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Switching and Signaling at the Telomere

Review

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This review describes the structure of telomeres, the protective DNA-protein complexes at eukaryotic chromosomal ends, and several molecular mechanisms involved in telomere functions. Also discussed are cellular responses to compromising the functions of telomeres and of telomerase, which synthesizes telomeric DNA.

Introduction

Telomeres are specialized functional complexes that protect the ends of eukaryotic chromosomes. This capping function of telomeres was originally deduced from classic cytogenetic studies. McClintock and Muller, using maize and flies, respectively, functionally defined capping as protection from chromosomal fusion (end-to-end joining) and its consequence: deleterious genomic instability (for references, see Levis, 1989). Because they contain DNA ends, telomeres must avoid eliciting the downstream events that are normally set in train by the cellular alarm bells that sound when DNA is broken. Instead, telomeres actually exploit some of the same cellular machinery that responds to such a threat to the genomic DNA, harnessing it to serve their own maintenance.

Telomeres must also be fully replicated. Linear DNA molecules such as those of eukaryotic chromosomes require mechanisms besides the conventional DNA polymerases to complete the replication of their ends. It was predicted that without such a mechanism, terminal attrition of chromosomal DNA would eventually lead to loss of genetic information and prevent cells from multiplying (cellular "senescence"). In most eukaryotes, this problem is solved by having the ribonucleoprotein enzyme telomerase, which lengthens terminal regions of telomeric DNA by RNA-templated addition of tandemly repeated, often G-rich, telomeric sequences (Greider and Blackburn, 1985; Yu et al., 1990). Although telomeric sequences vary between species, fundamentally, telomeres and telomerase function very similarly throughout the eukaryotes. Telomeres typically consist of a tandem array of telomeric DNA repeats, specified by copying of the template sequence within the telomerase RNA. These DNA repeats bind a set of sequence-specific DNA binding proteins that, through separate domains, bind additional proteins to assemble an inferred higher-order complex nucleated on the telomeric DNA repeats. By now, many molecular components of telomerase and telomeres are known. The list is still growing, however, with increasing examples of homologs of proteins that were previously found in only one group of eukaryotes now being found to be widespread.

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This review will focus on the telomere, and how it accomplishes its essential protective functions. The components of telomeres are reviewed first. Then, current information from a variety of experiments on the mechanisms that protect telomeres is discussed. The actions of telomerase and other enzymes that act on telomeres have to be controlled, and functional telomeres prevent, or strictly control, at least four distinct biochemical processes that happen at telomeric DNA in vivo: (1) fusion with another telomere or DNA end; (2) replication of telomeric sequences by telomerase; (3) degradation of telomeric DNA; and (4) recombination at and near a telomere. Such controls are integral to functional telomere capping. An overall theme that has emerged from many studies is that capping and protection of telomeres are provided by a set of multiple interactions, involving different kinds of molecular components that include: (1) the higher-order DNA protein complex structure on the entire telomeric repeat tract, which is influenced by telomere length; (2) subtelomere heterochromatization involving nucleosomal chromatin; (3) the protein complex on the most terminal double-stranded telomeric DNA repeats; (4) the DNA-protein complexes formed on the single-stranded overhang of the telomere; (5) looping back of the telomeric DNA into the duplex telomeric DNA tract; and (6) the components or structure of telomerase itself.

A defining feature of these experimentally separable contributors to telomere protection and regulation is that they mutually reinforce one another: compromising one can be harmless, but disrupting two or more can cause catastrophic loss of telomere function. The relative importance of any one component depends on the status of the others. Thus, as is now increasingly being found for other cellular and biological processes, the contributors to telomere functions normally act in a partially overlapping or mutually reinforcing manner.

Components of the Telomere/Telomerase Functional Complex

Telomeric DNA

The essential telomeric DNA sequences at each end of the eukaryotic linear chromosomes are, in most species, tandem repeats of a short sequence unit. Telomerase adds multiple copies of this DNA unit to the terminal portion of one strand of the repeat tract. Some telomeric repeats, like those of vertebrates, plants, certain molds, and some protozoans, are perfect repeats, such as the T_2AG_3 repeats of human or slime mold telomeres. Others, such as those in budding and fission yeasts and certain protozoans and slime molds, are irregular, an example being the irregular $G_{1\text{-}3}T/C_{1\text{-}3}A$ repeat sequences of the budding yeast *Saccharomyces cerevisiae* (reviewed in Blackburn, 1998).

The telomeric DNA in ciliates, a slime mold, yeast, and humans ends in a 3′ single-stranded overhang, comprising a few to several repeats of the telomerase-synthesized DNA strand (Hemann and Greider, 1999; and references therein). In *S. cerevisiae*, the length of

the overhang has been shown to be cell-cycle regulated, and increases in S phase (Wellinger et al., 1993). When human or trypanosome telomeric DNA is extracted and purified away from its endogenously bound proteins, the 3′ single-stranded overhang of a fraction of the telomeres is found tucked far back into the telomeric tract, by base pairing into the duplex DNA, forming a loop in vitro ("T-loop") (Griffith et al., 1999; Muñoz-Jordán et al., 2001).

In dividing cells with telomerase, the end of the telomeric DNA is in a state of flux, and turns over because of continuous additions and losses; factors affecting the balance between the two are discussed below. Such turnover was directly demonstrated by altering the template sequence within the telomerase RNA in the budding yeast *Kluyveromyces lactis*. This caused the corresponding altered telomeric DNA sequence to be added to telomeric ends in vivo. With a phenotypically silent template mutation, it was possible to monitor the normal action of telomerase over many cell divisions and to observe a gradual replacement of the terminal region of telomeres, at an average rate of 1–3 bp per generation (McEachern and Blackburn, 1995; Roy et al., 1998).

Subtelomeric sequences next to the terminal telomeric repeats often are also tandemly repeated and shared between telomeres, but can differ greatly between species. Homogeneity of tandem subtelomeric repeats within a species is apparently maintained by unequal crossing-over events. Telomere-adjacent sequences can take many forms: long complex tandem repeats, elements that have their apparent origins as transposable elements (e.g., the Y′ elements of *S. cerevisiae*) (Pryde et al., 1997), simple perfect repeats such as the ~1 kb of tandem T_3G_4 repeats adjoining the germline nuclear telomeres in the ciliate *Tetrahymena*, or degenerate variant telomeric repeat sequences that concentrate toward the centromere-proximal border of the repeat tracts in telomeres of the plant *Arabidopsis* and humans (reviewed in McEachern et al., 2000b).

Some eukaryotic species have apparently completely lost the telomerase-mediated mode of telomeric DNA maintenance during evolution, yet they have functional telomeres. In these organisms, the telomeric DNA is composed of other types of sequences, which provide exceptions to the usual type of canonical telomeric repeats. In one such well-studied case, the fruit fly *Drosophila melanogaster*, telomeres are primarily composed of a complex mosaic of large, non-LTR-type retrotransposons called HeT-A and TART elements (reviewed in Pardue and DeBaryshe, 1999). Sporadically, one of these retroposons is added onto the very termini of chromosomes by a variant retrotransposition mechanism, counteracting over time the gradual sequence loss from chromosome ends. A different telomerase-independent mechanism may normally be used to maintain telomeres in the midge *Chironomus tentans*, the mosquito *Anopheles gambiae*, and the onion *Allium cepa* (reviewed in McEachern et al., 2000b). Their telomeric regions lack the short telomerase-type telomeric repeats and instead appear to consist only of large arrays of complex-sequence tandem repeats. These repeats extend to the very end of the telomere and have the characteristics expected if they are maintained by recombination involving unequal homologous crossing-over and/or gene conversion: rapid species divergence and homogenization of repeats within an array.

Telomeric DNA Binding Proteins

In canonical telomeres (those maintained by telomerase), the terminal portion of the double-stranded telomeric DNA repeat tract exists within a nonnucleosomal DNA-protein complex, as judged by micrococcal nuclease digestion and salt disruption properties in *Tetrahymena*, *S. cerevisiae*, and vertebrates, including humans (Cohen and Blackburn, 1998; and references therein). A minority of the telomeres of *Tetrahymena*, however, package the inner region of the repeat tract as tightly spaced nucleosomes. In vertebrates, the nonnucleosomal portion of the telomeric repeat tract may also be confined to the distal end, since most of the telomeric repeat DNA is packaged as tightly spaced nucleosomes.

Various telomeric DNA binding structural proteins have been identified, and evolutionarily conserved classes are emerging (Figure 1). In budding yeasts such as *S. cerevisiae*, the nonnucleosomal complex includes the proteins Rap1p and Tel2p, which bind sequence specifically to the double-stranded telomeric repeat DNA (reviewed in Evans and Lundblad, 2000). Duplex telomeric repeats in the fission yeast *Schizosaccharomyces pombe* are bound by Taz1, and in mammals by TRF1 and TRF2 (see Li et al., 2000 for references). In vitro, the human telomeric sequence-specific binding protein TRF2 binds to the "T-loop". It has been proposed that this binding helps protect the telomeric end in vivo (Griffith et al., 1999).

