

Beginning to Understand the End of the Chromosome

Review

Thomas R. Cech*

Howard Hughes Medical Institute
Department of Chemistry and Biochemistry
University of Colorado
Boulder, Colorado 80309

In their 1985 *Cell* paper, Greider and Blackburn announced the discovery of an enzyme that extended the DNA at chromosome telomeres in the ciliate, *Tetrahymena*. Since then, there has been an explosion of knowledge about both the RNA and protein subunits of this unusual ribonucleoprotein enzyme in organisms ranging from the ciliates to yeast to humans. The regulation of telomerase is now understood to take place both at the level of synthesis of the enzyme and via the state of its substrate, the telomere itself. The roles of telomerase in both cellular immortality and cancer are vibrant areas of current research.

It is unusual for an enzyme to be a topic of widespread conversation. While millions may marvel at the lower cholesterol levels they've achieved by taking a statin, few of them know that the drug inhibits their HMG-CoA reductase enzyme. Telomerase, on the other hand, is connected to notions of our mortality and longevity and has been popularized by articles and books including "Merchants of Immortality" (Hall, 2003). The purpose of this review is to celebrate the Greider and Blackburn paper that started it all and then to highlight some of the major advances that followed. My review will be highly selective, covering only about 1% of the hundreds of scientific papers written each year that deal with telomerase, and I refer the reader to other recent reviews for a more comprehensive treatment (e.g., Blackburn, 2001; Collins and Mitchell, 2002).

Telomere Terminal Transferase

Carol Greider, a graduate student in Liz Blackburn's group at the University of California, Berkeley, had chosen an ambitious PhD thesis project: identify the molecular entity responsible for replicating chromosome ends. There was a basis for thinking that such an activity would exist: when linear DNA molecules from ciliated protozoa were propagated in yeast, their ends were extended not by the ciliate telomeric DNA sequence (repeats of TTAGGG in *Tetrahymena* or T₄G₄ in hypotrichous ciliates), but instead by the more heterogeneous G₁₋₃T yeast telomeric sequence (Szostak and Blackburn, 1982; Pluta et al., 1984; Shampay et al., 1984). One reasonable interpretation was that the ciliate telomeres served as "seeds" for addition of a DNA sequence that was specified by a nucleotide addition activity intrinsic to yeast. Greider's project was to purify the corresponding *Tetrahymena* enzyme.

The identification and characterization of this new en-

zymatic activity was the subject of Greider and Blackburn (1985). The activity added TTAGGG repeats, one nucleotide at a time, to the ends of GT-rich primers that represented either the *Tetrahymena* or the yeast telomeric sequence. This paper marked the first appearance in the literature of the six nucleotide "ladder" of extension products that would appear in a hundred subsequent papers—a hallmark of telomerase activity not just in *Tetrahymena*, but in human extracts as well. The authors made the reasonable proposal that the activity might be related to known terminal transferases, such as the enzyme that adds CCA to the 3' ends of transfer RNAs. The real nature of the enzyme turned out to be much more novel.

Telomerase Contains an Essential RNA

In the following years, Greider and Blackburn found that the enzyme (now called telomerase) was even much more interesting than one might have thought. It contained an essential RNA component, a portion of which served as a template to specify the sequence that was added to the chromosome end (Greider and Blackburn, 1989; Figure 1). The rules were those of Watson-Crick base-pairing: C's and A's in the RNA template specified G's and T's, respectively, in the sequence that was laid down at chromosome ends. Indeed, the formal proof of this model was provided in an elegant paper from Blackburn's lab, in which site-specific mutagenesis of nucleotides in the RNA template led to the deposition of complementary nucleotides in *Tetrahymena* telomeres (Yu et al., 1990).

Subsequently, Greider's group at Cold Spring Harbor Laboratory collaborated with scientists at Geron Corporation to identify and sequence the telomerase RNAs from mouse and human—the ribonucleoprotein nature of telomerase was general (Feng et al., 1995). She also worked with Ron DePinho to construct a mouse knockout for the RNA. The homozygous null mice were remarkable in that they were viable for six generations (Blasco et al., 1997). The explanation is that the mice start with long telomeres (10–60 kb); the failure to replicate those telomeres leads to gradual loss of terminal DNA sequences, but it takes many cell divisions before the shortest telomeres reach a critical length. Embryonic fibroblasts cultured from the fourth generation of these mice onward showed aneuploidy and chromosome end-to-end fusions, indicative of failure to cap chromosome ends.

