PERSPECTIVES: CELL BIOLOGY

Telomere Capping—One Strand Fits All

Titia de Lange

Linear chromosomes confer both benefits (*1*) and burdens on eukaryotic cells. This new genome design has created two additional problems stemming from the fact that linear chromosomes have ends. Eukaryotes have had to evolve a strategy for the replication of terminal DNA, a task mostly executed by the enzyme telomerase (*2*). Furthermore, chromosome termini must be "capped," otherwise they will be identified as damaged DNA and subjected to DNA repair, an outcome that would cause loss of chromosome integrity and cell viability. In essence, the capping problem has been solved by the presence of telomeres, complexes of DNA and protein that protect the ends of chromosomes. However, the ways in which various organisms protect their telomeres appear to be remarkably idiosyncratic. Ciliates, budding yeast, and mammals each seem to have arrived at a different system (see the figure), providing a perplexing diversity of mechanisms to solve such a fundamental problem. Data presented by Baumann and Cech on page 1171 of this issue suggest that, in all cases, telomeres are protected by a capping protein that binds to the single-stranded DNA commonly found at the ends of chromosomes (*3*).

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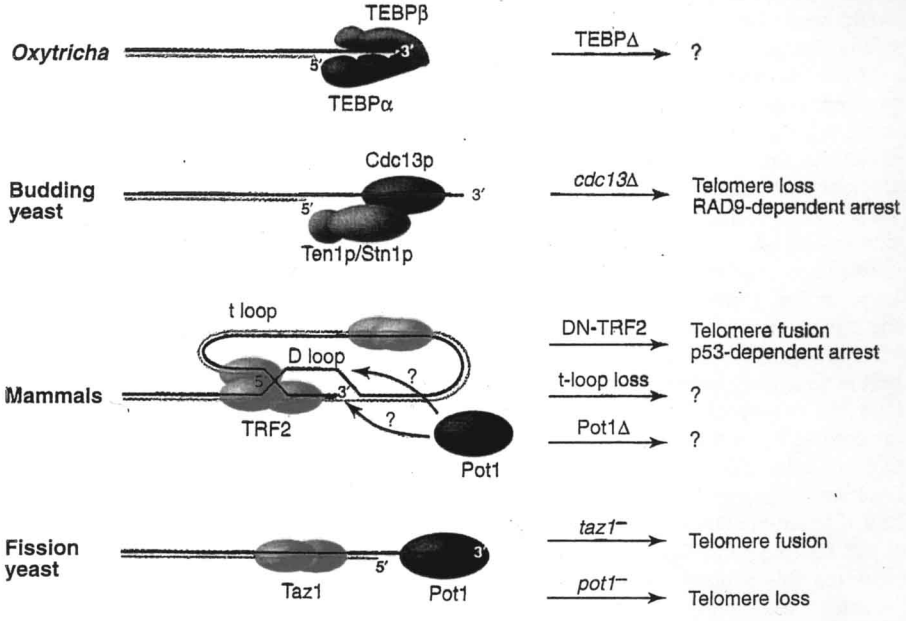
A first glimpse of the telomere nucleoprotein complex was afforded by the ciliate *Oxytricha nova* (*4*), whose genome is fragmented into gene-sized molecules, thus providing an abundant source of chromosome termini. Each *Oxytricha* telomere ends in a short 3′ overhang that is tightly bound by a single-stranded DNA binding protein, TEBP, composed of an α and β subunit (see the figure). TEBPα recognizes the telomeric repeat sequence in the overhang, and the β subunit stabilizes the DNA-protein complex. Together, α and β form an extensive interface along the overhang and bury the 3′ end of the telomere in a deep hydrophobic pocket (*5*). From its structure, this telomeric complex seems to provide an effective way to hide chromosome ends from DNA repair enzymes. The limitations of ciliates as an experimental system, however, prevent direct testing of this possibility.

No obvious orthologs of TEBP α and β have been found in the budding yeast genome. Instead, telomeres of budding yeast are capped by the Cdc13 protein. Yeast cells with an aberrant *CDC13* gene perceive their telomeres as damaged DNA (*6*). In these cells, one strand of the telomere is degraded resulting in RAD9-dependent cell cycle arrest, which also occurs in response to breaks in double-stranded DNA. Like the TEBPα factor, Cdc13p is a sequence-specific single-stranded DNA binding protein with a strong preference for telomeric repeats (see the figure) (*7*). But, Cdc13p has no obvious sequence similarity to TEBPα, and has no known β partner. Furthermore, the biochemical properties of Cdc13p indicate that it is unlikely to form a physical cap over the 3′ end of telomeres. Rather, Cdc13p is a recruitment factor bringing Stn1p to the telomere (*8*), which in turn binds a second capping protein, Ten1p (*9*). Indeed, mere tethering of Stn1p (via the DNA binding domain of Cdc13p) to the ends of telomeres is sufficient to fully protect the telomeres (*8*).

