From small beginnings, research on telomeres—the specialized structures at the chromosome ends—has exploded into the biotech realm

Chromosome End Game Draws a Crowd

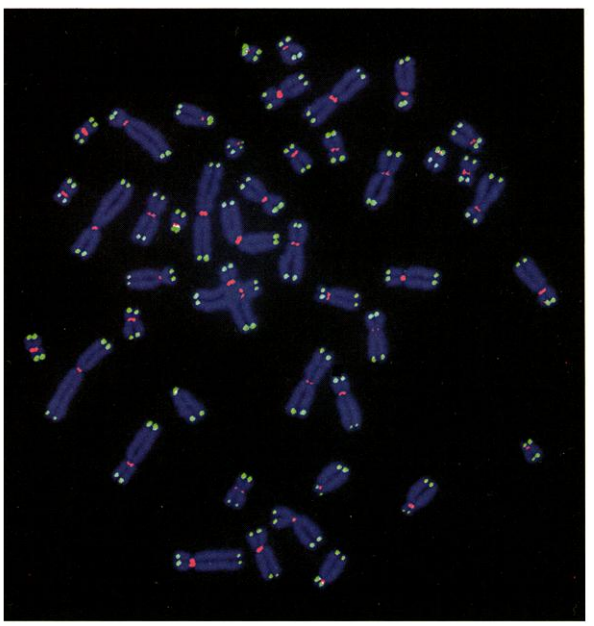
Even to a biologist, the tiny one-celled creature known as *Tetrahymena* can seem a bit odd. A ciliate, so called because it's propelled by hairlike projections called cilia, the single-celled *Tetrahymena* has not one but two nuclei. And the ciliate life cycle includes a phase during which the chromosomes of the larger of these two nuclei fragment into thousands or even millions of pieces, depending on the species. It was the latter quirk that caught the attention of cell biologist Elizabeth Blackburn.

In the mid-1970s, Blackburn embarked on a seemingly esoteric study of the ends of chromosomes—of which *Tetrahymena* and other ciliates offer an abundance. Now Blackburn and *Tetrahymena* find themselves center stage in one of the hottest areas in both biotechnology and basic cell biology: research into telomeres—the specialized structures that cap chromosome ends.

Telomeres perform the crucial job of protecting the chromosomes from erosion during cell division. Without them, essential genetic information would be lost. But telomeres are much more than mere passive caps: Researchers have linked them to both aging and cancer. Not surprisingly, these connections have attracted a broad range of researchers—among them an unusually large contingent of women who have emerged as leaders in the field. Telomeres have also enticed a slew of biotech and pharmaceutical firms eager to cash in on antiaging and anticancer drugs, some of which are moving into clinical trials (see sidebar, p. 2350).

It's a far cry from the field's beginnings, says Blackburn, who won the 2001 General Motors cancer award in basic science for her pioneering work on telomeres. Even as recently as the late 1980s, a meeting on, say, chromosomes might have a single session on the topic. Blackburn, now at the University of California (UC), San Francisco, recalls usually being the last speaker in the last session at Gordon conferences. Now "we have whole meetings on telomeres," she says.

The big influx of researchers has upped the level of competition, notes Carol Greider of Johns Hopkins University School of Medicine in Baltimore, Maryland, who entered the field in the mid-1980s as a graduate student in Blackburn's lab. "In the early days, we could think of an experiment and get a graduate student to do it," she says.



Hot spots. The green staining marks the telomeres on these human chromosomes.

CREDIT: J. W. SHAY AND W. E. WRIGHT; S. GRYAZNOV/GERON CORP.; Y. ZOU

"Now we have to worry about whether a company will put 10 postdocs on [the project] and scoop us." Even so, Greider and others say that having more competitors is, on balance, beneficial.