Additional DNA sequence-specific proteins also directly bind and protect the G-rich, single-stranded DNA 3′ overhang. These protective proteins include the α/β protein in ciliates, the α protein homolog Pot1 in fission yeast and humans, and Cdc13p in *S. cerevisiae* (see Hemann and Greider, 1999, for references; Baumann and Cech, 2001). Cdc13p has two functions: it binds the proteins Stn1p and Ten1p to protect the telomere from degradation in vivo, and also recruits telomerase by a direct interaction with the telomerase subunit protein Est1p (Pennock et al., 2001; and references therein).

The Higher-Order Telomeric DNA-Protein Complex

Proteins bound along the repeat tract of telomeric DNA assemble additional proteins to form a higher-order nucleoprotein complex (reviewed in Gasser, 2000) (Figure 1). The C-terminal domain of Rap1p is functionally separable from its DNA binding domain and interacts with the proteins Rif1p and Rif2p (Wotton and Shore, 1997). The Rap1p carboxy terminus also binds the proteins Sir3p and Sir4p, which in turn complex with Sir2 protein (Moazed et al., 1997). This Sir-containing complex mediates silencing of a gene placed near telomeres or near the silent mating type loci (Kyrion et al., 1992). Unlike yeast Rap1p, human Rap1p does not directly bind DNA in vitro, but appears to interact with telomeres via a protein-protein interaction with the telomeric DNA binding protein TRF2 (Li et al., 2000). Two other human telomeric proteins, TIN2 and the DNA repair protein Ku, interact with telomeres via TRF1 binding (Hsu et al., 2000; Kim et al., 1999).

Additional protein-protein interactions mediate the interactions of the telomere with structural components of the cell nucleus. Taz1 (and likely other proteins) medi-

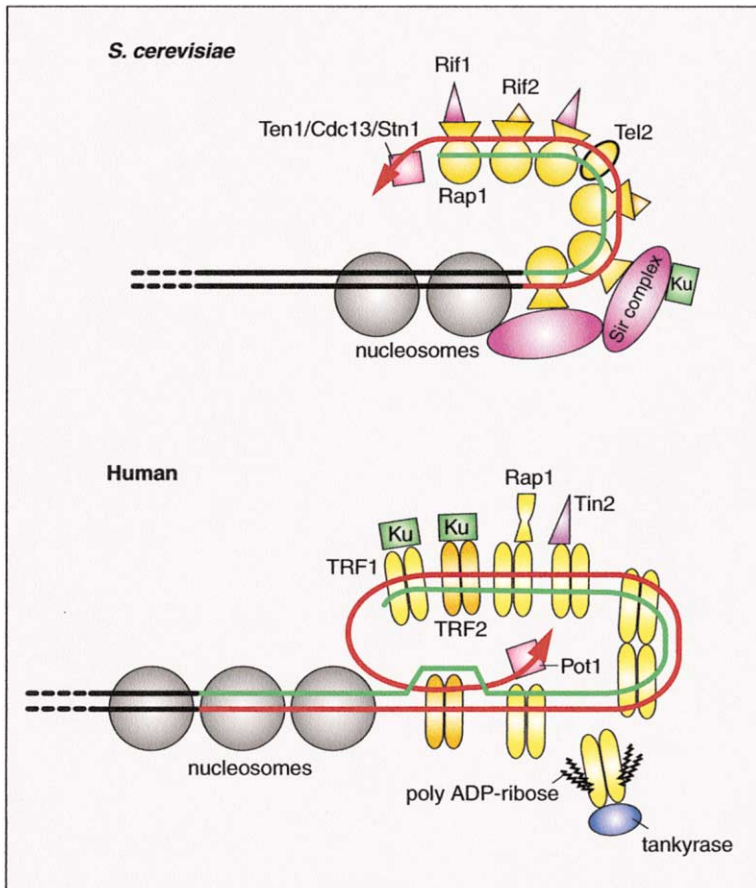

Figure 1. Examples of Demonstrated or Inferred Interactions among the Components of the Structural DNA-Protein Complexes Comprising the Telomeres in Budding Yeast (top) and Humans (bottom)

Red line: G-rich telomeric repeat strand synthesized by telomerase (telomerase, not shown here, interacts with and extends the 3′ single-stranded overhang indicated by the arrow); green line, complementary C-rich strand of the telomeric repeats; heavy black lines, subtelomeric DNA. The 3′ terminal single-stranded telomeric DNA in human telomeres might be in alternative forms: either bound by Pot1 protein or engaged in T-loop formation. See text for further description.

ate the meiosis-specific interaction of telomeres with the spindle pole body in *S. pombe* (Chikashige et al., 1994; Cooper et al., 1998). In budding yeast, Sir proteins mediate the localization of telomeres to the nuclear periphery; Sir4p interacts with Ku, and Ku interacts with the nuclear pore protein Mlp2 (Galy et al., 2000; and references therein).

Telomerase and Associated Proteins

Essential conserved core components of telomerase include the reverse transcriptase protein family member TERT and the telomerase RNA TER (reviewed in Blackburn, 2000b). Although recombinant TERT plus TER are sufficient to reconstitute activity from a rabbit reticulocyte lysate system in vitro, other proteins associate with telomerase and at least some of them are required for its action in vivo (reviewed in Evans et al., 1999). In yeast, the interaction of the telomerase RNP with telomeres is mediated by the RNA binding protein Est1p, which interacts with the essential single-stranded telomeric DNA binding protein Cdc13p to recruit telomerase to the telomere (reviewed in Evans and Lundblad, 2000). Telomerase is minimally dimeric, with two active sites and two functionally interacting RNAs (Prescott and Blackburn, 1997a; Wenz et al., 2001). The dimeric structure of telomerase might hold two chromatids or chromosomes together or act on two newly replicated chromatids. How the higher-order structure of the RNP helps coordinate telomerase action at the telomere with other in vivo processes, including telomeric DNA replication, is a question of current interest.

The La protein that matures various small RNAs is present in a ciliate telomerase; Sm proteins, which stabilize RNA-protein interactions (Zhang et al., 2001), and Est3p are associated with budding yeast telomerase RNP; and Tep1, dyskerin, a Staufen homolog, ribosomal L22 protein, and foldasome pathway proteins are all found to associate with mammalian telomerase or TER, although it is not clear if they all bind at the same time (reviewed in Blackburn, 2000b). The variety of these telomerase-associated factors, which are apparently involved in the biogenesis and assembly of telomerase, contrasts with the evolutionary conservation of the structural motifs in TERT and TER.

Telomere Length Homeostasis through Dynamic Switching of Telomeres between Two States

In cells containing active telomerase, although the length of the telomeric DNA repeat tract in a population of telomeres is heterogeneous even within a single cell (Lansdorp et al., 1996), length is normally regulated and kept within well-defined limits (telomere homeostasis) (Shampay and Blackburn, 1988). The following model has arisen from experiments described below: the higher-order telomeric DNA-protein complex normally rapidly interconverts, in a regulated and dynamic way, between two physical states. One (temporarily uncapped) can become competent for telomerase action, and the other (capped) is inaccessible to telomerase. Rounds of incomplete replication and/or degradation at

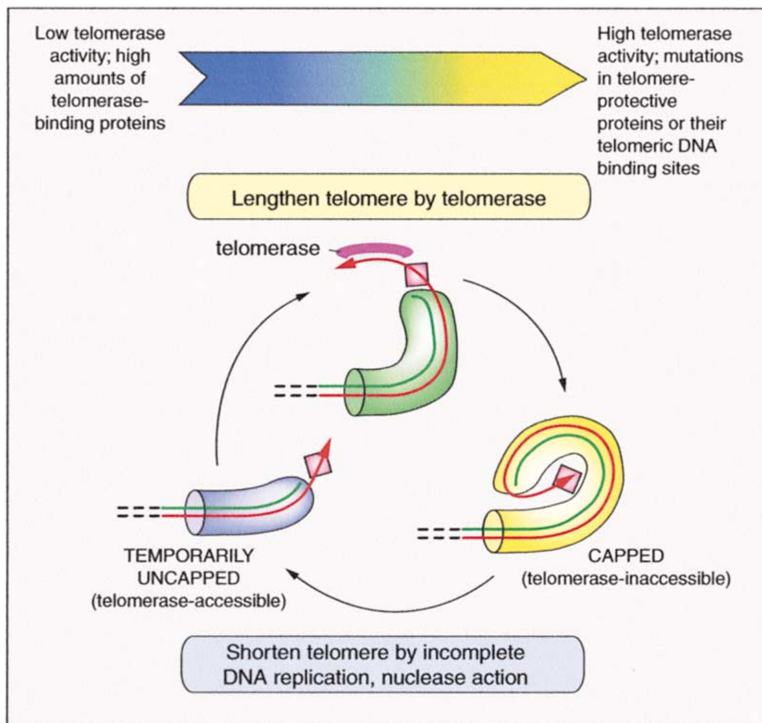

Figure 2. Changes Accompanying the Cycle of Capping (telomerase-inaccessible state) and Temporary Uncapping (telomerase-accessible state) of Telomeres in Dividing Cells with Telomerase (McEachern and Blackburn, 1995)

A shortened, temporarily uncapped telomeric DNA-protein complex (lower left) becomes competent for telomerase action (telomerase-accessible). The telomeric complex then becomes lengthened by telomerase (top) until it can assume a telomeric structure that is telomerase-inaccessible and therefore capped (lower right). Such capping can persist for many cell divisions even without further lengthening of the telomere by telomerase, until the telomere shortens enough to temporarily uncap again. In a normal situation, in a cell with telomerase, such temporary uncapping elicits a response involving ATM (Tel1) kinase, which acts on the telomere to render it competent for telomerase action (at least in part through phosphorylating the Rad50/Mre11/Xrs2 (NBS1) complex). See text for further description.

chromosome ends result in telomere shortening, and increase the probability of uncapping. A shortened telomere temporarily uncaps and is acted on by telomerase. Once lengthened by telomerase, the telomere has an increased probability of switching to the capped structural state. This uncapping-capping cycle keeps telomere length distributions confined within upper and lower limits (Figure 2).