A completely distinct telomere-capping strategy has been discovered in mammals, which require the telomeric protein TRF2 to protect their chromosome ends (see the figure). Inhibition of TRF2 induces the immediate activation of the ATM/p53 DNA damage checkpoint pathway and cell cycle arrest, analogous to the RAD9-dependent arrest of Cdc13p-deficient cells (*10*). Although TRF2-depleted telomeres are not degraded, they form covalent telomere-to-telomere fusions, suggesting that they too are



A common theme in telomere capping. Diverse telomere-capping strategies have in common a single-stranded telomeric DNA binding protein. Telomeres of the ciliate *Oxytricha nova* contain a 16-nucleotide 3′ overhang bound by the single-stranded DNA binding protein TEBPαβ. The function of this telomeric complex has not yet been tested. Budding yeast telomeres are protected by the single-stranded telomeric DNA binding protein Cdc13p, which recruits the capping proteins Stn1p and Ten1p. Loss of any component of this complex results in degradation of 5′ chromosome ends and cell cycle arrest. Mammalian telomeres are protected by TRF2, perhaps through its ability to form t loops. Loss of TRF2 results in cell cycle arrest and end-to-end ligation of telomeres. Fission yeast telomeres are protected by Taz1, an ortholog of TRF2. The newly discovered capping factor Pot1 binds to single-stranded telomeric DNA and protects fission yeast telomeres from degradation. A human ortholog of fission yeast Pot1 has been identified. Because this protein binds to single-stranded G-rich telomeric DNA in vitro, its in vivo binding sites could be the 3′ overhang (if it is unpaired) or the D loop (see arrows) of human telomeres. In all telomeres, the dark line indicates the G-rich telomeric repeat strand that extends as a 3′ overhang beyond the double-stranded region of the telomere.

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substrates for the cell's DNA repair machinery (*11*).

An evolutionarily related protein with a very similar function, Taz1, has been identified in fission yeast (see the figure) (*12*). TRF2 and Taz1 have no sequence similarity to TEBP α or β, and no TRF2/Taz1 ortholog has been found in the budding yeast genome. Indeed, unlike Cdc13p and TEBP, TRF2 has no affinity for single-stranded DNA and binds to the double-stranded region of telomeres. How could such a protein protect the telomere terminus? It turns out that TRF2 can remodel telomeric DNA into a duplex lariat structure called the t loop (see the figure) (*13*). T loops are formed by the single-stranded telomeric overhang invading the double-stranded region of the telomere. These t loops are found at high frequency in the telomeres of mammals and protozoa. Obviously, invasion of the duplex telomeric tract by the 3′ overhang provides an efficient way to protect chromosome ends. The cell cycle arrest of cells in which TRF2 is inhibited could result from inappropriate unfolding of the t loops.

Such diverse solutions to a common problem create the unsettling feeling that some critical feature, common to all three strategies, has somehow escaped notice. Baumann and Cech now provide evidence for a common theme in telomere protection among ciliates, yeast, and mammalian cells. Their findings indicate that all eukaryotes use a single-stranded DNA binding protein to cap the telomere. Apart from telomere synthesis by telomerase, this may be the first truly conserved aspect of eukaryotic telomeres. Taking advantage of the complete fission yeast genome, the authors found a distant ortholog of TEBPα. Deletion of this gene resulted in complete loss of telomeric DNA and reduced growth of the fission yeast. They called the gene encoding this capping factor *pot1*$^+$ (protection of telomeres). Fission yeast have the unique ability to live without telomeres altogether by circularizing each of their three chromosomes, and this is what happens in a *pot1*$^-$ strain. In vitro, Pot1 protein binds specifically to the single-stranded G-rich telomeric overhang of fission yeast; biochemical analysis suggests that this capping factor might bind along the length of the single-stranded DNA tail of the telomere, as well as at its end.

Using human sequence databases, Baumann and Cech identified a human ortholog of Pot1 and found that, like its fission yeast counterpart, this protein binds specifically to the G-rich DNA overhang of human telomeres in vitro. Mammalian telomeres end in a very long 3′ overhang (up to 300 nucleotides), so that multiple copies of Pot1 may have to bind along the single-stranded tail. In addition, Pot1 could bind to the G-rich telomeric DNA in the D loop and stabilize the t loop configuration (see the figure).