Finally, the Protein: Telomerase Reverse Transcriptase

Greider and Blackburn (1985) provided indirect evidence that telomerase contained at least one essential protein component, which presumably provided the catalytic center for nucleotide addition. The protein proved elusive, however, and it took ten years for two different approaches to converge on its identification. Joachim Lingner purified active telomerase from *Euplotes*, a ciliated protozoan with an extraordinary number of telo-

*Correspondence: thomas.cech@colorado.edu

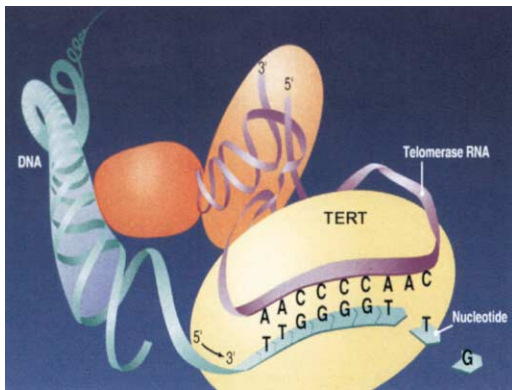


Figure 1. Telomerase in Action

The RNA subunit (purple) has the template sequence of *Tetrahymena* telomerase. Proteins include the catalytic subunit, TERT (yellow), and additional less-conserved proteins (orange). Reprinted with permission from Cech (1994). Illustration by K. Sutliff. Copyright 1994 AAAS.

meres per macronucleus (ca. 10^6) and a correspondingly high dose of telomerase (Lingner and Cech, 1996). It contained the telomerase RNA and two proteins, p123 and p43. At the same time, a genetic screen in yeast in Vicki Lundblad's lab identified three genes whose deletion resulted in an EST (Ever Shorter Telomeres) phenotype (Lendvay et al., 1996). It turned out that the best sequence match to *Euplotes* p123 was Lundblad's Est2p.

Better yet, both p123 and Est2p contained the amino acid sequence hallmarks of reverse transcriptases. It had long been thought that telomerase resembled a reverse transcriptase (RT), in that it synthesized DNA using an RNA template. It now appeared that it was directly related to other RTs in terms of its protein structure and evolution. The critical evidence came from a collaboration between the Lundblad and Cech groups, in which mutations of amino acids implicated in RT activity were shown to eliminate telomerase activity both in vivo and in vitro (Lingner et al., 1997).

As often happens in these days of the human genome project, the human version (*hTERT*) was found shortly thereafter (Meyerson et al., 1997; Nakamura et al., 1997). It was expressed in a variety of transformed cells but not detectable in primary cultures of human somatic cells, already giving a simple answer as to why telomerase activity was deficient in somatic cells. Thus, over a short time span, we went from having no telomerase protein to a whole family of TERTs (Telomerase Reverse Transcriptases).

TERT is now known to have several functions. The reverse transcriptase motifs, present in the C-terminal half of the protein, provide the active site for catalysis. Several conserved amino acid sequence motifs in the N-terminal half "rivet" the RNA component to the protein, assuring maintenance of a stable RNP while allowing the template to move through the active site (Bryan et al., 2000; Figure 2). Movement is essential, as a single active site (the triangle in Figure 2) must accommodate addition of multiple nucleotides, after which translocation of the template relative to the DNA

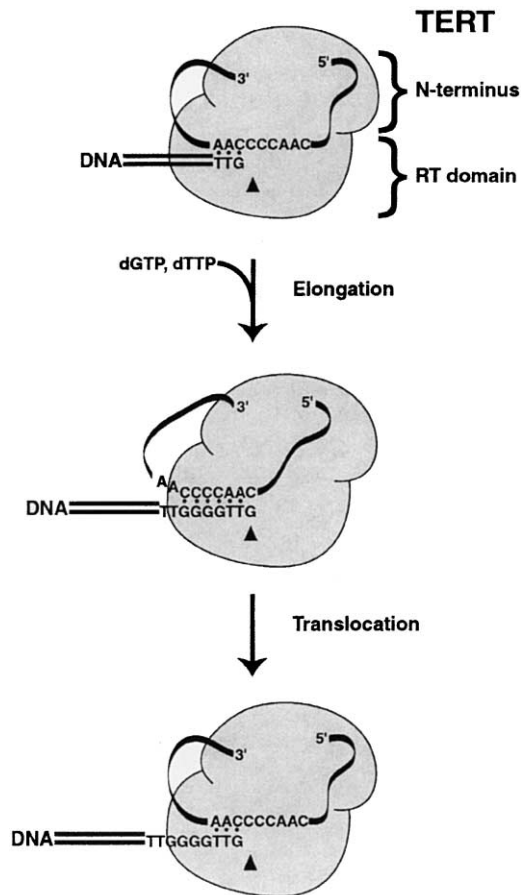


Figure 2. Telomerase Reaction Cycle

The RNA subunit is held into the complex by interactions with the N-terminal domain of TERT. This leaves the template (CA-containing sequence) free to move through the RT domain so that a single active site (triangle) can catalyze nucleotide addition at multiple positions.

product is necessary for multiple rounds of addition. Finally, the very N terminus of yeast TERT recruits another telomerase subunit, Est3p, to the complex (Friedman et al., 2003), and additional protein-protein interactions may remain to be discovered.

Current Picture of Telomerase

We currently view telomerase as composed of an RNA molecule with a well-defined secondary structure, best characterized in ciliates and vertebrates (Chen et al., 2000); the conserved TERT catalytic subunit; and a number of additional protein subunits, only some of which are conserved phylogenetically.