The balance between lengthening and shortening that determines the mean length and length distribution of telomeres is influenced by multiple factors: genetic, developmental (reviewed in Blackburn, 2000a), and physiological (von Zglinicki et al., 2000). At least two kinds of factors—telomerase activity and components of the telomere itself—have been investigated experimentally. For example, the balance is influenced by changing the level of telomerase: cells of mice with one copy of the TERT gene knocked out show shortened telomeres (Liu et al., 2000). Also, partially crippling telomerase in vivo, either by use of nucleoside analog inhibitors (in *Tetrahymena* and human lymphoid cell lines) (Strahl and Blackburn, 1994, 1996) or by certain mutations in the RNA component of telomerase (in *K. lactis* and *S. cerevisiae*) (Prescott and Blackburn, 1997b; Roy et al., 1998), causes the telomeres to shorten initially, although then they are stably maintained at a new equilibrium length. Conversely, ectopic overexpression of hTERT can cause telomere lengthening or shortening, and then reequilibration about a new mean length, with telomeres becoming resilient to further length change as the cells continue to divide (Bodnar et al., 1998; Counter et al., 1998; Yang et al., 1999; Zhu et al., 1999). The concept that has emerged is that in dividing cells with telomerase, the telomere-telomerase complex is a dynamic organelle, always being built up and shortened in a regulated way that maintains its length homeostasis.

Telomeres and Components of DNA Damage Response Pathways

Certain components of DNA damage response pathways, originally defined by their roles in the repair of chromosomal DNA breaks, are found at telomeres and are necessary for normal telomere maintenance and functions. Breaks in chromosomal DNA can be repaired by nonhomologous end-joining (NHEJ) or by homologous recombination. The protein Ku, involved in NHEJ, is localized to telomeres in budding yeasts and humans. In yeast, Ku is required for the normal localization of telomeres at the nuclear periphery (Galy et al., 2000) and for normal telomere maintenance; by genetic criteria, Ku acts, by a still unknown mechanism, to assist telomerase action (reviewed in Gasser, 2000; Peterson et al., 2001). Lack of Ku function leads to telomere-telomere fusions in fission yeast and mammals (Baumann and Cech, 2000; Samper et al., 2000).

Other components of DNA damage response pathways needed for telomere maintenance are the RAD50, MRE11, and XRS2 (yeast)/NBS1 (human) gene products. These form a complex thought to process broken DNA ends in preparation for their repair. In *S. cerevisiae*, mutating any of these genes leads to telomere shortening (Le et al., 1999). Human Rad50p is present at telomeres, whereas NBS1 is enriched during S phase, the cell cycle stage at which telomerase is thought to act (Zhu et al., 2000). The ATM (ataxia telangiectasia mutated) DNA damage response kinase (Tel1p in yeast) is another key component of telomere maintenance. In yeasts, a tel1 mutation causes marked telomere shortening; simultaneously mutating both Tel1p and the DNA damage response kinase Mec1p (or their homologs in *S. pombe*) produces cell senescence and telomere shortening like that seen in cells lacking active telomerase (Naito et al., 1998; Kim et al., 2000). Genetic experiments indicate that

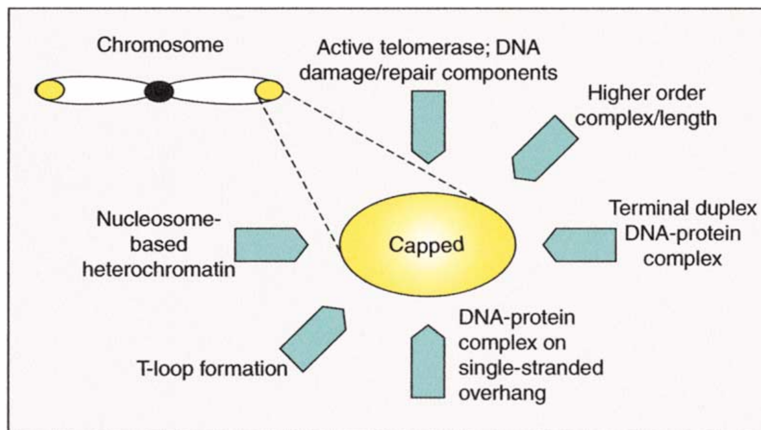

Figure 3. Mutually Reinforcing Mechanisms of Telomere Capping

Six factors contribute to the functional and structural capping of telomeres. See text for description.

Tel1p acts through the Rad50p-Mre11p-Xrs2p complex, which is needed for the telomerase-mediated pathway of telomere maintenance (Kim et al., 2000). In human cells, ATM kinase phosphorylates NBS1 (Lim et al., 2000). Hence, a plausible model is that ATM (Tel1p) targets Xrs2p/NBS1, thereby promoting telomerase action at the telomere, perhaps by activating processing of the DNA at the terminus of the telomere to make it an efficient substrate for telomerase. Consistent with such a model, in *S. cerevisiae*, the loss of ATM kinase function in telomere maintenance can be bypassed by mutations that compromise the normal telomeric DNA-protein complex in various ways, and the ATM kinases appear not to primarily affect the activity of the telomerase ribonucleoprotein enzyme itself (Chan et al., 2001). Finally, in *Caenorhabditis elegans*, the Mrt-2 gene, a homolog of the *S. cerevisiae* DNA damage checkpoint gene RAD17, is required for germline telomere maintenance; Mrt-2 mutant worms suffer progressive telomere shortening and fusions (for reviews see Blackburn, 2000a; Evans et al., 1999; Gasser, 2000).

All these results suggest that, in dividing cells with telomerase, a shortened telomere continually undergoes some regulated degree of temporary uncapping, provoking its being sensed as a DNA break. What is unknown is how this normally elicits telomerase action on that telomere, rather than NHEJ or recombination. It has been proposed that this exploitation of the DNA damage repair machinery allows cell cycle checkpoint control so that telomere integrity can be monitored (reviewed in Gasser, 2000), with the cell cycle being able to proceed only when telomere replication has been completed.

The structure of telomeres, and their ability to be functionally capped, both show meta-stable, epigenetic-like behavior. As described below, abrupt stochastic switches in telomere length regulation—between well-regulated and completely deregulated states—have been experimentally observed to be accompanied by changes in capping function. The sometimes extreme and irreversible changes seen in telomere behavior have underscored the mutually reinforcing nature of six experimentally distinguishable molecular players described next, which together determine the functionality of a telomere (Figure 3).

The Higher-Order DNA-Protein Complex on the Entire Telomeric Repeat Tract

In budding yeasts, Rap1p negatively regulates telomere length (Conrad et al., 1990; Krauskopf and Blackburn, 1996). The Rap1p C-terminal binding proteins Rif1p and Rif2p are important for this negative regulation; when they are deleted, telomeres lengthen considerably (Wotton and Shore, 1997; and references therein) in a telomerase-dependent manner (Chan et al., 2001; Teng et al., 2000). Mutations in the conserved C-terminal domain of Rap1p increase mean telomere length and accelerate telomerase action at telomeres (Krauskopf and Blackburn, 1996, 1998; Kyrion et al., 1992). Hence, the higher-order telomeric DNA-protein complex described above, which is nucleated on repeat-bound Rap1p molecules and therefore influenced by telomere length, negatively controls telomerase action at a telomere. In the budding yeast *K. lactis*, a small C-terminal tail truncation of Rap1p accelerated the turnover of the terminal telomeric repeats, but the slow turnover of the internal portion of the repeat tract remained unchanged (Krauskopf and Blackburn, 1998). It has been suggested that Sir proteins (whose deletion only slightly shortens telomeres (Palladino et al., 1993) bind Rap1p molecules on the inner portion of the telomeric tract, while Rif1 and Rif2 bind the Rap1p molecules occupying the outer portion (Wotton and Shore, 1997).

Similar negative regulation of human telomeres appears to be mediated by the binding of TRF1 and TRF2 (Smogorzewska et al., 2000) and their associated proteins. For example, overexpression of TRF1 in cultured telomerase-positive cells causes telomeres to shorten (Li et al., 2000). Overexpression of partially deleted, dominant-negative forms of TIN2 protein causes telomerase-dependent telomere lengthening, presumably because formation of the normal higher-order complex is blocked (Kim et al., 1999). The telomeric repeat tract may fold back on itself, as suggested by the ability of TRF1 and TIN2 to induce intertelomeric clustering in vitro and in vivo (Griffith et al., 1998; Kim et al., 1999). As described earlier, the telomere ends in a single-stranded overhang (of the DNA strand made by telomerase). It has been proposed for human and trypanosome telomeres that this overhang can insert into the inner region of the telomere (the T-loop model). The binding of each

yeast Rap1p or human TRF1 monomer induces DNA bending (Bianchi et al., 1997; Vignais and Sentenac, 1989). Thus, a bound array of tandem repeats would be predicted to be folded or coiled overall, potentially promoting and stabilizing looping back of the telomeric terminus.

