End Games

Tips of chromosomes may contain secrets of cancer and aging

By JOHN TRAVIS

Until the last few decades, *Tetrahymena thermophila*, a single-celled microorganism bristling with cilia, the hairlike appendages that enable it to flit around ponds, was an obscure creature. But once researchers isolated a chromosomal structure called a telomere in these protozoa, things began to change.

Telomeres, which cap the ends of chromosomes, and telomerase, the odd enzyme that constructs those caps, may hold the key to understanding what determines the life span of human cells—and how cancer cells get around that limit, achieving a deadly immortality. If so, *T. thermophila* could find a place in the annals of science alongside other unknowns thrust into medical prominence, such as the bread mold that gave the world penicillin.

In *T. thermophila*, telomerase maintains telomeres at a stable length; this makes the microorganism immortal, or capable of dividing indefinitely. In humans, however, most cells appear to stop making telomerase shortly after an embryo is fully developed.

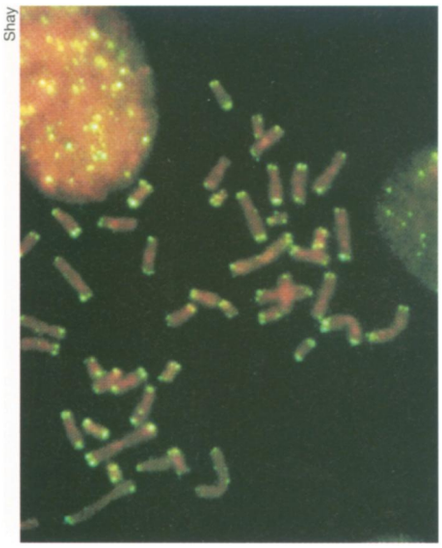
A bit of every telomere is lost whenever a cell divides, so the telomeres in most human cells gradually shorten. Consequently, investigators suspect that human telomere length marks both the age of cells and how long those cells will continue to divide.

Furthermore, the evidence indicates that many cancers keep growing because tumor cells renew production of telomerase, which stabilizes telomere length and allows the malignant cell to divide indefinitely.

These recent findings have spurred interest in telomeres beyond the small core of specialists who first studied them. "The telomere field has been on an exponential rise," says Carol W. Greider of Cold Spring Harbor (N.Y.) Laboratory.

In fact, numerous companies, such as Geron Corp. in Menlo Park, Calif., are exploring whether inhibition of human telomerase may prove an effective treatment for cancer. "I think telomerase's chances of being a specific, universal cancer target are very good, much better than anything we have now," says Gregg B. Morin of Geron.

Scientists' knowledge of telomeres predates *T. thermophila*'s involvement. As early as the 1930s, biologists knew that the tips of chromosomes had specific roles. Like aglets, the short pieces of plastic that protect the ends of shoelaces, the tips seemed to preserve the integrity of chromosomes. Without these structures, which became known as telomeres, chromosomes would fuse to each other or go astray when a cell replicated itself.



Shay

Telomeres, imaged in green, protect the ends of chromosomes.

Telomeres also appeared to protect against a potentially damaging quirk in the way cells copy DNA. In this process, the proteins that duplicate a cell's chromosomes do so incompletely, shaving off a piece at the end of each chromosome with every cell division. Without the telomeric cushion at the ends, this copying problem would slice off necessary genes after a number of cell divisions.

It wasn't until the late 1970s that biologists got a firm handle on the composition of telomeres. In most organisms, the scarcity of the structures makes them difficult to isolate. Human cells, for example, normally contain only 46 chromosomes each. That provides a paltry 92 telomeres per cell.

T. thermophila, in contrast, offers a telomeric jackpot. At points in its life cycle, the organism's five chromosomes fragment into tens of thousands of DNA scraps, all of whose ends are quickly capped by telomerase. Consequently, "a single cell has more than 20,000 telomeres," says Greider.

In 1978, that unusual abundance helped Yale University investigators Joseph G. Gall and Elizabeth Blackburn, who is now at the University of California, San Francisco, discover the makeup of the chromosomal aglets. Telomeres were a genetic stutter, a brief sequence of DNA repeated over and over again.

DNA is built from molecular units called nucleotides, which come in four varieties, abbreviated as A, C, G, and T. Long, complex nucleotide sequences, packaged into units called genes, tell cells how to construct proteins. But when Gall and Blackburn isolated *T. thermophila*'s telomeres, they found that each one consisted of little more than a strip of six nucleotides, TTGGGG, duplicated many dozen times.