Three classes of proteins in addition to TERT are now known to be associated with various telomerases. Est1p was first found in yeast (Lundblad and Szostak, 1989); it is essential for activity in vivo, but seems entirely dispensable for enzyme activity per se as judged by in vitro assays. Est1p interacts directly with the yeast telomere DNA end binding protein, Cdc13p (Pennock et al., 2001); this interaction appears to recruit telomerase to the chromosome end (Pennock et al., 2001) and somehow activate telomerase that is already associated

with the telomere (Taggart et al., 2002). A human Est1 ortholog, EST1A, is associated with most or all active telomerase in human cell extracts and is involved, either directly or indirectly, in chromosome end-capping and telomere elongation (Reichenbach et al., 2003; Snow et al., 2003). Another yeast subunit, Est3p, is similarly important for activity in vivo but not in vitro, with its specific function unknown.

The two subunits of the Ku heterodimer comprise the second class of telomerase proteins. Ku is responsible for nonhomologous end-joining of broken chromosomes, and initially it appeared odd or perhaps even dangerous that it would be telomere associated; after all, telomeres protect chromosome ends from fusion events that result in genomic instability. A solution to this conundrum was recently provided by Stellwagen et al. (2003), who showed that Ku binds directly to telomerase RNA and promotes the de novo addition of telomeres to broken chromosome ends, thereby helping heal DNA damage by capping the broken end with telomeric DNA.

Finally, a large variety of proteins contribute to the assembly and maturation of the telomerase RNP, and these vary much more in evolution than TERT and the other proteins listed above. Budding yeast telomerase RNA is an RNA polymerase II transcript, and its intracellular transport and assembly are mediated by the same Sm proteins found in the small nuclear RNPs involved in RNA splicing (Seto et al., 1999). Current evidence suggests that the RNA may be made in the nucleus, exported to the cytoplasm to pick up protein components, and then reimported into the nucleus where it functions (Ferrezuelo et al., 2002; Teixeira et al., 2002). Human telomerase RNA, also a pol II transcript, has a snoRNP (small nucleolar RNP) domain, appears to be matured in the nucleolus, and binds dyskerin and other snoRNP proteins (Mitchell et al., 1999; Pogacic et al., 2000). Defects in the RNA or the dyskerin protein that interrupt this maturation can lead to a human disease, dyskeratosis congenita (Mitchell et al., 1999; Vulliamy et al., 2001). Ciliate telomerase RNA is transcribed instead by pol III and, at least in *Euplotes*, is bound by a telomerase-specific La-motif protein p43 that may shepherd its maturation or confer nuclear localization (Aigner et al., 2003).

Returning to the RNA component, it is now seen to provide much more than the template. One class of additional functions is to provide specific binding sites for many of the proteins listed above. All telomerases exist as stable RNPs, and the RNA sequences responsible for TERT binding have been identified in several organisms (Mitchell and Collins, 2000; Chen et al., 2002; Livengood et al., 2002). In yeast, the Ku protein binds to one RNA secondary structure element (Stellwagen et al., 2003) and Est1p to a separate bulged stem (Seto et al., 2002) (Figure 3). RNA binding sites for the yeast Sm proteins and the human snoRNP proteins have similarly been defined. In the second class of functions, the RNA may be acting directly to promote a specific feature of catalysis. Clear examples are the base-paired RNA elements that form template boundaries to terminate each cycle of reverse transcription in yeast and human telomerase (Tzfati et al., 2000; Chen and Greider, 2003).

One key question about telomerase RNA concerns

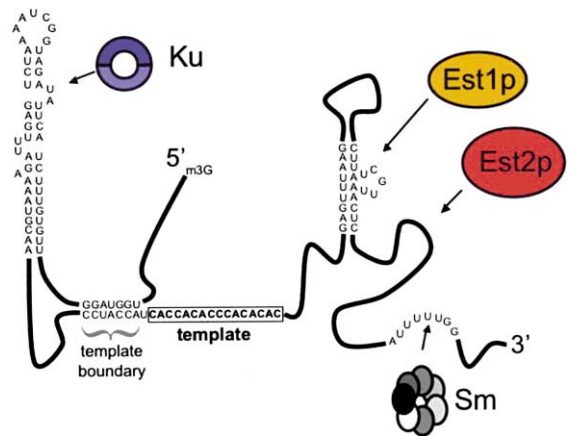


Figure 3. Telomerase RNA Functions

The *S. cerevisiae* telomerase RNA (1.2 kb) functions in part to bring proteins into the complex. Curved lines represent regions where the RNA secondary structure has not yet been reported.

how the template portion is identified by TERT. After all, the RNA subunits contain hundreds or even a thousand nucleotides, depending on the organism, and the template is not the only single-stranded region in the RNA that could in principle make a few base pairs with a DNA primer and be reverse transcribed. This problem has been most successfully tackled in *Tetrahymena*, where a short “template-recognition” sequence element directs the use of 5' adjacent nucleotides as the template for DNA synthesis (Miller and Collins, 2002). Whether this template-recognition element directly binds to TERT or interacts with another portion of the RNA remains a question for future research.

The yeast and human telomerases have been observed as dimers containing two functionally interacting RNA molecules (Ly et al., 2003 and references therein). Thus, telomerase and retroviruses resemble each other, not just in their reverse transcriptase proteins, but also in their “packaging” of their RNA template as a dimer. Because recombinant *Tetrahymena* telomerase is active as a monomer (one RNA + one TERT), dimerization is not always required for core enzymatic activity (Bryan et al., 2003).

