

Chromosome Ends Catch Fire

The limited life span of normal cells and the ability of cancer cells to transcend it may both depend on the telomeres, the structures that cap the chromosomes

To a living cell, few jobs are more important than protecting the integrity of its chromosomes. When these libraries of genetic information are damaged, the cell's own survival is in jeopardy, to say nothing of its progeny's. Ever since the work of pioneering geneticists Hermann Muller and Barbara McClintock more than 50 years ago, cell biologists have believed that at least part of the job of chromosomal protection falls to their ends—the so-called telomeres. McClintock's work suggested, for example, that the telomeres keep chromosomes from fusing end-to-end, which could lead to chromosome breakage and loss as cells divide.

That might seem a big enough assignment in the life of a cell. But a recent spate of research has indicated that telomeres, now known to consist of short repeated DNA sequences plus associated proteins, play many additional roles that touch on both the normal control of cell proliferation and the abnormal growth of cancer. "From both the scientific and practical standpoint, there's been a huge amount of progress," says Virginia Zakian of the Fred Hutchinson Cancer Research Center in Seattle, whose own work focuses on yeast telomeres.

The newer roles being assigned to telomeres include aiding in gene regulation and possibly serving as a "mitotic clock" for the cells of higher animals. Researchers have shown that the telomeres shorten slightly every time the chromosomes replicate in preparation for cell division, suggesting that cells become senescent and die when the telomeres have shortened beyond a certain point. That shortening takes place because most normal cells apparently do not make telomerase, the special enzyme needed to synthesize telomeres. But in cancer cells, telomerase synthesis somehow becomes reactivated, an event that may contribute to the cells' ability to divide continually. This finding, reported earlier this year by Calvin Harley, Silvia Bacchetti, and their colleagues at McMaster University in Hamilton, Ontario, suggests that telomerase may be a good target for anti-cancer drugs.

These findings mark the arrival of telomere research into the broad mainstream of biomedical research. When the field opened

up in the 1970s, researchers studied telomeres in protozoan ciliates, single-celled organisms that propel themselves with hairlike projections called cilia, rather than in mammalian cells. At the time, ciliates were much easier to work with because they have many more telomeres per cell than do mammalian cells. These organisms have two nuclei, and during the formation of the larger of these, the so-called macronucleus, the chromosomes break up into fragments that then replicate, producing from 20,000 to as many as 10 million pieces of DNA, each of which becomes capped at both ends by telomeres. In contrast, a human cell has but 92 telomeres, two for each of the 46 chromosomes.

The ciliates quickly began yielding surprises. Elizabeth Blackburn of the University of California, San Francisco, recalls that when she isolated the first telomere from the ciliate *Tetrahymena thermophila* in the early 1970s, "I realized [immediately] that there

TG-rich sequences repeating over and over. And as telomere researchers began broadening their view to include other kinds of organisms, they began accumulating evidence that these special structures do indeed help to maintain the chromosomes, just as McClintock's work had suggested. In the early 1980s, for example, Blackburn, working with Jack Szostak at the Dana-Farber Cancer Institute in Boston, showed that DNA strands equipped with *Tetrahymena* telomeres survive inside yeast cells; ordinarily, yeast cells quickly degrade foreign DNA. And Zakian and her colleagues obtained similar findings with telomeres from another ciliate, *Oxytricha fallax*.

A pleasing resemblance

These yeast experiments helped allay one worry: that the telomeric sequences detected on the chromosome fragments in the ciliate macronucleus might not be at all representative of the telomeres of the more standard chromosomes of other organisms. "The ciliates are a great paradigm," Zakian says, "but there's always the concern that they are abnormal." But the finding that ciliate telomeres protect DNA in yeast, as they apparently do in the ciliates themselves, suggests that telomeres must have been highly conserved during evolution, Blackburn says. Yeast and *Tetrahymena* are very distantly related; they are not even members of the same kingdom.

Confirmation of just how conserved telomeres are came later, in 1988, when Robert Moysis and his colleagues at Los Alamos National Laboratory isolated the first human telomeres and showed that they also consist of a repeating sequence, TTAGGG (where A stands for the base adenine). And since then researchers have found the same repeating sequence at the chromosome tips in every vertebrate they have examined.

As the yeast work progressed, it showed that telomeres not only prevent the immediate degradation or loss of the chromosomes, but they also protect chromosomes in a more subtle way. By extending the ends of the chromosomes with repetitive, non-coding DNA, they prevent the gradual loss of genetic information that would otherwise



A shade of difference. As the cells in the right yeast colony divided, they lost their red color as the result of the loss of a chromosome made unstable by removal of a telomere. Normal colony is at left.

was something quite peculiar about these molecular regions." The work, which was done while she was a postdoc in Joseph Gall's laboratory at Yale University, showed that the *Tetrahymena* telomere consists of a short sequence, TTGGGG (where T and G denote thymine and guanine, two of the four bases whose sequences spell out the genetic information in DNA), repeated some 50 to 70 times. The structure of the telomeric DNA came as a surprise because at the time the only DNAs that had been examined closely came from viruses and bacteria, which do not have such repeated sequences.

