The Telomerase Picture Fills In

Researchers working with protozoans and yeast have identified a protein that appears to be the long-sought catalytic component of telomerase, the enzyme that synthesizes the chromosome ends

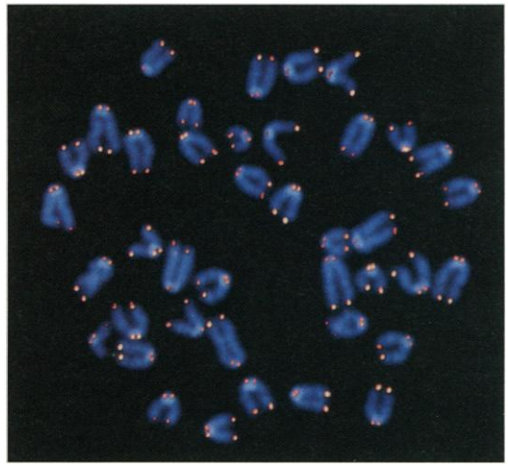
When Vicki Lundblad and Tom Cech sat down to talk about their common interest last August at a conference in Hawaii, neither realized how fruitful that chat would be. They both were in hot pursuit of one of the most elusive prizes in cell biology: the key protein in the enzyme, called telomerase, that rebuilds the chromosome ends after each cell division and may play a role in both cancer and aging.

Cech's and Lundblad's labs had each found telomerase proteins in one of two very divergent organisms—the ciliated protozoan *Euplotes aediculatus* and the brewer's yeast *Saccharomyces cerevisiae*. But neither knew whether they had found the crucial component of the complex enzyme. As a result of their talk, however, they swapped their protein sequences and quickly discovered that Cech's team at the University of Colorado, Boulder, and Lundblad's at Baylor College of Medicine in Houston had zeroed in on the same protein.

Within months, the two teams had used a mix of biochemistry and yeast genetics to show that the protein has features expected of the telomerase's catalytic component, and that altering those features abolishes the enzyme's activity. Theirs is not the first candidate for the catalytic role. But the evidence, reported on page 561 of this issue, constitutes "the tightest case for having a catalytic activity that is part of the telomerase," says Dan Gottschling, who studies telomeres at the Fred Hutchinson Cancer Research Center in Seattle.

If the new protein is indeed the catalytic component of telomerase, the discovery will provide a boost to researchers trying to understand how the enzyme does its crucial job. Without telomerase, the chromosomes would shorten with every cell division, eventually disrupting the genes. Understanding telomerase activity and how it falters might lead to insights into aging, which some researchers have linked to telomere loss. The enzyme is also a potential target for cancer therapy, as its activity is high in many types of cancer cells, the continuous division of which may demand good telomere maintenance.

The protein has been the biggest mystery in telomerase since Elizabeth Blackburn and then-graduate student Carol Greider, at the University of California (UC), Berkeley, showed in 1987 that the enzyme contains both protein and RNA. Blackburn's team, which moved to UC San Francisco (UCSF) during the course of the work, later showed that the RNA acts as a template from which the telomerase makes the DNA it adds to the chromosome tip. "That would make the enzyme officially a reverse transcriptase," notes Titia de Lange, who studies telomeres at Rockefeller University in New York City. But while the RNA part of telomerase could be fished out of cells by searching for RNAs with sequences complementary to those of telomeric DNA, the search for the protein responsible for that reverse transcriptase (RT) activity proved difficult, mainly because the enzyme is in such scarce supply in most cells.



Telo-glow. The bright orange stain marks the telomeres of these mouse chromosomes.

PETER LANSDORP AND MARK ZIJLMANS/*HUMAN MOLECULAR GENETICS* 5, 685 (1996)

To get around that problem, Lundblad took an approach that did not require isolating telomerase proteins directly. Three years ago, her team began a search for yeast mutants in which telomeres shorten over time—a defect that could result from a faulty telomerase. They found mutations in four genes, including one Lundblad had identified while a postdoc with Jack Szostak at Harvard Medical School. Yeast cells with mutations in any of the four genes, known as *EST* genes for *ever-shorter telomeres*, were indistinguishable from each other and from cells mutant in the telomerase RNA. That suggested the *EST* genes encode proteins necessary for telomerase function. "The problem came when we cloned the genes," says Lundblad. They bore no resemblance to known genes in the database, and so there were no clues as to what their biochemical function might be.

