CELL BIOLOGY

Cells Count Proteins to Keep Their Telomeres in Line

Dividing cells have to take good care of their telomeres. These stretches of repetitive, apparently nonsensical DNA at the end of the chromosomes make up for a quirk in the enzymes that replicate DNA: The enzymes can't reproduce the very ends of the DNA strands, so a cell's chromosomes get shorter each time it divides. Without the telomeres to act as buffers, essential genes could be lost. Indeed, in most cells of higher organisms, even this buffer is eventually exhausted, a development associated with aging and ultimately death.

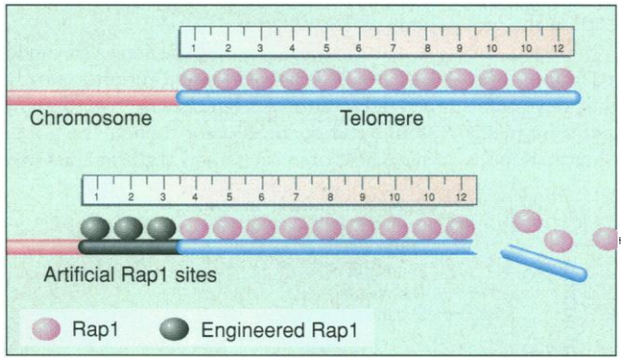
But cells that divide repeatedly, such as cancer cells, germ-line cells, and microorganisms like yeast, restore their telomeres by adding back DNA each time they divide. New results described on page 986 by a research team led by molecular biologist David Shore, of the University of Geneva, provide insight into a crucial part of this process.

The puzzle Shore and his colleagues, Stéphane Marcand and Eric Gilson of the École Normale Supérieure in Lyon, France, have addressed is what tells the telomere-restoring enzyme, telomerase, how much DNA to add back. They report that in baker's yeast (*Saccharomyces cerevisiae*), cells measure telomeres by counting the copies of a protein called Rap1 bound to the telomere, and shut off telomere growth when a standard length is reached.

The work is "an elegant series of studies that really sheds light on part of the telomeric sizing machinery," says Art Lustig, who studies telomeres at Tulane University in New Orleans. Cells seem to have several ways to measure telomeres, adds Lustig, who studies one of the alternate systems. But Shore's team, he says, has shown that protein counting seems to be one key way. And that is likely to be true for more than yeast. The Shore team notes that Titia de Lange's group at Rockefeller University in New York City has evidence that a similar counting system may operate in human cells, although de Lange declined to discuss the as-yet-unpublished work.

Telomerase itself was eliminated as a possible telomere measuring stick years ago. The enzyme has been hard to study directly, because researchers have not yet isolated the catalytic protein component, although they have candidates, one of which is described on page 973 by Lea Harrington's team at the University of Toronto. But despite the problems in purifying the enzyme, test-tube experiments with partially pure telomerase have shown that the enzyme alone "can't sense telomere length, and that implies that in vivo, the enzyme has to be told what to do," says telomerase researcher Gregg Morin, of Geron Corp. in Menlo Park, California.

In the early 1990s, it began to look like those instructions come from Rap1, which had been discovered in *S. cerevisiae* in the mid-1980s and shown to bind to telomeres. Shore's and Lustig's groups, as well as Virginia Zakian's team, then at the Fred Hutchinson Cancer Research Center in Seattle, found that mutations in Rap1 or alterations in its levels can change telomere lengths. The most dramatic result came in 1992, when Lustig's group reported that mutations that remove part of Rap1 cause runaway telomere growth. "That was the first clear demonstration that Rap1 is a negative regulator of telomerase elongation," says Shore. In 1995, Elizabeth Blackburn's team at the University of California, San Francisco, extended the findings beyond *S. cerevisiae*, with the discovery that mutations in the telomere sequence that hinder Rap1 binding produce longer telomeres in a related yeast, *Kluyveromyces lactis*.



Fooled. The yeast cell counts artificial Rap1 binding sites (black) as part of the telomere and shortens the true telomere accordingly.

While all this implied that Rap1 puts the brakes on telomere growth, how the protein accomplishes that was left wide open. Shore suspected the cell might be counting Rap1 molecules bound to the telomeres. To test that idea, his team decided to play with the number of Rap1 binding sites on an *S. cerevisiae* telomere—roughly 15 on the 300-base-pair stretch of DNA—and watch the effects on telomere length.

They used an assay developed by molecular geneticist Dan Gottschling, now at Seattle's Hutchinson center, to follow telomere restoration. The assay replaces the end of a yeast chromosome, including the telomere, with a stretch of DNA that ends in a short "seed" sequence of telomere-like DNA. Left on its own, the yeast adds to that seed, producing a telomere of normal length.

In one experiment, Shore's team inserted an 80-base-pair piece of telomere DNA between the chromosome and the seed, separated from the seed by a short nontelomere stretch of linker DNA. When the cell then elongated the seed, it grew to only 220 base pairs, not 300. That meant the cell had counted the inserted DNA as part of the telomere. What's more, Shore says, the enzyme "didn't particularly care which direction the [inserted] sequence was in." That is just what you would expect if the cell were monitoring bound proteins rather than the telomere sequence itself, because a protein would bind to double-stranded DNA regardless of which way it is facing.

To see whether Rap1 was key to the length measurement, the team created an engineered Rap1 protein that would bind to artificial sites made of nontelomeric DNA. They added some of these sites between the chromosome and the telomere sequence and found that this shortened the amount of telomere sequence the cells added to the chromosome, as if "the machinery that regulates telomere length is fooled," says Shore, into counting the extra Rap1 molecules as part of the telomere.

"It is clear from this experiment that Rap1 is being counted," Gottschling agrees. "But then that raises the question of how you count proteins." The Shore team suggests that the binding of 15 or so Rap1s alters the shape of the telomere so that telomerase can't bind to the end. When the number drops to fewer than that, they propose, the enzyme can bind and elongate the telomere again.

Whether that model turns out to be right or not, the counting seems to require other proteins. Shore's group found two proteins they call Rap1 interacting factors (Rifs) that bind to Rap1 and help regulate the telomeres. Deleting either of the *RIF* genes produces longer telomeres, as does a mutation of Rap1 that keeps it from binding to Rif1. In cells missing both *RIF* genes, the telomeres grow completely out of control, suggesting that the Rifs play a vital role in counting. Shore suspects there are more such helper-proteins.

Shore and others will be eagerly seeking those proteins, as well as answers to the obvious next questions: How are the Rap1 proteins actually counted, and how similar is the system in mammals? "We are just starting to get an idea [of] who the players are," he says. "How they work is another question."

–Marcia Barinaga