Telomere lengths change rapidly over small numbers of cell generations, even in normally regulated telomeres (Shampay and Blackburn, 1988; Vermeesch et al., 1993), suggesting that at least some components of the protein complexes at telomeres are dynamically assembled and disassembled. Several studies suggest that this may be controlled through the cell cycle. First, occupancy of telomeres by the structural proteins Rap1p, Sir3p, Sir4p, Rif1p, and Rif2p is lower in late G2/M than in G1 or S (Laroche et al., 2000). Second, telomerase acts in late S or G2/M but not in G1, with its action apparently being coupled with DNA replication (Diede and Gottschling, 1999; Marcand et al., 2000; Vermeesch et al., 1993). The domains in the Rap1p C-terminal region that interact with Rif1p, Rif2p, and Sir proteins overlap (Wotton and Shore, 1997). Hence, these proteins may antagonize each other in vivo by competing for Rap1p. Similarly, the homodimerization domain of human TRF1 overlaps with the TIN2-interacting domain (Kim et al., 1999). Covalent modifications of telomeric DNA proteins may regulate their dynamic interactions. The poly (ADP-ribose) polymerase activity of human tankyrase, which promotes telomere elongation, modifies TRF1 in vitro, inhibiting its binding to telomeric DNA (Smith et al., 1998). This also potentially removes the negative length regulator TIN2 from telomeres, to improve accessibility to telomerase. Finally, Sir2p has NAD-dependent ADP ribosylation and histone deacetylase activities in vitro (Imai et al., 2000; Tanny et al., 1999). The latter is thought to affect the neighboring subtelomeric heterochromatin (see below).

Without telomerase, the cycle of alternating lengthening and shortening of telomeres in dividing cells is broken. As a result, telomeres progressively shorten, and as the cells divide, a gradually increasing fraction of the cells exit the cell cycle until the cell population senesces (reviewed in Blackburn, 2000a). The efficiency of forming a higher-order telomeric complex that can support capping apparently drops as the telomere shortens. Depending on the type of cell, this loss of telomere capping in the absence of telomerase elicits a cellular response. In yeasts and mammalian cells, the cell cycle arrests, and recombination among the telomeres (which in some cases lengthens the telomeric repeat tracts) or telomere fusions can ensue (Baumann and Cech, 2000; Hackett et al., 2001; McEachern and Blackburn, 1996; Romanov et al., 2001; reviewed in McEachern et al., 2000b). In some mammalian cells, apoptosis is also provoked (Hahn et al., 1999; Kondo et al., 1998; Zhang et al., 1999).

Longer telomeres have more DNA binding sites for sequence-specific, double-stranded DNA binding proteins such as Rap1p. The number of telomere-bound Rap1p molecules (and specifically of bound Rap1p C-terminal domains) influences whether a telomere will become lengthened by telomerase (Marcand et al., 1997; Ray and Runge, 1999). However, the "counting" of bound Rap1p molecules (and possibly of Rif1p and Rif2p) may also require the correct geometry of assembly of the Rap1p-nucleated complex. This was deduced because certain mutant telomeric DNA repeats that bind Rap1p with normal affinity in vitro nevertheless cause loss of length control once the bulk of the tract is replaced by such repeats (Krauskopf and Blackburn, 1996, 1998; McEachern and Blackburn, 1995; Smith and Blackburn, 1999). Apart from there being fewer repeats, pinpointing the nature of the structural change that defines such uncapping is a major challenge in the field; uncapping upon telomere shortening can occur even though hundreds or thousands of base pairs of telomeric repeat DNA still remain, depending on the species or type of cell.

Heterochromatic Properties of Telomeric Regions

Cytologically, telomeric regions in a variety of plants and animals are heterochromatic, implying increased local DNA folding (reviewed in Dernburg et al., 1995). Positioning a gene in a telomeric heterochromatic region in *D. melanogaster*, or up to several kb away from the telomeric repeat tract in *S. cerevisiae*, can impose position effect variegation on that gene (Aparicio et al., 1991; Gottschling et al., 1990; Pardue, 1995; Renauld et al., 1993). Position effect variegation is characterized by epigenetic heterochromatin states that are semistable for several cell divisions, with a gene under its influence switching between silenced or nonsilenced states. Telomeric silencing in yeast shares several molecular genetic requirements with silencing of the chromosome-internal mating type loci (Aparicio et al., 1991), including the C-terminal domain of Rap1p and the Sir proteins (Kyrion et al., 1992).

Experimental evidence suggests a possible model in which folding of the terminal region of the telomere into a more internal subtelomeric region can help protect telomeres. In chromatin immunoprecipitation experiments done in yeast, proteins bound to the telomere such as Rap1p are crosslinked not only to telomeric repeat tract DNA, but also to several kb of the subtelomeric DNA sequences (de Bruin et al., 2000; Grunstein, 1998). A yeast transcriptional activation element, UAS_G, placed downstream of, or even somewhat distant from, a reporter gene, activated transcription of this gene if it was present in a subtelomeric, but not a nontelomeric, location (de Bruin et al., 2001). This suggested that folding back within the subtelomeric chromatin in effect positioned the element "upstream" of its associated gene, consistent with the chromatin immunoprecipitation findings.

When telomerase function is lost or compromised, heterochromatization of the subtelomeric region emerges as a potential mechanism for telomere protection. In *Drosophila*, the tandem repetition of a sequence is sufficient for it to gain heterochromatic properties (Dorer and Henikoff, 1997). Hence the long, complex-sequence repeats at telomeric regions in *D. melanogaster*, *Drosophila yakubi*, the midge *C. tentans*, and certain mosquitoes and onions are thought to form a nucleosomally based local heterochromatin structure that stabilizes telomeres (Figure 2D, top) (reviewed in Pardue and DeBaryshe, 1999 and McEachern et al., 2000b). In a similar way, the sub-telomeric Y′ tandem repeats in *S. cerevisiae* may also contribute to telomere capping. This poten-

tial role for Y′ repeats is only unmasked after compromise of some aspect(s) of telomere maintenance. Normally, one to four tandem copies of the subtelomeric Y′ elements (5–6 kb elements flanked by $G_{1\text{-}3}T$ repeat tracts) are present at some telomeres, and several subtelomeres ("X telomeres") completely lack Y′ elements. In experiments in which telomeres were impaired by mutation of Tel1p as well as Rif1p and/or Rif2p, the length of Y′-containing telomeres was more tightly regulated than that of the natural X telomeres that lacked subtelomeric Y′ elements (Craven and Petes, 1999).

Consequences of Altering the DNA-Protein Complex at the Extreme Terminus of the Telomere

Altering the sequence of only the few distal-most telomeric DNA repeats can strongly influence telomerase, degradation, recombination, and NHEJ actions on telomeres. This was shown in experiments in which the telomerase RNA template was mutated, causing mutant telomeric DNA repeats to be added to telomeric termini. Often, phenotypes were seen as soon as a small number of mutant repeats had been added to the end of the tract of wild-type repeats. First, certain mutant sequences caused net overelongation of the telomeres by telomerase. This did not require telomeric recombination—which can also elongate terminal telomeric repeat tracts (McEachern and Blackburn, 1996; Teng and Zakian, 1999)—because the same results were obtained in rad52 mutant cells (Chan et al., 2001; Prescott and Blackburn, 2000). Second, some mutations of the terminal repeats caused the telomeric DNA to become highly degraded and single-stranded, reminiscent of a DNA damage response (Chan et al., 2001; McEachern and Blackburn, 1995; Smith and Blackburn, 1999). Length regulation, control of nuclease activity, and functional capping could be simultaneously restored to such telomeres containing long tracts of mutant telomeric repeats by addition of just a few (3-4) functionally wild-type repeats to the telomeric tips (Krauskopf and Blackburn, 1998; Smith and Blackburn, 1999). Thus, normally capped telomeres limit nucleolytic activities at telomeres. Third, with certain *K. lactis* telomerase RNA template mutants, incorporation of only a few of the mutant repeats onto the telomeric termini induced a high frequency of subtelomeric homologous recombination events, even though no cellular phenotypes were observed and telomere maintenance was otherwise generally undisturbed (McEachern and Blackburn, 1995, 1996; McEachern and Iyer, 2001). Finally, mutations in the telomerase RNA template of *Tetrahymena* or *K. lactis* caused telomeres to fuse and fail to separate from each other in anaphase (Kirk et al., 1997; Lee et al., 1993; McEachern et al., 2000a; Ware et al., 2000). Molecular analyses indicated that in the mutant cells, only the tips of the telomeres had incorporated mutant repeats, with the bulk of the telomeric tracts still being of wild-type sequence. Again, the same mutant repeats in *Tetrahymena*, which caused deleterious effects when at the ends of telomeres (Yu et al., 1990), were harmless when capped by distal wild-type repeats (Yu and Blackburn, 1991).

Together, these findings demonstrated the critical nature of the most distal few repeats on the telomere. The key finding was that all these perturbations disappeared when as few as 3–4 wild-type repeats were added back to the telomeres that still contained tracts of mutant (Krauskopf and Blackburn, 1996; Smith and Blackburn, 1999). Three possible components of the telomere are potentially affected by mutating the terminal few telomeric repeats: the complex on the terminal double-stranded DNA, the complex on the terminal single-stranded DNA, and the ability to form a T-loop.