A slow start

Telomeres have long had their followers. Decades ago, they piqued the interest of Nobel Prize–winning geneticists Barbara McClintock and Hermann J. Muller. Their work in the 1930s and 1940s indicated that telomeres prevent chromosomes from fusing end to end, an event that would have catastrophic consequences for the cell. But telomeres were hard to study directly, mainly because the material is so scanty. Human cells, for example, have just 92 telomeres—one on each end of their 46 chromosomes.

Consequently, telomeres remained little more than a curiosity until Blackburn took up the cause in the mid-1970s. She had just moved to the lab of Joseph Gall, then at Yale University, after completing a Ph.D. with Fred Sanger at the University of Cambridge, U.K. Sanger was a leader in efforts to develop DNA sequencing methods, and Blackburn and Gall were eager to try them out.

Telomere DNA was a good target because it could be labeled and thus easily isolated. That's when *Tetrahymena,* with its numerous minichromosomes and rapid life cycle, first entered the picture. "Joe knew the right beast to use," Blackburn recalls. Indeed, *Tetrahymena* and other simple organisms proved to be central to telomere research. "Almost all the major advances in the earlier studies have been based on *Tetrahymena* and yeast," says Jerry Shay of the University of Texas Southwestern Medical Center in Dallas.

Gall gets credit for influencing the field in another way, too. "For whatever reason, when there were few women in science, his lab always had a substantial number," says Virginia Zakian of Princeton University. And as those women established their own labs, they attracted more to the field. "There were a fairly large number of women to start with, and they spawned more," says Greider.

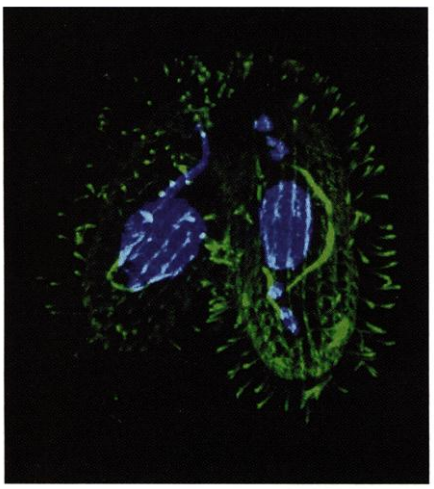
Blackburn puts the proportion of women at 50%. "This is a field that doesn't have a gender bias," says Vicki Lundblad, a prominent telomere researcher at Baylor College of Medicine in Houston. Few other research areas can claim such a high representation of women among their leaders today, let alone 15 to 20 years ago. "Did it help to have women who were mentors? I hope it did," says Blackburn.

The search for telomerase

As Blackburn and Gall laboriously sequenced *Tetrahymena* telomere DNA, they found that it was "very strange," recalls Blackburn. Unlike the few viral genomes then sequenced, it consisted of a short sequence—the six nu-

cleotides CCCCAA—repeated some 20 to 70 times. These researchers and others soon realized that the DNA repeats and length variation are a telomere trademark. This is true for telomeres of the yeast *Saccharomyces cerevisiae*, as Blackburn, who had by then joined the faculty at UC Berkeley, and Jack Szostak at Harvard's Dana-Farber Cancer Institute in Boston found in 1984. And in 1988, Robert Moyzis and colleagues at Los Alamos National Laboratory showed that human telomeres consist of a six-nucleotide repeat: TTAGGG.

Still, no one knew exactly what telomeres did until 1982, when Blackburn and Szostak confirmed McClintock and Muller's proposal that the telomeres protect the chromosomes. Normally, foreign DNA introduced into yeast is rapidly broken down. But Blackburn and Szostak found that they could prevent that by first attaching *Tetrahymena* telomeres to the ends of the foreign DNA. Zakian, then at the Fred Hutchinson Cancer Research Center in Seattle, Washington, obtained a similar result with telomere DNA from another ciliate, *Oxytricha fallax*.



Good starters. Studies of *Tetrahymena*, two of which are shown here, touched off the telomerase field.