Regulation of Telomerase

In the most general sense, telomere length either is maintained at a steady-state distribution or undergoes progressive shortening or lengthening depending on at least two considerations: the level of the telomerase RNP and the state of the telomere itself.

Human telomerase is regulated during development by the first of these factors—telomerase expression is dramatically reduced in many somatic cells during embryonic development, and therefore chromosome ends shrink with successive cell divisions (Wright et al., 1996). In these cells, the limiting component is hTERT, and the transcriptional repression of the *hTERT* gene leads to a loss of telomerase activity. An unidentified repressor encoded on chromosome 3 controls the state of *hTERT* chromatin, leading to transcriptional silencing (Szutorisz et al., 2003). Specifically, three tumor suppressor path-

ways have been identified as negative regulators of *hTERT* transcription: Mad1, a repressor of c-Myc; TGF- β , acting through SIP1; and Menin, binding directly to the *hTERT* promoter (Lin and Elledge, 2003). Human cells that retain readily detectable telomerase activity include some proliferating epithelial cells, lymphocytes, and testis. Stem cells have weak telomerase activity (reviewed by Collins and Mitchell, 2002). Even in somatic cells where the level of telomerase activity is undetectable by standard assays, one needs to be aware of the limit of detection. Recently, immunopurification has been used to reveal that there is in fact some expression of *hTERT* and telomerase activity in cycling human fibroblasts, and that this low level of activity has biological consequences (Masutomi et al., 2003).

Some cells that lack telomerase activity, on the other hand, still have a high level of *hTERT* transcription. In these cases, regulation at the level of alternative splicing leads to skipping of exons that encode reverse transcriptase function, so any translation product would not give an active enzyme (Ulaner et al., 1998).

In addition to the developmental regulation mentioned above, telomere length regulation in all organisms from yeast to human involves the accessibility of the telomere to telomerase. This appears to occur at four different levels, as follows:

(1) Double-stranded telomeric DNA binding proteins such as Rap1p in budding yeast are involved in telomere length regulation (e.g., Marcand et al., 1997; Ray and Runge, 1999). Data support the “protein counting model,” in which Rap1p binds Rif1p and Rif2p to nucleate the formation of a folded chromatin structure at the telomere, thereby preventing access by telomerase. As the telomere shortens due to incomplete replication, the number of protein binding sites decreases and the chromatin opens up to restore access to telomerase. The human telomeric dsDNA binding proteins, TRF1 and TRF2 (Smogorzewska et al., 2000), may act by a similar mechanism. TRF1 recruits TIN2 (Kim et al., 1999) and TRF2 recruits hRAP1 (Li et al., 2000) through protein-protein interactions. Similarly, in fission yeast, the dsDNA binding protein Taz1 recruits Rap1 and Rif1 (Kano and Ishikawa, 2001). Surprisingly, some aspects of telomere chromatin structure and function, including the binding of Taz1, are maintained in circular chromosomes that have no telomeric repeats (Sadaie et al., 2003).

(2) Long telomeres, including those in human cells, can form a “t loop” structure in which the entire telomeric DNA forms a large circle; the 3' single-stranded DNA tail invades the double-stranded telomeric DNA to form a D loop (Griffith et al., 1999). The t loop presumably provides chromosome end protection and also renders the DNA terminus inaccessible to telomerase. Double-stranded DNA binding proteins such as TRF1 and 2 might exert their effects on telomere length regulation in part by modulating t loop formation.

(3) Proteins that bind the 3' single-stranded DNA tail are involved in regulation of telomerase since the 3' end cannot simultaneously bind the protein and the alignment region of the telomerase RNA. The role of yeast Cdc13p in recruiting telomerase was described above. The Protection of Telomeres (POT1) protein appears to be the analog of Cdc13 in fission yeast, plants, mice, and humans (Baumann and Cech, 2001). A recent X-ray

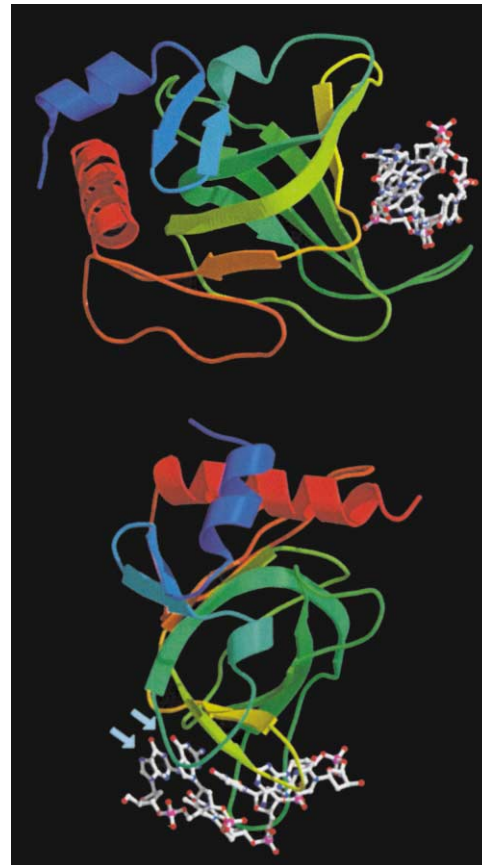


Figure 4. Pot1 (Protection of Telomeres) Protein from *S. pombe* Binds Single-Stranded Telomeric DNA (GGTTAC)