Sequence-Specific Double-Stranded DNA Binding Proteins at the Telomeric Tip

Mutant repeats are likely to have disrupted binding sites for telomeric protein(s) that normally protect the ends. A good candidate for such a protein in budding yeasts is Rap1p. In *K. lactis* and *S. cerevisiae*, one group of template mutations caused cellular growth and telomere lengthening phenotypes whose severity directly correlated with the degree to which the affinity of Rap1p binding was disrupted (McEachern and Blackburn, 1995, 1996; Prescott and Blackburn, 2000). However, the relative contributions to capping of proteins that bind the duplex terminal repeats, versus those that bind the 3′ single-stranded overhang, are not known.

A telomerase RNA template sequence mutation, which caused only a moderate reduction in Rap1p binding affinity of the mutant repeats added to *K. lactis* telomeres, by itself caused minimal effects on telomere length, capping, or stability. However, combining this type of mutation with another initially harmless mutation—a small truncation of the *K. lactis* Rap1p C-terminal tail—caused an abrupt switch to a deregulated state of the telomeres. They greatly elongated, while single-stranded, degraded telomeric DNA also accumulated and cell viability dropped (Krauskopf and Blackburn, 1996; Smith and Blackburn, 1999). Thus, simultaneously making modest changes in both the DNA-protein complex on the terminal repeats and the domain of Rap1p that organizes the higher-order protein complex led synergistically to catastrophic loss of telomere function and stability. Again, addition of only a few functionally wild-type repeats was sufficient to restore normal telomere stability (Krauskopf and Blackburn, 1996, 1998; Smith and Blackburn, 1999). These findings are indicative of mutual reinforcement between the higher-order complex (which was compromised by the Rap1p C-terminal domain mutation) and the complex on the terminal repeats (affected by the telomerase templating mutation).

The DNA-Protein Complex that Forms on the Single-Stranded Overhang of the Telomere

In the experiments described above in which the terminal telomeric sequences were mutated, the sequence of the 3′ overhang would also have been altered. In budding yeast, this could potentially have disrupted Cdc13p binding. A temperature-sensitive allele of this single-stranded telomeric DNA binding protein causes the C-rich strand of the telomeric DNA, together with its adjacent subtelomeric sequence, to become massively degraded at the nonpermissive temperature, presumably via unregulated nuclease or processing activities, and the single-stranded G-rich DNA extension becomes extremely long (Garvik et al., 1995). Since this mutant Cdc13p cannot bind telomeric DNA, it was thought that

the single-stranded terminal DNA constitutes the uncapping signal (Bertuch and Lundblad, 1998). The hypotrichous ciliate proteins (α/β heterodimer) that bind sequence specifically to the 3′ terminus of the single-stranded overhang protect that end from degradation and telomerase action in vitro (for references, see Baumann and Cech, 2001). These properties are consistent with a role as a telomere capping protein. In fission yeast, the α protein homolog Pot1 binds and protects telomeric single-stranded DNA and its absence in vivo causes telomere fusions. Hence, the properties of the DNA-protein complex on the single-stranded G-rich 3′ overhang of the telomere are important for regulating telomerase, nuclease, and end-to-end joining enzymes at telomeres. A human Pot1/α protein homolog has also been identified, but its function at telomeres has not yet been experimentally shown (Baumann and Cech, 2001).

The Overhanging G-Rich Strand and the T-Loop Structure of Telomeric DNA

Looping back of the G-strand telomeric DNA overhang into the duplex, inner portion of the telomeric DNA tract, by homologous strand invasion into the wild-type repeats, has been proposed to protect the telomeric end in vivo. This T-loop model predicts that altering the sequence of the 3′ G-rich strand overhang by telomerase RNA template mutations might prevent the terminal DNA nucleotides from such base pairing. The human telomeric DNA binding protein TRF2 preferentially binds at the junction of the T-loop in vitro (Figure 1). Overexpressing a dominant-negative mutant TRF2 depletes TRF2 from the telomeres in cells and causes apoptosis, with loss of a biochemically detectable G-strand overhang (Karlseder et al., 1999; van Steensel et al., 1998). A compromised ability to form a T-loop could underlie the telomere uncapping that occurs in these cells, as manifested by increased telomeric DNA end-to-end fusions. Conversely, deleting Ku, which binds broken DNA ends in vitro, increases the amount of G-strand 3′ overhang detected throughout the cell cycle in yeast (Gravel et al., 1998). However, while Ku knockout mice also show increased telomeric fusions and hence telomere uncapping, no overall change in the amount of G-strand overhang was detected (Samper et al., 2000). Therefore, the mechanism of the role of the single-stranded overhang in telomere capping remains a challenging problem.

Contributions of Active Telomerase to Telomere Capping

The most obvious function of active telomerase is to add telomeric DNA repeats to telomere ends, thus preventing telomere length from falling low enough to cause uncapping. However, recent findings have uncovered a potential additional role for active telomerase. These results were found by using telomerase alleles that were effectively hypomorphic, in the sense that although the core enzymatic activity of the telomerase was not obviously altered, telomeres became stably shortened. Several experiments indicated that active telomerase can permit continued proliferation of cells with short telomeres that, in the absence of functional telomerase, would be unstable. Human primary fibroblasts normally repress telomerase activity and eventually senesce in culture. Initially, Bodnar et al. (1998) showed that ectopically overexpressing the protein reverse transcriptase subunit of telomerase (hTERT) activated telomerase activity and indefinitely extended the proliferative lifespan of these cells. In these experiments, the telomeres lengthened. Similarly, telomeres also lengthened and crisis was bypassed in human fibroblasts that were transformed with SV40 T antigen and ectopically expressed hTERT (Counter et al., 1998). However, by ectopically expressing hypomorphic hTERT constructs, senescence or crisis could also be efficiently averted and cellular lifespan extended without net telomere lengthening. Two models were proposed to explain these findings (Zhu et al., 1999): either telomerase lengthened the shortest telomeres (although not the telomere population as a whole), or active telomerase plays a role in allowing cell proliferation and protection from telomere fusion even when telomeres are very short.

Similar results have been obtained with SV40 T antigen transformed human fibroblasts, normal human diploid fibroblasts, and endothelial cells; telomeres at first continued to shorten for several tens of population doublings, then eventually stabilized at a new, shorter mean length (Yang et al., 1999; Zhu et al., 1999). Shortened and stable telomeres were also seen in some spontaneously immortalizing SV40 T antigen transformed human fibroblast lines, in which the endogenous telomerase had been activated (Ducray et al., 1999). Ectopic hTERT expression also extended the life span of T cells without net telomere lengthening (Hooijberg et al., 2000). Finally, comparable results were obtained in budding yeasts expressing various hypomorphic but enzymatically active mutant telomerases, generated by mutating the telomerase RNA (Prescott and Blackburn, 1997b, 2000). A notable feature of all these experiments was that the new stable telomere length was considerably shorter than the telomere length in the control telomerase-negative cells at the point when they had stopped dividing. This was especially clear in the yeast experiments, where the ability to detect individual telomeres and to quantitatively monitor cell proliferation is enhanced. These results indicated that functional rescue by telomerase can be experimentally uncoupled from net lengthening of telomeres.

Telomere fusions, which become prevalent as human fibroblast cells transformed with SV40 T antigen enter crisis (Counter et al., 1992), were also reduced by the ectopic expression of wild-type or hypomorphic telomerase constructs (Zhu et al., 1999). This reduction was seen both with net telomere elongation (Counter et al., 1998) and net shortening (Zhu et al., 1999). In telomerase-deleted *K. lactis*, recombination events between the telomeric repeat tracts, which resulted in lengthening of the tracts, increased in frequency even in healthy cells when the telomeres were still relatively long (McEachern and Blackburn, 1996). This again suggested that telomerase helps to cap telomeres, since recombination in the telomeric regions is normally repressed.

To date, this function of the telomerase RNP defined by these results—the protection of very short telomeres from behaving as though they are uncapped—has only become manifest when telomeres shorten, and its