Investigators then found that telomeres of other species had similar structures, though the exact DNA sequence and number of times it repeats varies by species. For example, human telomeres contain a few hundred copies of the DNA sequence TTAGGG.

Another significant step came in 1985, when Blackburn and Greider described *T. thermophila*'s telomerase.

The two investigators found that telomerase has a peculiar, mongrel structure. Unlike most enzymes, which are simply proteins, telomerase is a mixture of a few proteins along with a piece of RNA, a DNA-like molecule. The RNA sequence provides a template for that species' telomeric DNA repeat, the researchers concluded. When investigators changed the RNA sequence of *T. thermophila*'s telomerase, the enzyme placed a different DNA sequence onto the chromosome ends.

Telomere research burst into the arena of human biology with discoveries in 1988 and 1989. In 1988, Robert Moysis and his colleagues at Los Alamos (N.M.) National Laboratory unraveled the sequence of human telomeres. A year later, Morin discovered active telomerase in HeLa cells, an immortal cell line derived from human tumor cells.

Knowing the human telomeric sequence, scientists could finally examine closely the lengths of telomeres in various human cells and tissues. Researchers recently found that the cells of older people generally have much shorter telomeres than those of younger individuals. And people with diseases that cause premature aging have dramatically shorter telomeres than other individuals the same age.

Investigators have made another curious observation in their test tubes and petri dishes: The length of the telomeres in a human cell can predict how long that cell will continue to divide in culture.

That cells in culture have a finite capacity to double is an old notion. In 1962, Leonard Hayflick of the Wistar Institute in Philadelphia reported that human cells maintained in a test tube undergo a finite number of doublings, about 50, before they enter a nondividing stage of life known as senescence. In senescence, cells change their personality, subtly altering the pattern of genes they had expressed earlier in life. "If you look at them in the microscope, [senescent cells] look very different. They get very large and flat," says Greider.

Though it is difficult to determine whether senescence occurs in the same way in the body as it does in laboratory cells, researchers speculate that the accumulation of senescent cells may account for a variety of age-related changes, from the wrinkling of skin to cardiovascular disease to memory loss. Senescence may have evolved as a defense against cancer in long-lived animals, some investigators suggest. The amassing of mutations might otherwise prompt long-lived cells to divide uncontrollably.

Although researchers such as Greider quickly recognized the apparent connection between human telomere shortening and senescence, they weren't the first. In 1970, Russian scientist Alexy M. Olovnikov heard a lecture by Hayflick and later speculated that the gradual trimming of chromosome ends might be the counter governing how many divisions a cell could undergo.

The notion that telomere shortening eventually triggers senescence is intellectually appealing, says Greider, but it has not yet been proved. Investigators have simply shown a correlation between the two phenomena. Just as graying hair accompanies aging but doesn't cause it, telomere shortening may not induce senescence, she explains.

To resolve the issue, researchers would like to use telomerase to lengthen telomeres in normal human cells and observe whether that delays senescence. That's easier said than done. "You can't do it without having all the components in hand," says Greider.

Though she and Blackburn have found the genes that encode the RNA and protein portions of *T. thermophila*'s telomerase, they've had less success in mammals. This summer, Greider and colleagues at Cold Spring Harbor and Geron reported in the Sept. 1 SCIENCE that they had finally found the genes for the RNA component of mouse and human telomerase. Their search for the protein ingredients continues.

As investigators examined the role of telomere shortening in human cells, they made a potentially more significant discovery. Though few normal human cells appear to make the enzyme, human telomerase seems essential to the growth of cancer cells.

That observation emerged from the study of immortal cells, which have escaped the Hayflick limit and can proliferate indefinitely in test tubes. Occasionally, a few genes go awry in a normal cell and eliminate the checks that would drive it into senescence after 50 doublings. Still, cells that avoid senescence generally survive for only another 50 divisions or so before they reach what biologists call crisis and commit a form of cellular suicide.

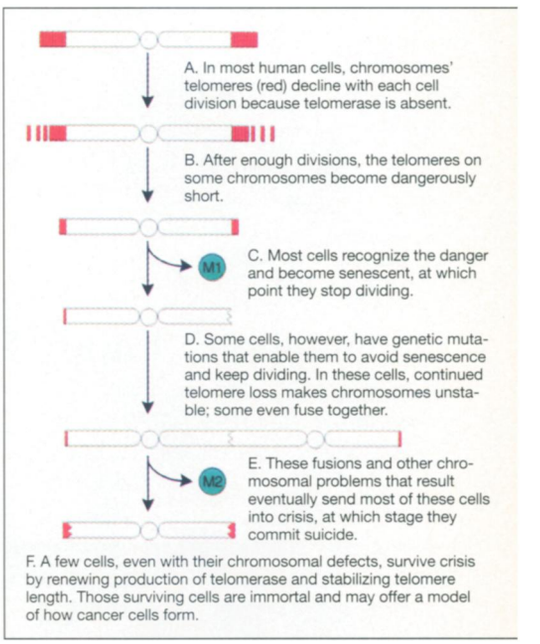
A few cells may weather even this crisis stage and become immortal. But, says Greider, "in order to divide indefinitely, they have to do something about their telomeres."