And both teams found that while in yeast, the ciliate telomeres grew longer by adding yeast telomeric sequences. "Yeast has the ability to add its DNA to something that looks like a telomere," Blackburn says. "That got us to thinking that there must be a [telomere-synthesizing] enzyme."

Blackburn, by now a tenured professor at UC Berkeley, set out to find it. In 1984, Greider, a new grad student, began searching for the enzyme, now known as telomerase, in extracts of *Tetrahymena* cells. She picked up the first signs of the enzyme's existence on Christmas day 1984. One of her gels showed signs of a ladder of telomeric repeats that had been added to a telomere by the elusive enzyme. "I was very excited but immediately started thinking of what could give me a result that *looked* like telomerase but was really something else," Greider says. Not until spring did she confirm that she had spotted the activity of a new enzyme that synthesizes telomere DNA and not, for example, a known DNA polymerase.

One sign that Greider had the right enzyme was her discovery that, unlike ordinary polymerases, telomerase contains an RNA component that is necessary for the enzyme's activity. "The last part of Carol's thesis was to nail down that RNA," says Blackburn. Greider did, showing that it is the template from which those short repeated telomere sequences are copied.

As Blackburn credits Greider, others salute Blackburn's vision. Thomas Cech, head of the Howard Hughes Medical Institute in Chevy Chase, Maryland, says, "She was so far ahead of her time, that it was years before anyone knew if [telomere synthesis] was just something that *Tetrahymena* did or would have broader significance." Lundblad agrees: "The field was really dominated by Liz Blackburn's insights. She provided the molecular framework—what a telomere looks like—and then discovered telomerase with Greider."

New fields to conquer

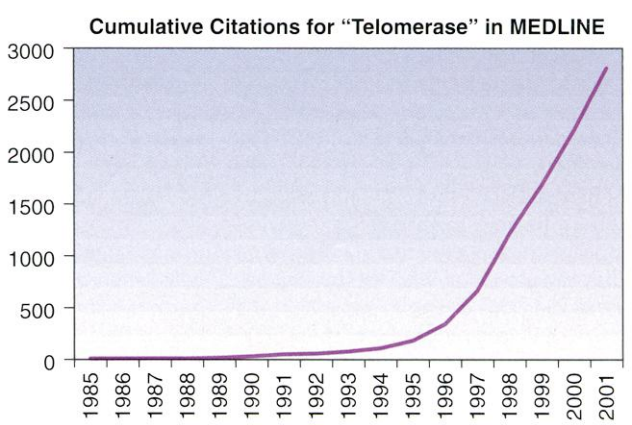
Identification of telomerase provided a solution to a long-standing mystery in cell biology—the so-called end-replication problem. Researchers had known for decades that the polymerase that replicates the DNA before cells divide can't make it all the way to the end of the strand it's copying. Without an enzyme such as telomerase, the DNA would erode with every cell division. Indeed, Titia de Lange, now at Rockefeller University in New York City, says that discovery helped attract her to the field in the early 1980s. "It was intriguing that there was a solution [in telomerase]," she recalls.

Within a few more years, researchers turned up even more attention-grabbing results—forging the first links between telomeres and aging. Some of this work came from Lundblad, then in Szostak's lab.

Ordinarily, cultures of yeast and other one-celled organisms can divide indefinitely. But these researchers identified a mutant yeast, called *est1* (for *ever shorter telomeres 1*), in which the telomeres progressively shortened and which also eventually stopped dividing, undergoing what is called replicative senescence. "If you knock out telomere replication, you confer senescence," Lundblad says. Shortly thereafter, Blackburn's group demonstrated that tinkering with telomere structure also leads to senescence in *Tetrahymena*. "This told us cells need telomerase for their normal maintenance," Blackburn says.