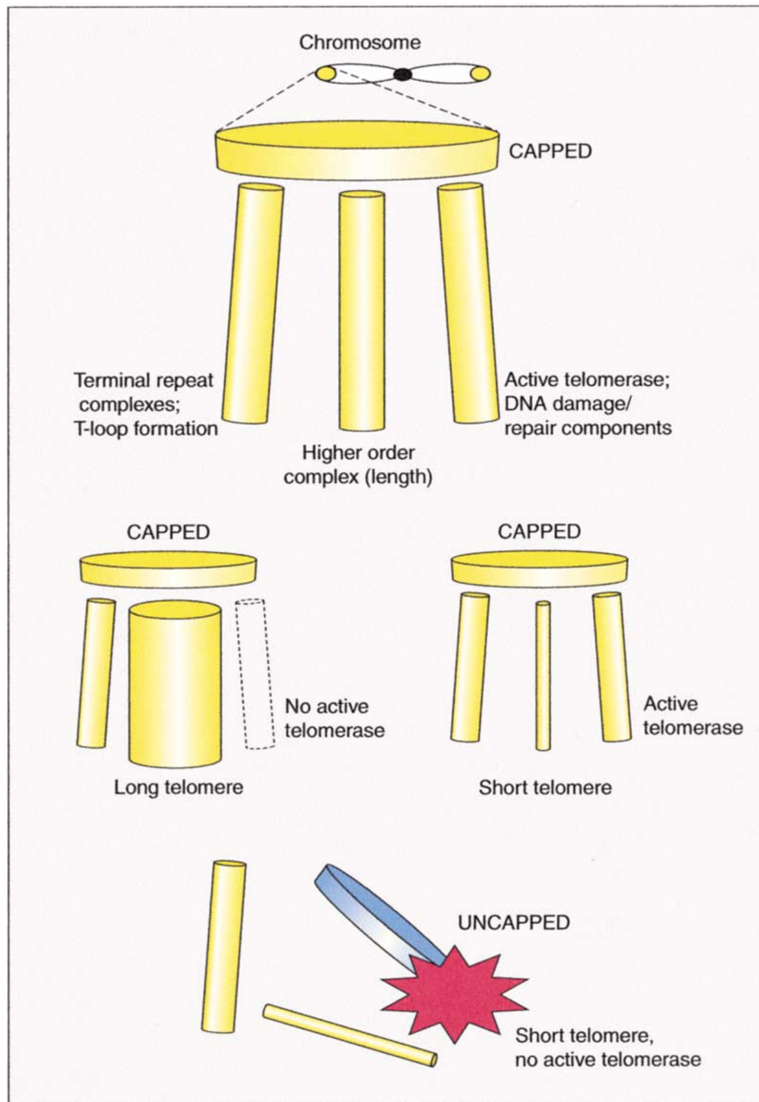

Figure 4. An Example of the Mutual Reinforcement among the Different Molecular Mechanisms that Contribute to Telomere Function

Three such molecular mechanisms are depicted as legs that together support a footstool (the capped state). When only one mechanism is compromised or abrogated, such as lack of functional telomerase (middle, left) or short telomeres (middle, right), the other components of the capping system can compensate for its absence. For example, long telomeres compensate for lack of telomerase (middle, left), and telomerase can compensate for short telomeres (middle, right). Capping functions often fail only when two or more mechanisms are compromised, such as in cells with both short telomeres and no telomerase (bottom). See text for description.

mechanism is unknown. As the presence of a stable but inactive telomerase RNP particle is not sufficient (Prescott and Blackburn, 1997b), it can be speculated that the enzymatically competent conformation of telomerase might signal to the cell to keep dividing. In this light, it is of interest that, in cancer cells, telomerase levels typically are significantly elevated, potentially conferring a selective proliferative advantage on the cells despite their often short telomeres. A small (10 amino acid) N-terminal deletion of Est2p in yeast produces a stable though enzymatically inactive protein, yet has an unexpected effect: cell growth is slowed immediately (Xia et al., 2000). This unexpected phenotype may be explained by an abnormality in this putative signaling function of telomerase. It is also possible that a physical interaction of an active form of telomerase with the telomere protects the telomere at a critical time in the cell cycle. This possibility was suggested because in vitro, telomerases from *S. cerevisiae* and *K. lactis* remain stably associated with their telomeric DNA extension products following a round of polymerization (Fulton and Blackburn, 1998; Prescott and Blackburn, 1997a).

Cells without Telomerase

As shown in Figure 4 (top), the ability to sustain telomere capping can be metaphorically likened to the combined action of the legs supporting a footstool. The situations with and without telomerase provide one example of the interaction and mutual reinforcement among the different determinants of capping. In cultured mammalian cells or yeasts lacking telomerase activity, telomeres progressively shorten, but the cells can proliferate typically for many tens of population doublings. The longer the telomere length when telomerase is first inactivated, the greater the delay before obvious senescence (Hahn et al., 1999; McEachern and Blackburn, 1996). Hence, sufficiently long telomeres can compensate for a lack of active telomerase, so that the telomeres are still functionally capped (Figure 4, middle left). However, as telomeres shorten, as described above, their capping status critically depends on whether active telomerase is pres-

ent (Figure 4, middle right and bottom). Hence, all other factors being equal, it is the combination of telomere length and the presence or absence of telomerase that determines whether a telomere is capped.

The point at which a shortening telomere becomes uncapped also appears to depend on multiple other interacting, species- and cell-type-specific factors. As well as telomerase, these are likely to include, minimally, some combination of the levels and variants of telomere components. The complex developmental control of mammalian telomerase, and doubtless of these other factors, results in telomeres that can vary considerably in length among different cell types yet still be functional, especially in the presence of telomerase (Weng et al., 1998). Thus, predicting a telomere's functionality solely on the basis of its length can be problematic. Dolly, the first cloned sheep, had shorter telomeres than her mother (Shiels et al., 1999), fueling speculation that this might presage Dolly's premature aging. However, cloned cows showed the opposite phenomenon—telomeres in the calves were longer than those of the parental somatic cells taken from the older animal used as the source of nuclei for the cloning (Lanza et al., 2000). The telomeres in cloned sheep and cows almost certainly are acted upon by telomerase during embryogenesis, when telomerase activity is widespread and relatively high. Therefore, the shortened and lengthened telomeres in Dolly and the cloned cows, respectively, may indicate neither an early demise nor an increased potential lifespan.

Summary and Conclusions

This review has focused upon the telomere as a nucleoprotein complex with multiple stabilizing molecular determinants. A number of molecular processes needed for functional telomeres have been defined experimentally, and they have also revealed that the telomeric complex can switch between physical states associated with functional capping and uncapping. Depending on the species and cell type, environment, or stage, some combination of multiple and distinct molecular mechanisms collaborates in telomere function. These include the telomere length-dependent higher-order complex, the ability of the whole telomeric region to form a heterochromatic domain, molecular components of the complex on the terminal double- and single-stranded telomeric DNA, T-loop formation, and the activity of telomerase itself. A defining feature of telomere behavior in vivo is mutual reinforcement between these different molecular players. Thus, as described above for some examples, lack of one of these aspects can be compensated for by the others.

Various factors can swing the balance between functional and nonfunctional telomeres, sometimes irreversibly. For telomerase-maintained telomeres, two kinds of situations can be envisaged. In dividing cells with telomerase, telomeres seem able to undergo reversible, cyclical uncapping and recapping. In these cells, a telomere can be envisaged as being poised between capped and uncapped states, regularly switching between the two, perhaps as often as each cell cycle, so that telomerase adds DNA repeat sequences in S or G2/M. However, in some situations, the balance swings completely to one or the other side. When telomeres are sufficiently long, they can remain stable or functional through multiple cell divisions even in the absence of telomerase. However, telomere shortening increases the probability of switching into the uncapped state, which can become irreversible if more than one of the molecular components of the telomere/telomerase functional complex are compromised. Thus, the multiple, mutually reinforcing mechanisms discussed in this review likely evolved to ensure against inappropriate irreversible uncapping. The presence of functional telomerase is one factor determining whether a telomere becomes recapped or irreversibly uncapped. In this light, the developmentally regulated shutdown of telomerase in humans and other metazoans (Weng et al., 1997) raises interesting questions regarding whether loss of telomere functionality is exploited to program organismal aging, or to create a natural barrier to prevent cancer. Understanding the interlocking mechanisms that regulate telomere functions may help answer these questions.

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References

Aparicio, O.M., Billington, B.L., and Gottschling, D.E. (1991). Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. Cell *66*, 1279–1287.

Baumann, P., and Cech, T.R. (2000). Protection of telomeres by the Ku protein in fission yeast. Mol. Biol. Cell *11*, 3265–3275.

Baumann, P., and Cech, T.R. (2001). Pot1, the putative telomere end-binding protein in fission yeast and humans. Science *292*, 1171–1175.

Bertuch, A., and Lundblad, V. (1998). Telomeres and double-strand breaks: trying to make ends meet. Trends Cell. Biol. *8*, 339–342.

Bianchi, A., Smith, S., Chong, L., Elias, P., and de Lange, T. (1997). TRF1 is a dimer and bends telomeric DNA. EMBO J. *16*, 1785–1794.

Blackburn, E.H. (1998). Telomerase RNA structure and function. In RNA Structure and Function, R.W. Simons and M. Grunberg-Manago, eds. (Cold Spring Harbor Laboratory Press), pp. 669–694.

Blackburn, E.H. (2000a). Telomere states and cell fates. Nature *408*, 53–56.

Blackburn, E.H. (2000b). The end of the (DNA) line. Nat. Struct. Biol. *7*, 847–850.

Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C., Morin, M., 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.

Chan, S., Chang, J., Prescott, J., and Blackburn, E.H. (2001). Altering telomere structure allows telomerase to act in yeast lacking ATM kinases. Curr. Biol. *11*, 1240–1250.

Chikashige, Y., Ding, D.Q., Funabiki, H., Haraguchi, T., Mashiko, S., Yanagida, M., and Hiraoka, Y. (1994). Telomere-led premeiotic chromosome movement in fission yeast. Science *264*, 270–273.

Cohen, P., and Blackburn, E.H. (1998). Two types of telomeric chromatin in *Tetrahymena thermophila*. J. Mol. Biol. *280*, 327–344.

Conrad, M.N., Wright, J.H., Wolf, A.J., and Zakian, V.A. (1990). RAP1 protein interacts with yeast telomeres in vivo: overproduction alters telomere structure and decreases chromosome stability. Cell *63*, 739–750.

