suitable telomerase inhibitor. Just as a specific key fits a certain lock, so the inhibitor will have to have a specific shape and construction in order to selectively inhibit the telomerase gene.

Approval for anti-telomerase therapies could take anywhere from five to 20 years, according to Meyerson.

Of all the potentials for the clinical use of telomerase research, the manipulation of the telomerase gene in aging is the most controversial. Some, like Meyerson, feel that "it is unclear what telomerase has to do with aging, if anything," while others, such as West, feel that the "burden of proof rests with those who say telomerase has only an indirect effect" on aging.

As clear-cut as it is that telomerase inhibition mortalizes cancer cells while telomerase activation immortalizes normal cells, it is unclear what effect manipulating the telomerase gene will have on the aging process. The issue remains: Aging is not as simple as an on/off switch. Research has shown that aging and age-related effects can be affected by such diverse actions as decreasing caloric intake, avoidance of sunlight, and ingestion of antioxidant foods and supplements.

Geron is evaluating PSC technology in the treatment of eye problems, heart disease and neurodegenerative disorders.

Aging also is much more than just deterioration of body systems. Aging can be affected by disease and mental state, and by exercise or the lack of exercise. And if telomeres are the control clock for aging, how do we reconcile the fact that nerve and muscle cells-cells that never divide and therefore are not regulated by telomere length-still age?

Regardless of the controversy, the data indicate that telomere shortening indeed plays some role in cell death, and that cell death is a process of aging. Activation of the telomerase gene does offer the potential to affect the life span of cells. Initially, it will most likely be used to rejuvenate specific tissues seriously affected by aging, such as heart, eye and nerve cells. Geron is evaluating the use of telomerase activation of cells specifically affected in Parkinson's disease, atherosclerosis, macular degeneration and congestive heart failure. Cells so affected could be removed from a patient, and replaced after immortalization through telomerase activation.

In the long run, the possibility exists that through telomerase activation, cells throughout the body could be treated to extend cellular life span, and delay the onset of cell senescence. Serious concerns must be addressed before this technique is possible, especially whether telomerase activation will increase the risk of cancer. Transient activation may address this concern; that is, the telomerase gene can be deactivated after telomere length is rebuilt.

But many questions still remain to be answered before telomerase can live up to the lofty expectations raised by some. It will be necessary to correlate cell aging in the test tube to cell aging in the body, since what happens in vitro is not always the same as what happens in vivo.

The effect of telomerase activation on the various types of cells that make up the body's systems needs to be determined before it can be administered.

Nevertheless, the research offers promise. While it may not allow humans to live indefinitely, at least it may allow us to live our mature years as more healthy members of society.

Back to the Magazine Forum