But for telomeres "peculiar" is in fact normal. Researchers soon found that the telomeres of other ciliates also consist of short

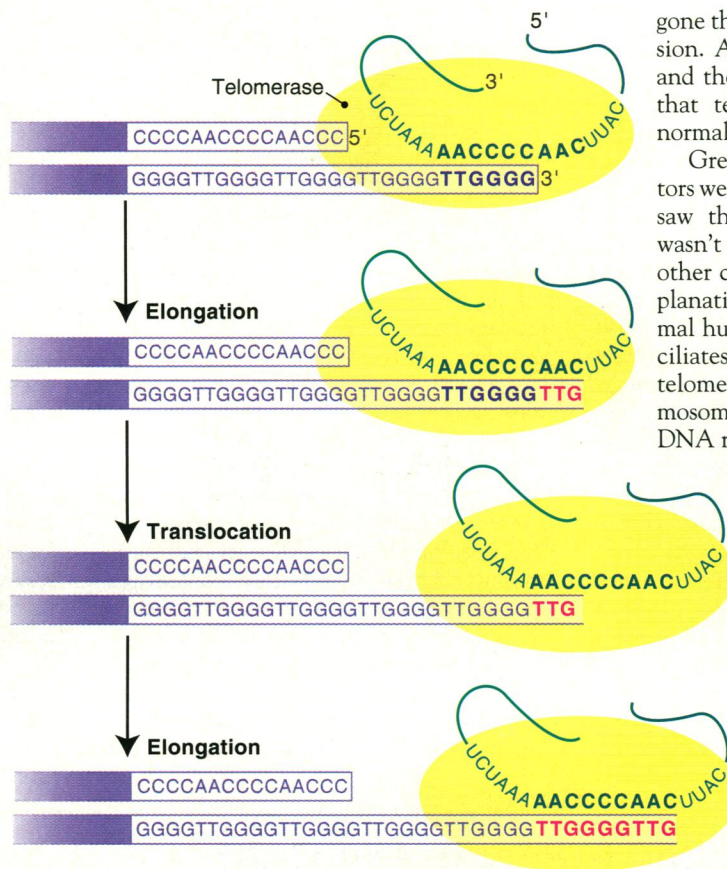
result from a quirk in the way DNA is replicated: The polymerase enzyme that copies the DNA can't reproduce both strands of the double-helical molecule all the way to the ends. As a result, chromosomes would get progressively shorter with every cell division—and essential genes would gradually be eroded. And in the lower organisms, at least, this doesn't happen because the cells can add telomeric DNA to the incompletely replicated ends of the chromosomes.

The yeast work showed that when linear DNAs tipped with ciliate telomere sequences are put into yeast cells, they subsequently acquire yeast telomere sequences. This indicated that the yeast was able to add sequences *de novo* to chromosomes, says Carol Greider of Cold Spring Harbor Laboratory, who was then working with Blackburn as a graduate student. To find the enzyme that might be tacking on these additional sequences, Greider and Blackburn again turned to the ciliate *Tetrahymena* because, with its horde of telomeres, it was likely to be a much richer source of the enzyme than yeast. By the mid-1980s they had succeeded. "We found telomerase," Greider says, "but it was hard to believe at first because it was such an unusual polymerase."

A most unusual enzyme

Indeed, telomerase is unlike nearly all other enzymes researchers have studied. It contains an essential RNA component in addition to the expected protein. Subsequent work by the Blackburn group showed that the RNA serves as the template for synthesizing the telomere repeats—and it may even play a key role in the enzyme's chemical activity. Mutations in the RNA alter the enzymatic properties of telomerase, says Blackburn, a finding that led the group to speculate that "the RNA and the protein may be collaborating in forming the active site [the part of the enzyme that carries out the catalysis]." Because RNA is thought to have played a dual role as both enzyme and repository of genetic information in a primordial "RNA world," the collaboration of an RNA with a protein in the telomerase suggests to Blackburn that the enzyme might be an intermediate in the evolution from the RNA world to the current world of DNA and protein.

Blackburn is the first to point out that that intriguing idea is strictly speculative. But it's not the only reason telomerase is attracting attention: It may play a role in overriding the processes that determine the proliferative life span of mammalian cells. Researchers have known since the work of



Adding the ends. Telomerase RNA binds to the DNA strand, where it serves as a template for addition of the telomeric repeats, sliding along the strand it is elongating. Another cell polymerase then synthesizes the second strand.