Other researchers, meanwhile, were extending this work to humans. For example, in the 1960s, Leonard Hayflick, then at the Wistar Institute in Philadelphia, had found that human cells, unlike those of one-celled organisms, have a limited capacity to divide in culture. After some 35 to 50 division cycles, they stop, going into replicative senescence. Greider, then at Cold Spring Harbor Laboratory on Long Island; Cal Harley, then at McMaster University in Hamilton, Ontario; and several colleagues wanted to know why. The answer seemed to lie in the telomeres, which they found to shorten with every division of normal human cells—an indication that they provide the clock that tells cells they are old and it's time to stop replicating. In these cells, the telomeres effectively sacrifice themselves to protect the genetic information, but ultimately get so short that division ceases.

An even more exciting discovery came in the early 1990s, when researchers—including Greider, Harley, Silvia Bacchetti, also at McMaster, and Shay and his Southwestern colleague Woodring Wright—linked telomerase to cancer. They showed that while the enzyme is not active in normal cells—thus allowing progressive telomere shortening—the gene for the enzyme somehow gets turned back on in cancer cells. Indeed, the enzyme is active in up to 90% of human cancers. That makes telomerase "an important target for cancer therapy," says cancer researcher Robert Weinberg of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts.



Exactly how the gene making telomerase gets turned back on in cancer cells isn't clear, although work by Shay and Wright provides some clues. On their route to becoming cancerous, cells first have to overcome the block to cell division normally imposed by short telomeres. And they can do this, the

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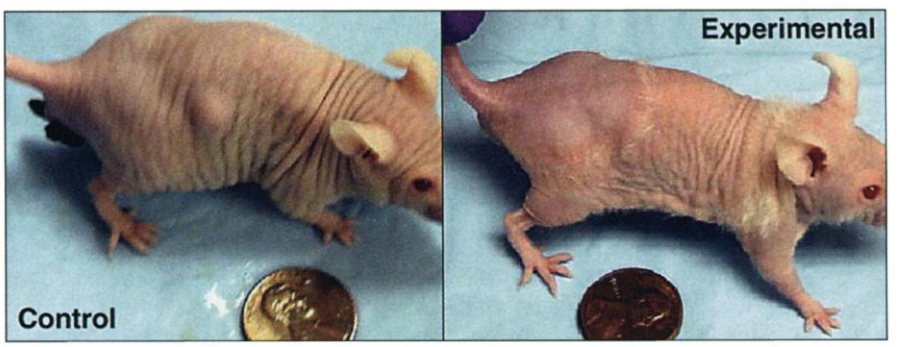
Tackling Cancer at the Telomeres

Telomeres, the end bits of chromosomes, can pull off some amazing feats. Not only do they protect the genetic material from loss during cell division, they also serve as a "mitotic clock," telling cells when it's time to stop dividing and go into senescence. And now, findings about how telomeres are maintained may point the way to new therapies to fight cancer.

One of many discoveries about telomeres over the past few decades (see main text) is that telomerase, the enzyme that synthesizes the telomere DNA, helps drive the growth of cancer cells. Because telomerase is not present in most normal cells, the enzyme could be an attractive target for therapies aimed at halting tumor growth. "We are really excited," says Murray Robinson of Amgen Inc. in Thousand Oaks, California, one of the companies trying to develop such therapies. Cancer therapies—if they can be developed—are likely years away, however.

Researchers are experimenting with two basic strategies. One aims to block the enzyme's activity, and the other uses telomerase as a tag to elicit cancer cell killing in other ways—for example, by enlisting the immune system to destroy the cells that make it.



Improvement. A human tumor implanted in a mouse and treated with a telomerase inhibitor plus an angiogenesis inhibitor (*right*) shrinks more than one treated with the angiogenesis inhibitor alone (*left*).