Cooper, J.P., Watanabe, Y., and Nurse, P. (1998). Fission yeast Taz1 protein is required for meiotic telomere clustering and recombination. Nature *392*, 828–831.

Counter, C.M., Avilion, A.A., LeFeuvre, C.E., Stewart, N.G., Greider, C.W., Harley, C.B., and Bacchetti, S. (1992). Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J. *11*, 1921–1929.

Counter, C.M., Meyerson, M., Eaton, E.N., Ellisen, L.W., Caddle, S.D., Haber, D.A., and Weinberg, R.A. (1998). Telomerase activity is restored in human cells by ectopic expression of hTERT (hEST2), the catalytic subunit of telomerase. Oncogene *16*, 1217–1222.

Craven, R.J., and Petes, T.D. (1999). Dependence of the regulation of telomere length on the type of subtelomeric repeat in the yeast *Saccharomyces cerevisiae*. Genetics *152*, 1531–1541.

de Bruin, D., Kantrow, S.M., Liberatore, R.A., and Zakian, V.A. (2000). Telomere folding is required for the stable maintenance of telomere position effects in yeast. Mol. Cell. Biol. *20*, 7991–8000.

de Bruin, D., Zaman, Z., Liberatore, R.A., and Ptashne, M. (2001). Telomere looping permits gene activation by a downstream UAS in yeast. Nature *409*, 109–113.

Dernburg, A.F., Sedat, J.W., Cande, W.Z., and Bass, H.W. (1995). Cytology of telomeres. In Telomeres, E.H. Blackburn and C.W. Greider, eds. (Plainview, NY: Cold Spring Harbor Laboratory Press).

Diede, S.J., and Gottschling, D.E. (1999). Telomerase-mediated telomere addition in vivo requires DNA primase and DNA polymerases alpha and delta. Cell *99*, 723–733.

Dorer, D.R., and Henikoff, S. (1997). Transgene repeat arrays interact with distant heterochromatin and cause silencing in cis and trans. Genetics *147*, 1181–1190.

Ducray, C., Pommier, J.P., Martins, L., Boussin, F.D., and Sabatier, L. (1999). Telomere dynamics, end-to-end fusions and telomerase activation during the human fibroblast immortalization process. Oncogene *18*, 4211–4223.

Evans, S.K., and Lundblad, V. (2000). Positive and negative regulation of telomerase access to the telomere. J. Cell Sci. *113*, 3357–3364.

Evans, S.K., Bertuch, A.A., and Lundblad, V. (1999). Telomeres and telomerase: at the end, it all comes together. Trends Cell. Biol. *9*, 329–331.

Fulton, T.B., and Blackburn, E.H. (1998). Identification of *Kluyveromyces lactis* telomerase: discontinuous synthesis along the 30-nucleotide-long templating domain. Mol. Cell. Biol. *18*, 4961–4970.

Galy, V., Olivo-Marin, J.C., Scherthan, H., Doye, V., Rascalou, N., and Nehrbass, U. (2000). Nuclear pore complexes in the organization of silent telomeric chromatin. Nature *403*, 108–112.

Garvik, B., Carson, M., and Hartwell, L. (1995). Single-stranded DNA arising at telomeres in *cdc13* mutants may constitute a specific signal for the *RAD9* checkpoint. Mol. Cell. Biol. *15*, 6128–6138.

Gasser, S.M. (2000). A sense of the end. Science *288*, 1377–1379.

Gottschling, D.E., Aparicio, O.M., Billington, B.L., and Zakian, V.A. (1990). Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. Cell *63*, 751–762.

Gravel, S., Larrivee, M., Labrecque, P., and Wellinger, R.J. (1998). Yeast Ku as a regulator of chromosomal DNA end structure. Science *280*, 741–744.

Greider, C.W., and Blackburn, E.H. (1985). Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell *43*, 405–413.

Griffith, J., Bianchi, A., and de Lange, T. (1998). TRF1 promotes parallel pairing of telomeric tracts *in vitro*. J. Mol. Biol. *278*, 79–88.

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.

Grunstein, M. (1998). Yeast heterochromatin: regulation of its assembly and inheritance by histones. Cell *93*, 325–328.

Hackett, J.A., Feldser, D.M., and Greider, C.W. (2001). Telomere dysfunction increases mutation rate and genomic instability. Cell *106*, 275–286.

Hahn, W.C., Stewart, S.A., Brooks, M.W., York, S.G., Eaton, E., Kurachi, A., Beijersbergen, R.L., Knoll, J.H., Meyerson, M., and Weinberg, R.A. (1999). Inhibition of telomerase limits the growth of human cancer cells. Nat. Med. *5*, 1164–1170.

Liu, Y., Snow, B.E., Hande, M.P., Yeung, D., Erdmann, N.J., Wakeham, A., Itie, A., Siderovski, D.P., Lansdorp, P.M., Robinson, M.O., and Harrington, L. (2000). The telomerase reverse transcriptase is limiting and necessary for telomerase function in vivo. Curr. Biol. *10*, 1459–1462.

Hemann, M.T., and Greider, C.W. (1999). G-strand overhangs on telomeres in telomerase-deficient mouse cells. Nucleic Acids Res. *27*, 3964–3969.

Hooijberg, E., Ruizendaal, J.J., Snijders, P.J., Kueter, E.W., Walboomers, 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.

Hsu, H.L., Gilley, D., Galande, S.A., Hande, M.P., Allen, B., Kim, S.H., Li, G.C., Campisi, J., Kohwi-Shigematsu, T., and Chen, D.J. (2000). Ku acts in a unique way at the mammalian telomere to prevent end joining. Genes Dev. *14*, 2807–2812.

Imai, S., Armstrong, C.M., Kaeberlein, M., and Guarente, L. (2000). Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature *403*, 795–800.

Karlseder, J., Broccoli, D., Dai, Y., Hardy, S., and de Lange, T. (1999). p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science *283*, 1321–1325.

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, B., Ritchie, K.B., and Petes, T.D. (2000). The Mre11p/Rad50p/Xrs2p complex and the Tel1p function in a single pathway for telomere maintenance in yeast. Genetics *155*, 475–479.

Kirk, K.E., Harmon, B.P., Reichardt, I.K., Sedat, J.W., and Blackburn, E.H. (1997). Block in anaphase chromosome separation caused by a telomerase template mutation. Science *275*, 1478–1481.

Kondo, S., Kondo, Y., Li, G., Silverman, R.H., and Cowell, J.K. (1998). Targeted therapy of human malignant glioma in a mouse model by 2–5A antisense directed against telomerase RNA. Oncogene *16*, 3323–3330.

Krauskopf, A., and Blackburn, E.H. (1996). Control of telomere growth by interactions of RAP1 with the most distal telomeric repeats. Nature *383*, 354–357.

Krauskopf, A., and Blackburn, E.H. (1998). Rap1 protein regulates telomere turnover in yeast. Proc. Natl. Acad. Sci. USA *95*, 12486–12491.

Kyrion, G., Boakye, K.A., and Lustig, A.J. (1992). C-terminal truncation of RAP1 results in the deregulation of telomere size, stability, and function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *12*, 5159–5173.

Lansdorp, P.M., Verwoerd, N.P., van de Rijke, F.M., Dragowska, V., Little, M.T., Dirks, R.W., Raap, A.K., and Tanke, H.J. (1996). Heterogeneity in telomere length of human chromosomes. Hum. Mol. Genet. *5*, 685–691.

Lanza, R.P., Cibelli, J.B., Blackwell, C., Cristofalo, V.J., Francis, M.K., Baerlocher, G.M., Mak, J., Schertzer, M., Chavez, E.A., Sawyer, N., et al. (2000). Extension of cell life-span and telomere length in animals cloned from senescent somatic cells. Science *288*, 665–669.

Laroche, T., Martin, S.G., Tsai-Pflugfelder, M., and Gasser, S.M. (2000). The dynamics of yeast telomeres and silencing proteins through the cell cycle. J. Struct. Biol. *129*, 159–174.

Le, S., Moore, J.K., Haber, J.E., and Greider, C.W. (1999). *RAD50* and *RAD51* define two pathways that collaborate to maintain telomeres in the absence of telomerase. Genetics *152*, 143–152.

Lee, M.S., Gallagher, R.C., Bradley, J., and Blackburn, E.H. (1993). *In vivo* and *in vitro* studies of telomeres and telomerase. Cold Spring Harbor Symp. Quant. Biol. *58*, 707–718.

Levis, R.W. (1989). Viable deletions of a telomere from a *Drosophila* chromosome. Cell *58*, 791–801.

Li, B., Oestreich, S., and de Lange, T. (2000). Identification of human Rap1: implications for telomere evolution. Cell *101*, 471–483.

Lim, D.S., Kim, S.T., Xu, B., Maser, R.S., Lin, J., Petrini, J.H., and Kastan, M.B. (2000). ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. Nature *404*, 613–617.

Marcand, S., Gilson, E., and Shore, D. (1997). A protein-counting mechanism for telomere length regulation in yeast. Science *275*, 986–990.

Marcand, S., Brevet, V., Mann, C., and Gilson, E. (2000). Cell cycle restriction of telomere elongation. Curr. Biol. *10*, 487–490.

McEachern, M.J., and Blackburn, E.H. (1995). Runaway telomere elongation caused by telomerase RNA gene mutations. Nature *376*, 403–409.