Leonard Hayflick in the 1960s that normal cells have a limited life span in culture, with cells taken from younger individuals dividing more times before they become senescent and die than do cells from older individuals. An indication that the telomeres might set this cellular clock came in the Blackburn group's experiments on the effect of mutations in the RNA of *Tetrahymena* telomerase. "One particular mutation changed the activity so badly that the telomeres got shorter and shorter until the cells died," she says. The presence of telomerase in the ciliates may thus make these unicellular organisms immortal by allowing them to keep replenishing the DNA that would otherwise be lost every time the cells divide.

But as Hayflick's experiments showed, normal mammalian cells are not immortal. And several research teams have found evidence that their limited life span may be due to telomere shortening. For example, Howard Cooke of the Medical Research Council's Human Genetics Unit at Western General Hospital in Edinburgh, Scotland, and teams led by Titia de Lange of Rockefeller University and by Robin Allshire and Nicholas Hastie, also in Edinburgh, found that human sperm cells—whose clocks have just started ticking—have much longer telomeres than ordinary tissue cells that have

gone through multiple rounds of division. And work by Greider, Harley, and their colleagues showed directly that telomeres shorten every time normal cells divide in culture.

Greider notes that the investigators were initially surprised when they saw this shortening, "because that wasn't seen in *Tetrahymena* and the other ciliates." The mostly likely explanation for this, she says, is that normal human cells, unlike those of the ciliates, seem to lack a functional telomerase that can replace the chromosome ends lost to incomplete DNA replication.

Exactly what might cause cells to stop dividing and die when their telomeres get too short is unclear, but recent results point to a couple of possibilities. Zakian and her graduate student Lisa Sandell, for example, found that removing a single telomere from a yeast chromosome temporarily blocked cell division, and that this effect depends on the activity of the RAD9 gene. Because RAD9 halts the growth of cells whose DNA has been damaged, allowing for repairs, the re-

sult suggests that telomeres may be part of the cell's damage sensing system.

Other groups, meanwhile, have evidence that loss of telomere DNA may lead to activation of the *p53* tumor suppressor gene, which serves the same function in mammalian cells as RAD9 does in yeast: arresting cell growth in response to DNA damage. Another possibility is suggested by work from researchers, including Zakian and Daniel Gottschling of the University of Chicago, who have shown that intact telomeres somehow repress the activity of nearby genes. Some of those genes might trigger cell death once the telomeres become short enough to allow their expression.

Immortal cells

But none of this means that it's good for mammalian cells to preserve their telomeres. Indeed, just the opposite may be true. Evidence is now accumulating that the presence of telomerase in cells that normally lack it may contribute to the uncontrolled cell growth of cancer. One hint that this may be the case is the fact that the human enzyme was first detected, by Gregg Morin of the University of California, Davis, in HeLa cells, a cultured cell line originally derived from a human cervical cancer. Following up on that 1989 observation, Harley, along with

McMaster's Bacchetti and Cold Spring Harbor's Greider, decided to see whether telomerase activation might allow cultured cells to escape the normal limits on their growth and become "immortalized." And it did.

When the researchers tried to mimic the tumor-forming process by introducing oncogenes from simian virus 40 or an adenovirus into cultured cells, they found that telomerase activation was a good predictor of immortality. The oncogenes stimulated cell growth, as expected, and most cells' telomeres continued to shorten with each division until the cells died. But some survived and continued to divide—and those cells, Harley says, had an active telomerase, which presumably stabilized their telomeres. Exactly what allowed the cells to make telomerase is unclear, although the Harley-Bacchetti group detected numerous abnormal chromosomes in cells that had extremely short telomeres. These chromosomal abnormalities, which are similar to those commonly seen in cancer cells, may have led to mutations in the telomerase gene and in other genes that contribute to tumor malignancy. "When cells lose telomeric DNA, it

may open the window for all kinds of horrible things to happen," says Zakian, who has noted similar chromosome abnormalities in her yeast experiments.

More recently, Harley, Bacchetti, and their colleagues have turned their attention to cells taken directly from cancerous tumors. They reported in the 14 April *Proceedings of the National Academy of Sciences* that telomerase is active in ovarian cancer cells, although not in normal ovarian tissue. The researchers have since gone on to survey telomerase activity in a variety of additional human cancers, and the preliminary results look promising.

Those findings, together with the signs that the enzyme is not active in normal cells, make it an important new target for cancer drugs. "We think [telomerase] will be a specific and probably universal target in tumorigenesis," says Harley, who last year moved to the biotech firm Geron Inc., in Menlo Park, California, to work on developing inhibitors of the enzyme. The hope is that if telomerase activity can be blocked, cancer cells won't be able to maintain adequate telomere length and will die. This

may be easier said than done, however.