But a solution to this dilemma was coming—along a very different path. About the same time that Lundblad's team began its screen, Joachim Lingner, a postdoc in Cech's lab, began to purify telomerase from *Euplotes*, a protozoan they chose because it has a specialized nucleus called a macronucleus that contains 40 million tiny chromosomes. And because all those chromosomes have telomeres, *Euplotes* needs buckets of telomerase to maintain its chromosome ends.

Lingner devised a way to snag the *Euplotes* telomerase RNA and purify the whole enzyme along with it. He found two proteins associated with the RNA, and fractions containing the RNA and proteins had telomerase activity, measured by their ability to add DNA to telomeres in the test tube. He couldn't isolate enough of the proteins to determine their amino acid sequences by the usual methods, however, so he and Cech teamed up with Matthias Mann at the European Molecular Biology Laboratory in Heidelberg, Germany.

Mann had developed a way to sequence tiny amounts of proteins (as little as 10^{-15} mole) by digesting them with enzymes, squirting them through a highly charged needle, and separating the fragments by mass. Andrej Shevchenko in Mann's lab got partial sequences from the proteins, and Lingner used the sequences to make DNA probes that enabled him to clone the corresponding genes.

Lingner searched the DNA databases for genes that resembled the proteins, and found a yeast gene that matched the larger protein, p123. No information was given about the identity of the matching yeast gene beyond its sequence. But Lingner, while poring over the p123 sequence, found scattered patches that match sequence motifs found in the active site of known reverse transcriptase enzymes. When he aligned the p123 and yeast-protein sequences, he found the RT motifs in the yeast protein, also. The parts the proteins had in common with RTs were small, and by themselves would not constitute a convincing match, but they were precisely the sequences that make up the active site of known RT enzymes.

Lingner decided to knock out the yeast gene to see if it affected telomerase activity. But Cech and Lundblad's conversation in Hawaii spared him the work. Lundblad offered her unpublished *EST* gene sequences for

comparison with those Lingner had found, and one of those genes, *EST2*, turned out to be the very gene that Lingner had pulled out of the yeast database. "That was very exciting," says Cech, because of the Lundblad group's genetic evidence that *EST2* is needed for telomere maintenance. It was looking like p123 and Est2 could be the catalytic components of their respective telomerases.

The yeast connection was a lucky break for his lab, says Cech, because it enabled them to test that hypothesis genetically. "You can do genetics so easily in yeast," he notes, "and in *Euplotes* you can't do it at all." The teams collaborated to mutate sites in Est2 that correspond to the RT active site. They found that the telomeres of yeast cells containing the mutant enzyme shortened just as they did in cells missing the whole *EST2* gene.

Lingner subsequently showed that telomerase partially purified from those mutant yeast cells could no longer elongate telomeres. From that, "it was a reasonable conclusion," says Lundblad, "that [the proteins] were the catalytic subunits of their respective enzymes." Many in the field agree. "Looking at the whole package, I find it very convincing," says Jef Boeke, who studies reverse transcriptases at Johns Hopkins University. "They mutated the [key RT] residues, and they killed an in vitro activity. That evidence is very strong."

The evidence also suggests that Est2 and p123 represent a new class of RT that is most closely related not to RTs from retroviruses like HIV, but to RTs coded for by transposable elements (segments of DNA that can move around in the genome) found in many cells. In that case, drugs that inhibit the HIV enzyme may not work on telomerase, Boeke says, so researchers wanting to develop anti-telomerase drugs for possible anticancer applications may have to start from scratch.

Despite the evidence that Est2 and p123 are telomerase catalysts, they still have rivals for that role: p95 and p80, telomerase-associated proteins purified and cloned 2 years ago from the ciliated protozoan *Tetrahymena thermophilia* by Greider, with Kathleen Collins and Lea Harrington, who were then postdocs in Greider's lab at Cold Spring Harbor Laboratory on Long Island. Collins proposed that p95 may be the catalytic component, based on resemblances it shows to enzymes that synthesize RNA or DNA. And in the past 3 months, Harrington's team at the University of Toronto and Fuyuki Ishikawa's at the Tokyo Institute of Technology have reported finding p80-like proteins in human, mouse, and rat telomerases.