McEachern, M.J., and Blackburn, E.H. (1996). Cap-prevented recombination between terminal telomeric repeat arrays (telomere CPR) maintains telomeres in *Kluyveromyces lactis* lacking telomerase. Genes Dev. *10*, 1822–1834.

McEachern, M.J., and Iyer, S. (2001). Short telomeres in yeast are highly recombinogenic. Mol. Cell *7*, 695–704.

McEachern, M.J., Iyer, S., Fulton, T.B., and Blackburn, E.H. (2000a). Telomere fusion caused by mutating the terminal region of telomeric DNA. Proc. Natl. Acad. Sci. USA *97*, 11409–11414.

McEachern, M.J., Krauskopf, A., and Blackburn, E.H. (2000b). Telomeres and their control. Annu. Rev. Genet. *34*, 331–358.

Moazed, D., Kistler, A., Axelrod, A., Rine, J., and Johnson, A.D. (1997). Silent information regulator protein complexes in *Saccharomyces cerevisiae*: a SIR2/SIR4 complex and evidence for a regulatory domain in SIR4 that inhibits its interaction with SIR3. Proc. Natl. Acad. Sci. USA *94*, 2186–2191.

Muñoz-Jordán, J.L., Cross, G.A., de Lange, T., and Griffith, J.D. (2001). T-loops at trypanosome telomeres. EMBO J. *20*, 579–588.

Naito, T., Matsuura, A., and Ishikawa, F. (1998). Circular chromosome formation in a fission yeast mutant defective in two ATM homologues. Nat. Genet. *20*, 203–206.

Palladino, F., Laroche, T., Gilson, E., Axelrod, A., Pillus, L., and Gasser, S.M. (1993). SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres. Cell *75*, 543–555.

Pardue, M. (1995). *Drosophila* telomeres: another way to end it all. In Telomeres, E.H. Blackburn and C.W. Greider, eds. (Plainview, NY: Cold Spring Harbor Laboratory Press).

Pardue, M.L., and DeBaryshe, P.G. (1999). *Drosophila* telomeres: two transposable elements with important roles in chromosomes. Genetica *107*, 189–196.

Pennock, E., Buckley, K., and Lundblad, V. (2001). Cdc13 delivers separate complexes to the telomere for end protection and replication. Cell *104*, 387–396.

Peterson, S.E., Stellwagen, A.E., Diede, S.J., Singer, M.S., Haimberger, Z.W., Johnson, C.O., Tzoneva, M., and Gottschling, D.E. (2001). The function of a stem-loop in telomerase RNA is linked to the DNA repair protein Ku. Nat. Genet. *27*, 64–67.

Prescott, J., and Blackburn, E.H. (1997a). Functionally interacting telomerase RNAs in the yeast telomerase complex. Genes Dev. *11*, 2790–2800.

Prescott, J., and Blackburn, E.H. (1997b). Telomerase RNA mutations in *Saccharomyces cerevisiae* alter telomerase action and reveal nonprocessivity *in vivo* and *in vitro*. Genes Dev. *11*, 528–540.

Prescott, J.C., and Blackburn, E.H. (2000). Telomerase RNA template mutations reveal sequence-specific requirements for the activation and repression of telomerase action at telomeres. Mol. Cell. Biol. *20*, 2941–2948.

Pryde, F.E., Gorham, H.C., and Louis, E.J. (1997). Chromosome ends: all the same under their caps. Curr. Opin. Genet. Dev. *7*, 822–828.

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.

Renauld, H., Aparicio, O.M., Zierath, P.D., Billington, B.L., Chhablani, S.K., and Gottschling, D.E. (1993). Silent domains are assembled continuously from the telomere and are defined by promoter distance and strength, and by SIR3 dosage. Genes Dev. *7*, 1133–1145.

Romanov, S.R., Kozakiewicz, B.K., Holst, C.R., Stampfer, M.R., Haupt, L.M., and Tlsty, T.D. (2001). Normal human mammary epithelial cells spontaneously escape senescence and acquire genomic changes. Nature *409*, 633–637.

Roy, J., Fulton, T.B., and Blackburn, E.H. (1998). Specific telomerase RNA residues distant from the template are essential for telomerase function. Genes Dev. *12*, 3286–3300.

Samper, E., Goytisolo, F.A., Slijepcevic, P., van Buul, P.P.W., and Blasco, M.A. (2000). Mammalian ku86 protein prevents telomeric fusions independently of the length of TTAGGG repeats and the G-strand overhang. EMBO Reports *1*, 244–252.

Shampay, J., and Blackburn, E.H. (1988). Generation of telomere-length heterogeneity in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *85*, 534–538.

Shiels, P.G., Kind, A.J., Campbell, K.H., Waddington, D., Wilmut, I., Colman, A., and Schnieke, A.E. (1999). Analysis of telomere lengths in cloned sheep. Nature *399*, 316–317.

Smith, C.D., and Blackburn, E.H. (1999). Uncapping and deregulation of telomeres lead to detrimental cellular consequences in yeast. J. Cell. Biol. *145*, 203–214.

Smith, S., Giriat, I., Schmitt, A., and de Lange, T. (1998). Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. Science *282*, 1484–1487.

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.

Strahl, C., and Blackburn, E.H. (1994). The effects of nucleoside analogs on telomerase and telomeres in Tetrahymena. Nucleic Acids Res. *22*, 893–900.

Strahl, C., and Blackburn, E.H. (1996). Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortalized human cell lines. Mol. Cell. Biol. *16*, 53–65.

Tanny, J.C., Dowd, G.J., Huang, J., Hilz, H., and Moazed, D. (1999). An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. Cell *99*, 735–745.

Teng, S.C., and Zakian, V.A. (1999). Telomere-telomere recombination is an efficient bypass pathway for telomere maintenance in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *19*, 8083–8093.

Teng, C.S., Chang, J., McCowan, B., and Zakian, A.V. (2000). Telomerase-independent lengthening of yeast telomeres occurs by an abrupt Rad50p-dependent, Rif-inhibited recombinational process. Mol. Cell *6*, 947–952.

van Steensel, B., Smogorzewska, A., and de Lange, T. (1998). TRF2 protects human telomeres from end-to-end fusions. Cell *92*, 401–413.

Vermeesch, J.R., Williams, D., and Price, C.M. (1993). Telomere processing in *Euplotes*. Nucleic Acids Res. *21*, 5366–5371.

Vignais, M.L., and Sentenac, A. (1989). Asymmetric DNA bending induced by the yeast multifunctional factor TUF. J. Biol. Chem. *264*, 8463–8466.

von Zglinicki, T., Serra, V., Lorenz, M., Saretzki, G., Lenzen-Grossimlighaus, R., Gessner, R., Risch, A., and Steinhagen-Thiessen, E. (2000). Short telomeres in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor? Lab. Invest. *80*, 1739–1747.

Ware, T.L., Wang, H., and Blackburn, E.H. (2000). Three telomerases with completely non-telomeric template replacements are catalytically active. EMBO J. *19*, 1–13.

Wellinger, R.J., Wolf, A.J., and Zakian, V.A. (1993). *Saccharomyces* telomeres acquire single-strand TG1–3 tails late in S phase. Cell *72*, 51–60.

Weng, N.P., Palmer, L.D., Levine, B.L., Lane, H.C., June, C.H., and Hodes, R.J. (1997). Tales of tails: regulation of telomere length and telomerase activity during lymphocyte development, differentiation, activation, and aging. Immunol. Rev. *160*, 43–54.

Weng, N.P., Hathcock, K.S., and Hodes, R.J. (1998). Regulation of telomere length and telomerase in T and B cells: a mechanism for maintaining replicative potential. Immunity *9*, 151–157.

Wenz, C., Enenkel, B., Amacker, M., Kelleher, C., Damm, K., and Lingner, J. (2001). Human telomerase contains two cooperating telomerase RNA molecules. EMBO J. *20*, 3526–3534.

Wotton, D., and Shore, D. (1997). A novel Rap1p-interacting factor, Rif2p, cooperates with Rif1p to regulate telomere length in *Saccharomyces cerevisiae*. Genes Dev. *11*, 748–760.

Xia, J., Peng, Y., Mian, S., and Lue, N.F. (2000). Identification of functionally important domains in the N-terminal region of telomerase reverse transcriptase. Mol. Cell. Biol. *20*, 5196–5207.

Yang, J., Chang, E., Cherry, A.M., Bangs, C.D., Oei, Y., Bodnar, A., Bronstein, A., Chiu, C.P., and Herron, G.S. (1999). Human endothelial cell life extension by telomerase expression. J. Biol. Chem. *274*, 26141–26148.

Yu, G.L., and Blackburn, E.H. (1991). Developmentally programmed healing of chromosomes by telomerase in *Tetrahymena*. Cell *67*, 823–832.

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 telomerase-inhibited human tumor cells. Genes Dev. *13*, 2388–2399.

Zhang, D., Abovich, N., and Rosbash, M. (2001). A biochemical function for the Sm complex. Mol. Cell *7*, 319–329.

Zhu, J., Wang, H., Bishop, J.M., and Blackburn, E.H. (1999). Telomerase extends the lifespan of virus-transformed human cells without net telomere lengthening. Proc. Natl. Acad. Sci. USA *96*, 3723–3728.

Zhu, X.D., Küster, B., Mann, M., Petrini, J.H., and Lange, T. (2000). Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. Nat. Genet. *25*, 347–352.