For one thing, researchers still have much to learn about telomerase. So far, they have not been able to isolate the protein portion from any organism, and they have obtained the RNA part only from ciliates. That may soon change, however. Both Blackburn and Gottschling have described candidates for yeast telomerase RNAs at meetings. And a yeast gene identified by Szostak and Victoria Lundblad, who's now at Baylor College of Medicine in Houston, may encode the protein component of yeast telomerase. Greider's group also has a candidate telomerase protein, although in this case from a ciliate.

Having the complete enzyme may aid researchers in designing inhibitors, but whether they will actually kill cancer cells remains to be established. Blackburn finds, for example, that some yeast cells are able to survive without telomerase. "There is life without telomerase," as she puts it. That may be true for cells, but for many researchers, the enzyme—along with the telomeres it synthesizes—is now an essential part of their scientific lives.

—Jean Marx

ASTRONOMY

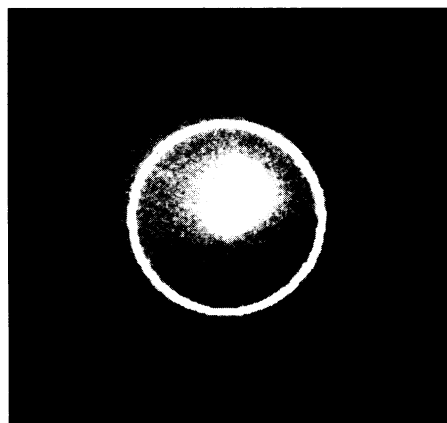
How Comets Stay Frisky in the Cold

Most comets spend nearly all their lives as inert lumps of ice and dust, enjoying brief bursts of activity only when they enter the inner solar system. Like animals emerging from hibernation, they are energized by the sun's heat, which turns their water ice to vapor in a process called sublimation. The vapor in turn blows dust and other gases outward to form a halolike coma and, often, a magnificent tail. When a comet swings back out among the outer planets, it usually goes back into hibernation.

Not always, though. In the far reaches of the solar system, where the temperatures are too low for ice to sublimate, some comets, including Halley's, experience brief flare-ups of activity, while others, such as Schwassmann-Wachmann 1 (SW1), sport persistent comae. Now, Matthew Senay and David Jewitt of the University of Hawaii in Manoa think they know one reason why. In yesterday's issue of *Nature*, the pair reports detecting radio emission from carbon monoxide (CO) molecules in SW1, which orbits well beyond Jupiter. Because solid CO sublimates at a much lower temperature than ice, they say, it could be what keeps some comets lively in the cold.

Astronomers had already guessed that the puzzling activity of distant comets is driven by the sublimation of molecules more volatile than water. "But up to now, we had no direct evidence as to what these molecules could be," says comet expert Jacques Cro-

visier of the Observatoire de Paris-Meudon in France. And there seemed to be little hope of finding out: In the cold, dim environment of the outer solar system, the radio emissions characteristic of the gas molecules would probably be so faint as to be undetectable.



Gas-powered. Dust envelops comet SW1, driven by the CO detected by radio observations (circle indicates the telescope's view).

But the quantity of gas ejected by SW1 made up for the feeble signal. Using the 15-meter James Clerk Maxwell Telescope on Mauna Kea in Hawaii, Senay and Jewitt were able to pick up submillimeter radio emissions from SW1 at a frequency characteristic of CO. Based on the strength of this spectral line, Senay and Jewitt estimate that the com-

et is emitting CO at a rate of about 2 tons per second. "This is remarkable," says Jewitt, "because it is comparable to the outgassing rates of comets [in the inner solar system]." The style of outgassing is comparable, too. A slight Doppler shift in the line, the researchers believe, implies that the gas is spewing out of SW1 in a jet like those produced by water vapor in comets closer to the sun.

To pin down the link between CO and the comet's visible activity, Senay and Jewitt measured the width of the CO line with the 10-meter submillimeter telescope at the California Institute of Technology. The line width indicates how fast molecules are darting around within the gas cloud surrounding the comet—and therefore how fast the cloud is expanding. The result agreed well with the observed expansion of SW1's dust coma. With all these pieces falling into place, says Crovisier, "we know that CO is at least one" of the gases driving distant activity.

The two researchers stress that their findings don't rule out a role for other highly volatile molecules such as nitrogen. But Crovisier notes that "we have no way to detect nitrogen at the present time," because its spectral fingerprint would be masked by those of other gases. For now, Senay and Jewitt are planning to study several other distant comets to see whether they, too, are gassed up by CO.

—Ray Jayawardhana

Ray Jayawardhana is a science writer based in Cambridge, Massachusetts.