Two views of the X-ray crystal structure of the complex are shown (Lei et al., 2003). The six bases are bound as three stacked pairs; arrows indicate the 5'-most pair (GG).

structure shows the ssDNA compacted and sequestered within the protein (Figure 4; Lei et al., 2003), consistent with human POT1 acting as a repressor of telomerase (Loayza and de Lange, 2003). Under other conditions, human POT1 can stimulate telomere elongation (Colgin et al., 2003), perhaps in analogy to yeast Cdc13p. Telomeric single-stranded DNA binding proteins may also control the action of nucleases that generate the G strand overhangs at chromosome ends (Jacob et al., 2003).

(4) Single-stranded telomeric DNA tails also become resistant to telomerase extension when they fold into quadruplex structures, a proclivity of guanine-rich sequences. The extent to which this occurs in vivo is unknown. However, small molecules that bind to quadruplex structures can push the equilibrium toward this folded form, providing a credible approach to telomerase inhibition (Kim et al., 2002).

Cellular Immortality

Telomeres shorten during serial passage of human fibroblasts in vitro (Harley et al., 1990). Early proposals that telomere length determines the number of cell divisions a cell can undergo—the Hayflick Limit—were based on

such correlation between telomere length and proliferative potential. The availability of *hTERT* allowed a direct test of this proposal. When *hTERT* was transfected into fibroblasts or retinal epithelial cells, they had a greatly extended lifespan, apparently limitless, while control cells transfected with empty vector underwent senescence at the Hayflick Limit as expected (Bodnar et al., 1998). This apparent immortalization differed from oncogenic transformation in that the *hTERT*-transfected cells did not develop chromosome abnormalities, were unable to grow on soft agar, and were not tumorigenic. T lymphocytes also achieved dramatic extension of their replicative lifespan upon ectopic expression of *hTERT* (Hooijberg et al., 2000; Rufer et al., 2001). Mammary epithelial cells and keratinocytes, on the other hand, required inactivation of the Rb/p16 tumor suppressor pathway in addition to activation of *hTERT* in order to achieve extended lifespan (Kiyono et al., 1998).

This simple picture—repression of human telomerase initiates telomere shortening, telomere length then serves as a yardstick for proliferative potential—now appears incomplete. For example, overexpression of *TRF2* in primary human fibroblasts uncouples telomere shortening from senescence (Karlseder et al., 2002). Moreover, dividing primary human fibroblasts, which show progressive telomere shortening, nevertheless have recently been shown to have low levels of *hTERT* expression and telomerase activity. Disruption of this activity by ectopic expression of a catalytically inactive mutant of *hTERT* (DN-*hTERT*) or by RNA interference (RNAi) leads to premature senescence (Masutomi et al., 2003).

Cancer

Human cancers are invariably associated with activation of some mechanism to maintain telomere length: approximately 85%–90% show reactivation of telomerase, while the remainder maintain telomeres by ALT (alternative lengthening of telomeres), which occurs by exchange of sequences between telomeres (Dunham et al., 2000). Hahn et al. (1999a, 2002) have shown that one pathway to transformation of cultured human cells involves three steps: activation of proliferation, e.g., induced by expression of a mutant *ras* oncogene and the SV40 small t antigen; inactivation of tumor suppressors p53 and Rb; and activation of telomerase by expression of *hTERT*. This differs from the situation with rodent cells, which can be transformed by the first two events alone. Lin and Elledge (2003) achieved transformation of human cells by a slightly different pathway, inactivating the *hTERT* repressor, Menin, instead of expressing *hTERT* ectopically. Thus, telomerase activation may not be just a marker for neoplastic growth in humans, but a causal event. This makes telomerase an attractive target for pharmaceutical development of anti-cancer chemotherapeutics.

As an initial “proof of principle” of the usefulness of telomerase inhibition, two groups have shown that ectopic expression of DN (dominant-negative) mutants of *hTERT* in transformed human cells lead to growth inhibition and apoptosis (Hahn et al., 1999b; Zhang et al., 1999). A very different result was obtained with a synthetic non-nucleoside drug candidate that inhibits telo-

merase. The drug induces senescence rather than apoptosis in human cancer cells, and the inhibition of both cell growth and telomerase are fully reversible upon removal of the drug (Damm et al., 2001). Although the difference in results could be due to the different cell lines used, it is also possible that the way in which telomerase is inhibited is the decisive factor. DN-TERT may inhibit by titrating telomerase or telomere components from the chromosome end, thereby perturbing capping, while the small molecule inhibitor may simply decrease the catalytic activity of the telomerase enzyme or its protein-protein contacts. For this reason, it will be interesting to test whether small molecule inhibitors of catalytic activity are less toxic for normal cells than DN-TERT or RNAi, which may be more likely to perturb chromosome capping.

Acknowledgments

I thank Bob Weinberg, Bill Hahn, and Carol Greider for helpful comments and Ming Lei and David Zappulla for preparation of illustrations.