The new work suggests that protecting chromosome ends with a single-stranded DNA binding protein may well be a universal principle. It is even possible that TEBPα, Pot1, and Cdc13p are all evolutionarily related. The structure of the TEBPα protein reveals the presence of three OB-folds, a β-barrel oligonucleotide/oligosaccharide binding motif present in a wide variety of nucleic acid binding proteins (for example, RPA subunits, gene V SSB, and S1 nuclease) (*5*). Pot1 has sequence similarity to TEBPα in a region that partially overlaps the first OB-fold. However, OB-folds cannot be predicted on the basis of the amino acid sequence of the protein, so that it is not possible to determine whether Pot1 or indeed Cdc13p contain the same motifs. We eagerly await the three-dimensional structures of both Pot1 and Cdc13p.

We still need to know whether human Pot1, like its fission yeast counterpart, actually binds and protects telomeres. Another question requiring urgent attention is whether fission yeast telomeres form t loops and whether such t loops predominate or alternate with an unfolded Pot1-capped state. Finally, it will be important to learn more about the possible activities of Pot1. Cdc13p not only caps telomeres but also recruits telomerase to the ends of chromosomes (*14*); perhaps Pot1 engages in this dual task. Now that a common theme has emerged, it is comforting that the otherwise divergent solutions to the telomere-capping problem are neither loopy nor potty.

References and Notes

1. F. Ishikawa, T. Naito, *Mutat. Res.* **434**, 99 (1999).
2. M. J. McEachern, A. Krauskopf, E. H. Blackburn, *Annu. Rev. Genet.* **34**, 331 (2000).
3. P. Baumann and T. R. Cech, *Science* **292**, 1171 (2001)
4. D. E. Gottschling, T. R. Cech, *Cell* **38**, 501 (1984); D. E. Gottschling, V. A. Zakian, *Cell* **47**, 195 (1986).
5. M. P. Horvath, V. L. Schweiker, J. M. Bevilacqua, J. A. Ruggles, S. C. Schultz, *Cell* **95**, 963 (1998).
6. B. Garvik, M. Carson, L. Hartwell, *Mol. Cell. Biol.* **15**, 6128 (1995).
7. J. J. Lin, V. A. Zakian, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13760 (1996); C. I. Nugent, T. R. Hughes, N. F. Lue, V. Lundblad, *Science* **274**, 249 (1996).
8. E. Pennock, K. Buckley, V. Lundblad, *Cell* **104**, 387 (2001).
9. N. Grandin, C. Damon, M. Charbonneau, *EMBO J.* **20**, 1173 (2001).
10. J. Karlseder, D. Broccoli, Y. Dai, S. Hardy, T. de Lange, *Science* **283**, 1321 (1999).
11. B. van Steensel, A. Smogorzewska, T. de Lange, *Cell* **92**, 401 (1998).
12. M. Godhino Ferreira, J. Promisel Cooper, *Mol. Cell* **7**, 55 (2001).
13. J. D. Griffith *et al.*, *Cell* **97**, 503 (1999).
14. S. K. Evans, V. Lundblad, *Science* **286**, 117 (1999).
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PERSPECTIVES: GEOPHYSICS

Oceanic Crust When Earth Was Young

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Oceanic lithosphere makes up about two-thirds of Earth's outer rigid shell and has probably done so since early in Earth's history (*1–4*). New oceanic crust and mantle are created by sea-floor spreading, which is controlled by thermally sensitive processes such as partial melting of the mantle, melt segregation and transport, and crustal magmatic construction. It has therefore been suggested that during the Archean (more than 2500 million years ago), when Earth's mantle may have been as much as three times hotter than today, a somewhat different oceanic crust should have been generated (*3–5*).

This hypothesis can be tested directly with surviving samples of Archean oceanic lithosphere. Oceanic lithosphere is usually "recycled" back into the mantle through subduction, but a few fragments of ancient oceanic lithosphere ("ophiolite complexes") survive in collisional mountain belts (*6*). The discovery of a ~2500-million-year-old ophiolite complex, reported by Kusky *et al.* on page 1142 of this issue (*7*), represents an important new chapter in this continuing line of inquiry.

There are two very good reasons why this area is steeped in controversy. First, we do not know all that much about contemporary oceanic crust and how it varies from one tectonic and magmatic setting to the next. Second, whether we recognize Archean ophiolites depends on what we are looking for, and this in turn depends on which of the various models we use to describe plate tectonics on early Earth.

Over the past few decades, studies of Phanerozoic (544 million years ago to present) ophiolite complexes and of contem-

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