Last year, evidence emerged that immortal cells maintain their telomere cushions by reactivating the genes that make telomerase. Researchers at Geron and the University of Texas Southwestern Medical Center at Dallas developed a sensitive assay for human telomerase and began testing immortal cells. Of 100 immortal cell populations derived from 18 different human tissues, 98 were positive for telomerase activity, Jerry W. Shay of Texas and his colleagues reported in the Dec. 23, 1994 SCIENCE.

Those results explained why investigators had previously found that telomeres in immortal cells, though typically shorter than those in normal cells, did not dwindle with each subsequent cell division.

Investigators have long tried to forge a link between the immortality of cells in test tubes and the unrestricted growth of cancerous cells in the body. "A lot of people equate tumor cells with immortal cells," notes Greider. That controversial link gained a measure of support from Shay's study. In addition to spotting telomerase in immortal cells, he and his coworkers found that 90 out of 101 biopsies of human tumors showed the presence of telomerase.

Other studies have confirmed this finding. In the March NATURE MEDICINE, for example, the Texas group joined with a team from Japan's Hiroshima University School of Medicine in reporting the presence of telomerase activity in 94 out of 100 cases of neuroblastoma, one of the most common childhood cancers. Furthermore, the amount of telomerase they detected seemed crucial. In some cases where telomerase activity was low or absent, children had spontaneous remissions, says Shay. In contrast, high telomerase activity showed up in the cancers that proved more difficult to treat.



Adapted from JOURNAL OF THE NATIONAL CANCER INSTITUTE

Proposed role of telomeres in human cells.

Shay believes that assaying telomerase activity "may tell surgeons whether they face a more malignant type of cancer." In breast cancer, for example, the amount of telomerase in a biopsy may help patient and physician decide between a lumpectomy or a combination of mastectomy and chemotherapy.

Evidence of telomerase activity in tumor cells also offers an increasingly persuasive argument to many investigators that inhibiting the enzyme will provide a way of slowing or eliminating cancer progression.

Recently, Geron and Cold Spring Harbor investigators tried to inhibit telomerase activity in HeLa cells. Since they knew the nucleotide sequence of the RNA component of the enzyme, the scientists created RNA strands with the opposite sequence. These so-called antisense molecules, they reasoned, would attach to

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the telomerase RNA in the HeLa cells and impede its work, resulting in a shortening of the cells' telomeres. Indeed, after 23 to 28 doublings, more than half of the HeLa cells injected with the antisense went into crisis and died, the collaboration reports in the Sept. 1 SCIENCE.

Few investigators expect that the antisense approach will develop into a practical method of inhibiting telomerase. Instead, they hope to find or create small molecules that also bind to the enzyme and would be easier to develop into drugs.

But, researchers caution, inhibiting telomerase throughout the human body may not be safe. If normal cells depend on the enzyme, the treatment could cause as many problems as the cancer it targets.

In the first studies of telomerase activity in normal human tissues, researchers found that the only tissues that tested clearly positive for the enzyme were the ovaries and testes. That didn't surprise investigators, because those sites presumably contain a reservoir of stem cells that provide the progenitors of sperm and egg cells. These stem cells are long-lived and divide relatively frequently, Shay explains, so they must have found a way to keep making telomerase after most other human cells turn off the necessary genes.

If those so-called germline cells were the only human cells that needed telomerase, says Calvin Harley of Geron, telomerase inhibitors could be an effective cancer treatment. Most people, he argues, would sacrifice their reproductive ability to rid themselves of cancer. Moreover, cancer tends to strike after an individual's reproductive years, he adds.

Recent work by Harley and others, however, establishes that some nongermline cells do in fact contain telomerase, though in much less abundance than tumor cells or germline cells.

"The most simple paradigm—yes in malignant cells and no in normal [nongermline] cells—is not absolutely true," says Richard J. Hodes, director of the National Institute on Aging in Bethesda, Md., "That doesn't mean that there is not an important biological difference between normal and malignant cells that couldn't be capitalized on. But it certainly calls for great caution in presuming one could, in a perfectly specific way, influence malignant cells, but not normal cells, by influencing telomerase activity."