References

- Aigner, S., Postberg, J., Lipps, H.J., and Cech, T.R. (2003). The *Euplotes* La motif protein p43 has properties of a telomerase-specific subunit. *Biochemistry* 42, 5736–5747.
- Baumann, P., and Cech, T.R. (2001). Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science* 292, 1171–1175.
- Blackburn, E.H. (2001). Switching and signaling at the telomere. *Cell* 106, 661–673.
- Blasco, M.A., Lee, H.W., Hande, M.P., Samper, E., Lansdorp, P.M., DePinho, R.A., and Greider, C.W. (1997). Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* 91, 25–34.
- Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S., and Wright, W.E. (1998). Extension of life-span by introduction of telomerase into normal human cells. *Science* 279, 349–352.
- Bryan, T.M., Goodrich, K.J., and Cech, T.R. (2000). Telomerase RNA bound by protein motifs specific to telomerase reverse transcriptase. *Mol. Cell* 6, 493–499.
- Bryan, T.M., Goodrich, K.J., and Cech, T.R. (2003). *Tetrahymena* telomerase is active as a monomer. *Mol. Biol. Cell* 14, 4794–4804.
- Cech, T.R. (1994). Chromosome end games. *Science* 266, 387–388.
- Chen, J.-L., and Greider, C.W. (2003). Template boundary definition in mammalian telomerase. *Genes Dev.* 27, 2747–2752.
- Chen, J.-L., Blasco, M.A., and Greider, C.W. (2000). Secondary structure of vertebrate telomerase RNA. *Cell* 100, 503–514.
- Chen, J.-L., Opperman, K.K., and Greider, C.W. (2002). A critical stem-loop structure in the CR4–CR5 domain of mammalian telomerase RNA. *Nucleic Acids Res.* 30, 592–597.
- Colgin, L.M., Baran, K., Baumann, P., Cech, T.R., and Reddel, R.R. (2003). Human POT1 is a positive regulator of telomere length. *Curr. Biol.* 13, 942–946.
- Collins, K., and Mitchell, J.R. (2002). Telomerase in the human organism. *Oncogene* 21, 564–579.
- Damm, K., Hemmann, U., Garin-Chesa, P., Huel, N., Kauffmann, I., Priepke, H., Niestroj, C., Daiber, C., Enekel, B., Guilliard, B., et al. (2001). A highly selective telomerase inhibitor limiting human cancer cell proliferation. *EMBO J.* 20, 6958–6968.
- Dunham, M.A., Neumann, A.A., Fasching, C.L., and Reddel, R.R. (2000). Telomere maintenance by recombination in human cells. *Nat. Genet.* 26, 447–450.