As with most searches for new pharmaceuticals, the gold standard would be a small molecule that is easy to make and administer. At first, researchers were optimistic, because telomerase is a reverse transcriptase, an enzyme that copies RNA into DNA. A number of small-molecule inhibitors have been developed against the AIDS virus's reverse transcriptase. But finding one for telomerase has proved harder than expected. Cal Harley of Geron Corp. in Menlo Park, California, recalls that in 1994, "I predicted that we would be in [clinical] trials in 3 to 4 years."

Although no small-molecule drugs have made it into clinical trials yet, at least one candidate is on the horizon: an inhibitor described in the 17 December issue of *EMBO Journal* by Klaus Damm of Boehringer Ingelheim Pharma KG in Biberach, Germany, and colleagues. In tests with human cancer cells in culture, the inhibitor led to telomere shortening and eventually caused the cells to stop dividing. It also inhibited the growth of human tumors transplanted into animals, without any apparent toxic side effects.

The Boehringer team's drug is directed at the catalytic protein of telomerase; other groups are targeting the enzyme's RNA, which serves as the template for the reverse transcriptase. Some, such as Seiji Kondo of Mount Sinai School of Medicine in New York City and his colleagues, are using an antisense approach to disable the RNA. This involves making an RNA whose sequence is complementary (or antisense) to that of human telomerase RNA and will thus bind to it, blocking its action. To be sure to take the telomerase RNA out of action, the Kondo team also attached the antisense RNA to another molecule that can attract an RNA-destroying enzyme. In test tube and animal studies, this construct can inhibit the growth of malignant gliomas—a particularly deadly type of brain cancer—says Kondo.

In a similar vein, Geron has developed a small modified nucleic acid that specifically binds to telomerase RNA near the active site of the enzyme. Harley says that this molecule has proved very effective in test tube and animal tests, and "assuming all goes well, we will have it in clinical trials in 2003."

All telomerase inhibitors have a possible drawback, however: They kill slowly, because tumor cells will continue to divide until the telomeres progressively shorten and trigger cell suicide. Several labs are exploring telomere-based immunotherapies that can kill much more quickly.

All cells display on their surfaces fragments of the proteins they make. This helps "train" the body's immune cells not to attack its own tissues. Robert Vonderheide, then working with Lee Nadler at the Dana-Farber Cancer Institute in Boston, found in 1999 that telomerase-making cancer cells carry fragments of the enzyme on their surfaces and that these can be recognized in cell culture by appropriately activated T cells. Indeed, because telomerase is present in the cells of as many as 90% of human cancers, it may come close to being the long-sought "universal tumor antigen," says Vonderheide.

The trick is to get patients' immune cells to recognize the telomerase fragments on tumor cells. One way to do this is to feed an antigenic telomerase peptide to a patient's dendritic cells in culture. Dendritic cells trigger immune responses by "presenting" antigens to T cells. When the cells exposed to the telomerase peptide are then infused back into the patient, they can thus spark an immune attack on telomerase-bearing tumor cells, says Vonderheide, who is now at the Abramson Family Cancer Research Institute of the University of Pennsylvania in Philadelphia.

Johannes Vieweg, Eli Gilboa, and colleagues at Duke University Medical Center in Durham, North Carolina, are using a similar approach: They introduce an RNA that directs telomerase synthesis into the dendritic cells in culture. These two teams and another led by Steven Rosenberg at the National Cancer Institute in Bethesda, Maryland, are beginning clinical trials of antitelomerase immunotherapy.

Another telomerase-based therapy that is being explored by several teams, including Kondo's and Harley's, aims to kill cancer cells by introducing genes encoding toxic proteins. Kondo and his colleagues are trying a gene encoding a caspase, an enzyme that brings about cell destruction. To ensure that the caspase is made only in cancer cells, the researchers attach the caspase gene to a regulatory sequence from the telomerase gene. The idea is that this compound gene will be turned on in cancer cells, just as the telomerase gene itself is. Studies with cells in culture and animal tumor models have been encouraging, Kondo reports.