But, so far, no one has shown either p95 or p80 to be essential for telomerase activity, and the current discovery casts more doubt on the role of those proteins. "The most straightforward scenario," says UCSF's Blackburn, is that the *Tetrahymena* and mammalian telomerases also have a p123-like protein as their catalytic unit, and it has somehow been missed. In that case, p80 and p95 may have an accessory role in the enzyme. "Given this [result], one should look very hard for RT motifs in all species of telomerases," Blackburn says.

Or it may be that either p80 or p95 will prove to be the catalytic part of *Tetrahymena* telomerase. But that would mean that the two ciliates, *Tetrahymena* and *Euplotes*, have evolved different enzymes to do the job—an idea that Gottschling describes as "sort of amazing, evolutionarily." Most researchers say they are reserving judgment about these possibilities until some of the remaining gaps in the story are filled. However, most also agree with telomere researcher David Shore of the University of Geneva that "the burden of proof is now on those with p80 and p95 in their hands" to show that those proteins are essential parts of telomerase.

Even if the new results do pinpoint the crucial actor in telomerase, the enzyme likely contains many other proteins, all with some role in its function, that vary from organism to organism. The picture looks confusing now, Greider says, because "we are interpolating between several different organisms. We need to solve the problem completely in each organism" before the true nature of telomerase is revealed.

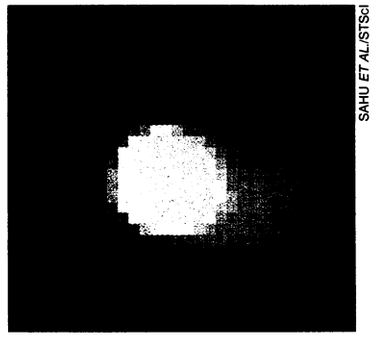
–Marcia Barinaga

GAMMA-RAY BURSTS

Visible 'Source' Teases Observers

Just when astronomers thought they might be solving one of their longest running mysteries, the story has taken another dizzying twist. The question is whether the flashes of gamma rays called gamma-ray bursts (GRBs) originate in or near our galaxy or billions of light-years away, at cosmological distances, which would make them the brightest outpourings in the universe.

Astronomers thought they were on the verge of an answer when the Italian-Dutch satellite Beppo-SAX saw a fading source of x-rays that seemed to be the afterglow of a GRB the satellite had detected on 28 February. Because x-ray detectors have much better spatial resolution than those for gamma rays, that helped pin down the burst's position for further observation. Hopes shot even higher when ground-based telescopes aimed at the spot then fished out both a point of light and a faint fuzzy patch next to it—possibly the GRB source and its host galaxy in the distant universe (*Science*, 21 March, p. 1738). The cosmological alternative seemed poised to carry the day. But then the orbiting Hubble Space Telescope (HST) got into the act.



SAHU *ET AL.*/STScI

Near or far? Hubble view of the point source and adjacent fuzzy patch.

The latest results of its scrutiny of the proposed source, reported on the Internet last week in International Astronomical Union (IAU) circulars, have thrown the debate wide open again. One group claimed that the pointlike burst source, if that's what it is, is moving noticeably across the sky and might be a nearby object. Another group—looking at the same data—saw nothing of the kind. Says Chryssa Kouveliotou of NASA's Marshall Space Flight Center in Huntsville, Alabama: "I'm more confused than anything."

This new chapter in what Princeton University's Bohdan Paczyński calls "the wonderful story of [gamma-ray burst] 970228" opened earlier this month when Kailash Sahu, Mario Livio, Larry Petro, and F. Duccio Macchetto of the Space Telescope Science Institute (STScI) in Baltimore, along with several collaborators including Kouveliotou, published a circular reporting HST observations of the optical counterpart 26 and 38 days after the burst. The observations confirmed the fading point source and the adjacent fuzzy patch. By prior agreement, the group immediately released its raw HST data to the entire astronomical community.

Then, on 17 April, a separate group, including Patrizia Caraveo at the Istituto di Fisica Cosmica (IFC) in Milan, Italy, and several collaborators, posted another circular reporting their own analysis of the data. They had found something startling: The point source was moving across the sky. The angular motion was so quick, says one of the collaborators, Marco Tavani of IFC and Columbia University, that the object might have to be within a few hundred light-years of Earth, much closer than even the proponents of a galactic origin for GRBs have been suggesting recently. The fuzzy object could then be anything from a transient cloud of gas associated with the burst to a background galaxy, aligned by chance with the pointlike object.

But the Sahu team's own analysis, completed after their first circular, shows that to