- Feng, J., Funk, W.D., Wang, S., Weinrich, S.L., Avilion, A.A., Chiu, C., Adams, R.R., Chang, E., Allsopp, R.C., Yu, J., et al. (1995). The RNA component of human telomerase. *Science* 269, 1236–1241.
- Ferrezuelo, F., Steiner, B., Aldea, M., and Futcher, B. (2002). Biogenesis of yeast telomerase depends on the importin Mtr10. *Mol. Cell Biol.* 22, 6046–6055.
- Friedman, K.L., Heit, J.J., Long, D.L., and Cech, T.R. (2003). N-terminal domain of yeast telomerase reverse transcriptase: recruitment of Est3p to the telomerase complex. *Mol. Biol. Cell* 14, 1–13.
- Greider, C.W., and Blackburn, E.H. (1985). Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* 43, 405–413.
- Greider, C.W., and Blackburn, E.H. (1989). A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. *Nature* 337, 331–337.
- Griffith, J.D., Comeau, L., Rosenfield, S., Stansel, R.M., Bianchi, A., Moss, H., and de Lange, T. (1999). Mammalian telomeres end in a large duplex loop. *Cell* 97, 503–514.
- Hahn, W.C., Counter, C.M., Lundberg, A.S., Beijersbergen, R.L., Brooks, M.W., and Weinberg, R.A. (1999a). Creation of human tumour cells with defined genetic elements. *Nature* 400, 464–468.
- Hahn, W.C., Stewart, S.A., Brooks, M.W., York, S.G., Eaton, E.N., Kurachi, A., Beijersbergen, R.L., Knoll, J.H.M., Meyerson, M., and Weinberg, R.A. (1999b). Inhibition of telomerase limits the growth of human cancer cells. *Nat. Med.* 5, 1164–1170.
- Hahn, W.C., Dessain, S.K., Brooks, M.W., King, J.E., Elenbaas, B., Sabatini, D.M., DeCaprio, J.A., and Weinberg, R.A. (2002). Enumeration of the simian virus 40 early region elements necessary for human cell transformation. *Mol. Cell Biol.* 22, 2111–2123.
- Hall, S.S. (2003). *Merchants of Immortality* (New York: Houghton Mifflin Company).
- Harley, C.B., Futcher, A.B., and Greider, C.W. (1990). Telomeres shorten during ageing of human fibroblasts. *Nature* 345, 458–460.
- Hooijberg, E., Ruizendaal, J.J., Snijders, P.J., Kueter, E.W., Wal-boomers, J.M., and Spits, H. (2000). Immortalization of human CD8+ T cell clones by ectopic expression of telomerase reverse transcriptase. *J. Immunol.* 165, 4239–4245.
- Jacob, N.K., Kirk, K.E., and Price, C.M. (2003). Generation of telomeric G strand overhangs involves both G and C strand cleavage. *Mol. Cell* 11, 1021–1032.
- Kanoh, J., and Ishikawa, F. (2001). spRap1 and spRif1, recruited to telomeres by Taz1, are essential for telomere function in fission yeast. *Curr. Biol.* 11, 1624–1630.
- Karlseder, J., Smogorzewska, A., and de Lange, T. (2002). Senescence induced by altered telomere state, not telomere loss. *Science* 295, 2446–2449.
- Kim, S.H., Kaminker, P., and Campisi, J. (1999). TIN2, a new regulator of telomere length in human cells. *Nat. Genet.* 23, 405–412.
- Kim, M.Y., Vankayalapati, H., Shin-Ya, K., Wierzb, K., and Hurley, L.H. (2002). Telomestatin, a potent telomerase inhibitor that interacts quite specifically with the human telomeric intramolecular G-quadruplex. *J. Am. Chem. Soc.* 124, 2098–2099.
- Kiyono, T., Foster, S.A., Koop, J.I., McDougall, J.K., Galloway, D.A., and Klingelutz, A.J. (1998). Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature* 396, 84–88.
- Lei, M., Podell, E., Baumann, P., and Cech, T.R. (2003). DNA self-recognition in the structure of Pot1 bound to telomeric single-stranded DNA. *Nature* 426, 198–203.
- Lendvay, T.S., Morris, D.K., Sah, J., Balasubramanian, B., and Lundblad, V. (1996). Senescence mutants of *Saccharomyces cerevisiae* with a defect in telomere replication identify three additional EST genes. *Genetics* 144, 1399–1412.
- Li, B., Oestreich, S., and de Lange, T. (2000). Identification of human Rap1: implications for telomere evolution. *Cell* 101, 471–483.
- Lin, S.-Y., and Elledge, S.J. (2003). Multiple tumor suppressor pathways negatively regulate telomerase. *Cell* 113, 881–889.
- Lingner, J., and Cech, T.R. (1996). Purification of telomerase from *Euplotes aediculatus*: Requirement of a primer 3' overhang. *Proc. Natl. Acad. Sci. USA* 93, 10712–10717.
- Lingner, J., Hughes, T.R., Shevchenko, A., Mann, M., Lundblad, V., and Cech, T.R. (1997). Reverse transcriptase motifs in the catalytic subunit of telomerase. *Science* 276, 561–567.
- Livengood, A.J., Zaug, A.J., and Cech, T.R. (2002). Essential regions of *Saccharomyces cerevisiae* telomerase RNA: separate elements for Est1p and Est2p interaction. *Mol. Cell Biol.* 22, 2366–2374.
- Loayza, D., and de Lange, T. (2003). POT1 as a terminal transducer of TRF1 telomere length control. *Nature* 424, 1013–1018.
- Lundblad, V., and Szostak, J.W. (1989). A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell* 57, 633–643.
- Ly, H., Xu, L., Rivera, M.A., Parslow, T.G., and Blackburn, E.H. (2003). A role for a novel “trans-pseudoknot” RNA-RNA-interaction in the functional dimerization of human telomerase. *Genes Dev.* 17, 1078–1083.
- Marcand, S., Gilson, E., and Shore, D. (1997). A protein-counting mechanism for telomere length regulation in yeast. *Science* 275, 986–990.
- Masutomi, K., Yu, E.Y., Khurts, S., Ben-Porath, I., Currier, J.L., Metz, G.B., Brooks, M.W., Kaneko, S., Murakami, S., DeCaprio, J.A., et al. (2003). Telomerase maintains telomere structure in normal human cells. *Cell* 114, 241–253.
- Meyerson, M., Counter, C.M., Eaton, E.N., Ellisen, L.W., Steiner, P., Caddle, S.D., Ziaugra, L., Beijersbergen, R.L., Davidoff, M.J., Liu, Q., et al. (1997). *hEST2*, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 90, 785–795.
- Miller, M.C., and Collins, K. (2002). Telomerase recognizes its template by using an adjacent RNA motif. *Proc. Natl. Acad. Sci. USA* 99, 6585–6590.
- Mitchell, J.R., and Collins, K. (2000). Human telomerase activation requires two independent interactions between telomerase RNA and telomerase reverse transcriptase *in vivo* and *in vitro*. *Mol. Cell* 6, 361–371.
- Mitchell, J.R., Wood, E., and Collins, K. (1999). A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* 402, 551–555.
- Nakamura, T.M., Morin, G.B., Chapman, K.B., Weinrich, S.L., Andrews, W.H., Lingner, J., Harley, C.B., and Cech, T.R. (1997). Telomerase catalytic subunit homologs from fission yeast and human. *Science* 277, 955–959.
- Pennock, E., Buckley, K., and Lundblad, V. (2001). Cdc13 delivers separate complexes to the telomere for end protection and replication. *Cell* 104, 387–396.
- Pluta, A.F., Dani, G.M., Spear, B.B., and Zakian, V.A. (1984). Elaboration of telomeres in yeast: recognition and modification of termini from *Oxytricha* macronuclear DNA. *Proc. Natl. Acad. Sci. USA* 81, 1475–1479.
- Pogacic, V., Dragon, F., and Filipowicz, W. (2000). Human H/ACA small nucleolar RNPs and telomerase share evolutionarily conserved proteins NHP2 and NOP10. *Mol. Cell Biol.* 20, 9028–9040.
- Ray, A., and Runge, K.W. (1999). The yeast telomere length counting machinery is sensitive to sequences at the telomere-nontelomere junction. *Mol. Cell Biol.* 19, 31–45.
- Reichenbach, P., Hoss, M., Azzalin, C.M., Nabholz, M., Bucher, P., and Lingner, J. (2003). A human homolog of yeast est1 associates with telomerase and uncaps chromosome ends when overexpressed. *Curr. Biol.* 13, 568–574.
- Rufer, N., Migliaccio, M., Antonchuk, J., Humphries, R.K., Roosnek, E., and Lansdorf, P.M. (2001). Transfer of the human telomerase reverse transcriptase (TERT) gene into T lymphocytes results in extension of replicative potential. *Blood* 98, 597–603.
- Sadaie, M., Naito, T., and Ishikawa, F. (2003). Stable inheritance of telomere chromatin structure and function in the absence of telomeric repeats. *Genes Dev.* 17, 2271–2282.
- Seto, A.G., Zaug, A.J., Sobel, S.G., Wolin, S.L., and Cech, T.R. (1999).