Still, a lingering concern is that both the immunotherapies and those involving the toxic genes could harm tissues other than their tumor targets. Although most cells don't make telomerase, stem cells, such as those in the bone marrow and the lining of the gastrointestinal tract, do. Researchers suspect they can get around that problem, but the safety and efficacy of telomerase-based therapies remain to be established.

–J.M.

CREDITS: SHAY-WRIGHT LAB/UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

Texas scientists found, if they have also lost active copies of the *p53* and *Rb* tumor suppressor genes—mutations known to contribute to cancer development.

Pioneer. Elizabeth Blackburn is still a leader in telomere research.

The telomeres of cells that then continue to divide get progressively shorter, leading to still more chromosomal abnormalities, apparently including, in rare cases, reactivation of the telomerase gene. And when that happens, the cells can keep on growing, forming a cancerous tumor.

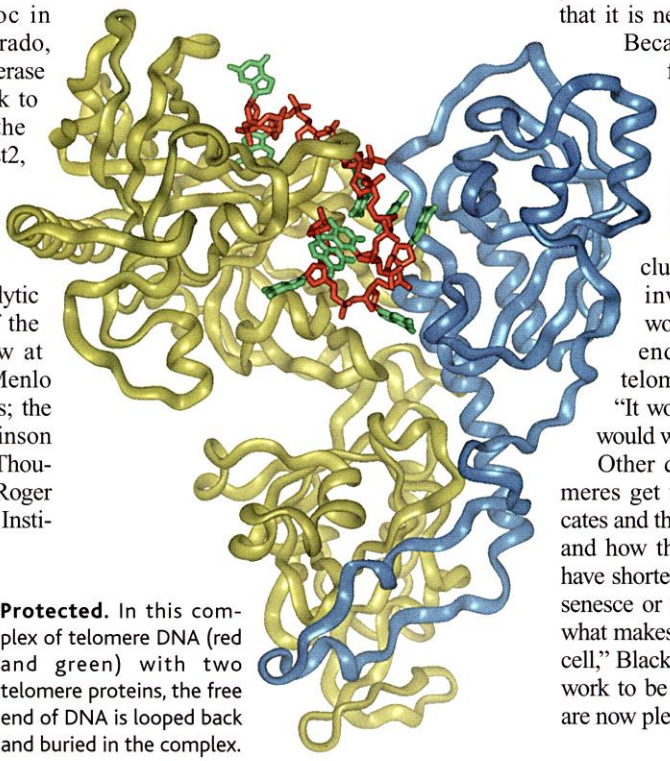
In the wake of these discoveries, the telomere field exploded. When Greider plotted the number of papers mentioning the word "telomerase," she found a dramatic increase beginning in 1994, and the plot is still going up (see graph, p. 2349). The discoveries also gave increased impetus to the search for the gene for the catalytic protein of telomerase, which was still at large, a situation that was a handicap to a full understanding of how the enzyme works—not to mention to efforts to develop inhibitors for use in cancer therapy. A ciliate, this time *Euplotes aediculatus*, again provided the first opening.

Joachim Lingner, then a postdoc in Cech's lab at the University of Colorado, Boulder, was able to isolate the telomerase protein from *Euplotes* in 1996. A link to yeast came with the discovery that the protein is the *Euplotes* equivalent of Est2, a protein made by a gene that Lundblad had identified in yeast as being necessary to maintain telomere length. By 1997, four groups had cloned the gene for the telomerase catalytic protein (also called hTERT). One of the groups included Cech, Harley—now at Geron Corp., a biotech company in Menlo Park, California—and their colleagues; the other three were led by Murray Robinson and Lea Harrington of Amgen Inc. in Thousand Oaks, California, Weinberg, and Roger Reddel of Children's Medical Research Institute in Sydney, Australia.