The nongermline cells that appear to use the enzyme come from so-called renewal tissues—blood, skin, and intestinal lining. The body must constantly supply new cells in these tissues, which means the tissues may have evolved means of avoiding the normal limits on cell division.

The renewal tissues probably contain a small reservoir of stem cells that can produce telomerase intermittently, suggests Shay. He believes that telomerase inhibitors, though they could affect those stem cells, may still offer a cancer treatment option that is far less dangerous than traditional chemotherapy, which kills all dividing cells.

Studies in mice over the next year or so should resolve many of the questions about the importance of telomerase in normal mammalian cells. Now that investigators have the gene for the RNA component of mouse telomerase, they can knock it out in mouse embryos and observe whether the animals are born healthy and survive without the enzyme.

"That's a very important experiment. This will settle the whole issue," says Titia de Lange of Rockefeller University in New York City.

Researchers also plan to test whether tumor growth differs between mice that can't make telomerase and those that can, says Greider.

If these efforts do provide a new strategy for combating tumors, or if they simply explain why a cell stops dividing in its old age, it will be a stunning example of the value of basic research, telomere investigators say.

"Telomere research started out as a novelty, with people focusing on these strange organisms you find in ponds," says Morin. "Now, all of a sudden, this backwater field of research is offering a potential cure for cancer."

Environment

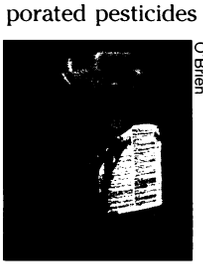
Janet Raloff reports from Vancouver, British Columbia, at the annual meeting of the Society of Toxicology and Environmental Chemistry

Recycling pesticide bottles: A risk?

Manufacturers bottle many toxic pesticides in high-density polyethylene (HDPE). Because such contents can permeate this plastic, labels instruct consumers not to reuse the bottles. Yet people dispose of so many plastic pesticide containers—1 million a year in Canada alone—that waste managers have begun recycling pesticide-impregnated HDPE into building materials, such as fence posts. Researchers at the University of Guelph in Ontario now report that a technique adopted recently to limit pesticide leaching from this plastic "is not as effective as it could be in limiting pesticide penetration."

Manufacturers usually dissolve oily pesticides in a solvent to ease their dispersal. However, the solvent degrades HDPE, helping pesticides enter and leach out of the plastic. To stem the resulting losses from evaporation, pesticide makers have turned to fluorination—where fluorine atoms swap places with hydrogen in the plastic's molecules, forming a solvent barrier.

However, notes Graham M. O'Brien, "the containers we looked at, the industry standard for packaging these pesticide formulations, didn't form a complete barrier." These plastics still incorporated pesticides at "quite high levels"—up to 1 gram of solvent-based herbicide, such as trifluralin or 2,4-D, per 350 grams of plastic. By contrast, HDPE produced by a more stringent, but expensive, fluorination technique allowed one-thousandth or less of that amount to permeate—yielding truly negligible levels.



O'Brien

Orange stains illustrate extensive pesticide (trifluralin) penetration of HDPE container.

Few companies use this more expensive technique for pesticide bottles, he noted, because the old type "appeared to be doing its job"—cutting product losses from evaporation.

Guelph studies published earlier this year found that plastic from recycled pesticide containers can leach detectable, albeit insignificant amounts, of the toxic compounds. However, O'Brien observes, pesticides that are more mobile or more highly concentrated than those studied may still present problems. That's why he argues that only the better-fluorinated pesticide containers should be recycled if any resulting products will be used in watery environments or where extensive human contact can occur.

Another source of lead in kids

Potentially toxic amounts of lead can leach from good crystal into any drink it holds (SN: 1/26/91, p. 54). But the heavy metal poses its biggest risk to children, who don't tend to sip their milk, juice, and sodas from crystal goblets. So Charles V. Shorten and Mary L. Glowacki of West Chester (Pa.) University asked whether some other use of crystal, such as vinegar stored in crystal cruets, might add lead to children's diets.

Their 42-day study of 13 different cruets showed that lead's passage into the acidic liquid started quite rapidly, reaching an average of 162 micrograms per liter (μg/l) within the first hour. By the end of the trial, lead concentrations in the vinegar had climbed to an average of 730 μg/l, or 8 times the level achieved in distilled water. These data suggest that a 16-kilogram child could thus acquire 15 percent of the federal government's provisional daily tolerable intake from all sources.