Saccharomyces cerevisiae telomerase is an Sm small nuclear ribonucleoprotein particle. *Nature* 401, 177–180.

Seto, A.G., Livengood, A.J., Tzfati, Y., Blackburn, E.H., and Cech, T.R. (2002). A bulged stem tethers Est1p to telomerase RNA in budding yeast. *Genes Dev.* 16, 2800–2812.

Shampay, J., Szostak, J.W., and Blackburn, E.H. (1984). DNA sequences of telomeres maintained in yeast. *Nature* 310, 154–157.

Smogorzewska, A., van Steensel, B., Bianchi, A., Oelmann, S., Schaefer, M.R., Schnapp, G., and de Lange, T. (2000). Control of human telomere length by TRF1 and TRF2. *Mol. Cell. Biol.* 20, 1659–1668.

Snow, B.E., Erdmann, N., Cruickshank, J., Goldman, H., Gill, R.M., Robinson, M.O., and Harrington, L. (2003). Functional conservation of the telomerase protein est1p in humans. *Curr. Biol.* 13, 698–704.

Stellwagen, A.E., Haimberger, Z.W., Veatch, J.R., and Gottschling, D.E. (2003). Ku interacts with telomerase RNA to promote telomere addition at native and broken chromosome ends. *Genes Dev.* 17, 1–11.

Szostak, J.W., and Blackburn, E.H. (1982). Cloning yeast telomeres on linear DNA plasmid vectors. *Cell* 29, 245–255.

Szutorisz, H., Lingner, J., Cuthbert, A.P., Trott, D.A., Newbold, R.F., and Nabholz, M. (2003). A chromosome 3-encoded repressor of the human telomerase reverse transcriptase (*hTERT*) gene controls the state of *hTERT* chromatin. *Cancer Res.* 63, 689–695.

Taggart, A.K.P., Teng, S.-C., and Zakian, V.A. (2002). Est1p as a cell cycle-regulated activator of telomere-bound telomerase. *Science* 297, 1023–1026.

Teixeira, M.T., Förstemann, K., Gasser, S.M., and Lingner, J. (2002). Intracellular trafficking of yeast telomerase components. *EMBO Rep.* 3, 652–659.

Tzfati, Y., Fulton, T.B., Roy, J., and Blackburn, E.H. (2000). Template boundary in a yeast telomerase specified by RNA structure. *Science* 288, 863–867.

Ulaner, G.A., Hu, J., Vu, T.H., Giudice, L.C., and Hoffman, A.R. (1998). Telomerase activity in human development is regulated by *hTERT* transcription and by alternate splicing. *Cancer Res.* 58, 4168–4172.

Vulliamy, T., Marrone, A., Goldman, F., Dearlove, A., Bessler, M., Mason, P.J., and Dokal, I. (2001). The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. *Nature* 413, 432–435.

Wright, W.E., Piatyszek, M.A., Rainey, W.E., Byrd, W., and Shay, J.W. (1996). Telomerase activity in human germline and embryonic tissues and cells. *Dev. Genet.* 18, 173–179.

Yu, G.L., Bradley, J.D., Attardi, L.D., and Blackburn, E.H. (1990). *In vivo* alteration of telomere sequences and senescence caused by mutated *Tetrahymena* telomerase RNAs. *Nature* 344, 126–132.

Zhang, X., Mar, V., Zhou, W., Harrington, L., and Robinson, M.O. (1999). Telomere shortening and apoptosis in telomere-inhibited human tumor cells. *Genes Dev.* 13, 2388–2399.