Since then, researchers have strengthened the link between telomerase and both aging and cancer. For example, in 1998, Shay, Wright, and their colleagues showed that they could extend the replicative life-span of human cells in culture by introducing the cloned *hTERT* gene. In addition, independent teams, one led by Kathleen Collins of UC Berkeley and the other by Tom Vulliamy at Hammersmith Hospital in London, have found that mutations that reduce, but don't eliminate, telomerase activity cause some cases of dyskeratosis congenita, a rare genetic disease whose symptoms include some that are consistent with premature aging. Blackburn describes the work as "fascinating. It argues that for a normal human life-span, [telomerase] dose is important."

And on the cancer front, Weinberg, William Hahn of the Dana-Farber Cancer Institute, Christopher Counter of Duke University Medical Center in Durham, North Carolina, and their colleagues reported in the 29 July 1999 issue of *Nature* that they could make human cells cancerous by introducing just a few genes: the T antigens of simian virus 40, which interfere with the tumor-suppressing activities of *p53* and *Rb*; the *Ras* oncogene; and *hTERT*. "No one had ever before put in defined genes and gotten a human cancer cell," Weinberg says. "We were excited."

Just as important, several researchers have demonstrated that they can inhibit tumor cell growth by blocking telomerase activity in the cells. Several teams, including researchers at biotech firms such as Geron and Amgen, as well as pharmaceutical giants such as Novartis and Boehringer Ingelheim, are now pursuing this lead to develop therapies for cancer.



Protected. In this complex of telomere DNA (red and green) with two telomere proteins, the free end of DNA is looped back and buried in the complex.

CREDITS: (TOP TO BOTTOM) UCSF PUBLIC AFFAIRS; BASED ON DATA IN M. P. HOWATH *ET AL.*, *CELL* **95**, 963 (1998)

Unanswered questions

Although the cancer connection is a huge draw, many researchers are still tackling fundamental questions of telomere biology. One is how telomeric DNA is protected. If telomeres were just bare DNA at the tips of the chromosomes, the cell would recognize them as damaged DNA and "repair" them, producing fused chromosomes and other damaging abnormalities. Telomeres have a couple of ways of avoiding this fate.

Using electron microscopy, Jack Griffith of the University of North Carolina, Chapel Hill, de Lange of Rockefeller, and their colleagues showed in 1999 that the bare ends of mammalian telomeres bend back and tuck into the double-stranded DNA. Since then, researchers have found similar "t-loops," as the group called them, in ciliates and trypanosomes. "This tucking in may be how telomeres protect their ends," de Lange says.

T-loops don't form on their own, however. Researchers including de Lange, Cech, and Lundblad have identified many proteins that bind to telomeric DNA. Indeed, says Cech, "you wonder how there is even room" for them all. De Lange's group has evidence that two of these proteins, TRF1 and TRF2, are involved in t-loop formation and telomere protection (see p. 2446).

And about a year ago, Cech and University of Colorado postdoc Peter Baumann identified the telomere end-binding protein, Pot1 (for protection of telomeres 1), present in organisms ranging from fission yeast to mammals (*Science*, 11 May 2001, pp. 1075 and 1171). Deletion of the *Pot1* gene in yeast results in rapid telomere loss, showing that it is needed for telomere maintenance. Because these proteins are essential for telomere maintenance, they, too, might be targets of anticancer therapies.

Researchers now want to unearth the functions of all those binding proteins. In addition to TRF1, TRF2, and Pot1, these include several proteins known to be involved in DNA repair. "Why would you put proteins involved in end-to-end [DNA] joining at the telomeres?" asks Princeton's Zakian. "It would seem to be the last place you would want them."

Other questions concern how the telomeres get uncapped when the DNA replicates and then recapped when that is finished, and how the cell knows that the telomeres have shortened to the point where it's time to senesce or die. "We don't have a clue about what makes a telomere look good or bad to a cell," Blackburn says. But while there's much work to be done, one thing is certain: There are now plenty of hands willing to tackle it.

–JEAN MARX