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REGULATION OF TELOMERASE BY TELOMERIC PROTEINS

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■ **Abstract** Telomeres are essential for genome stability in all eukaryotes. Changes in telomere functions and the associated chromosomal abnormalities have been implicated in human aging and cancer. Telomeres are composed of repetitive sequences that can be maintained by telomerase, a complex containing a reverse transcriptase (hTERT in humans and Est2 in budding yeast), a template RNA (hTERC in humans and Tlc1 in yeast), and accessory factors (the Est1 proteins and dyskerin in humans and Est1, Est3, and Sm proteins in budding yeast). Telomerase is regulated in *cis* by proteins that bind to telomeric DNA. This regulation can take place at the telomere terminus, involving single-stranded DNA-binding proteins (POT1 in humans and Cdc13 in budding yeast), which have been proposed to contribute to the recruitment of telomerase and may also regulate the extent or frequency of elongation. In addition, proteins that bind along the length of the telomere (TRF1/TIN2/tankyrase in humans and Rap1/Rif1/Rif2 in budding yeast) are part of a negative feedback loop that regulates telomere length. Here we discuss the details of telomerase and its regulation by the telomere.

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THE END REPLICATION PROBLEM

The advent of linear chromosomes created a significant challenge for DNA replication. The problem, referred to as the end replication problem (1, 2), originates from the use of short RNAs to prime DNA synthesis. Removal of these primers results in 8–12 nucleotide (nt) gaps that do not impede the duplication of circular genomes because each gap can be closed by extending a preceding Okazaki fragment. However, on a linear template, the last RNA that primed lagging-strand synthesis will leave a gap that can not be filled. In the absence of a telomere maintenance system, many eukaryotes (fungi, trypanosomes, flies, mosquitos) lose terminal sequences at ~3–5 bp/end/division, a modest rate predicted by the end replication problem (3–6; G. Cross, personal communication; J. Cooper, personal communication). Human and mouse telomeres shorten much faster (50–150 bp/end/cell division (7–9); this suggests that chromosome ends, in these organisms, might be actively degraded. If telomere erosion is not balanced by elongation, telomeres will progressively shorten, eventually leading to chromosome instability and cell death. Therefore, the long-term proliferation of all eukaryotic cells, including cells giving rise to the germline, requires a mechanism to counteract telomere attrition. Here we review the mechanisms by which telomeric DNA is maintained and discuss how telomere associated proteins regulate this process.

THE CONSEQUENCES OF TELOMERE DYSFUNCTION

The telomeric nucleoprotein complex allows cells to distinguish natural chromosome ends from DNA breaks [reviewed in (10, 11)]. Without telomere protection, chromosome ends activate DNA damage response pathways that signal cell cycle arrest, senescence, or apoptosis. Telomeres also prevent inappropriate DNA repair reactions, such as exonucleolytic degradation and ligation of one end to another. When telomere function is impaired, fusion of unprotected chromosome ends can generate dicentric chromosomes, which are unstable in mitosis and wreck havoc in the genome.

Telomeres have received considerable attention since the realization that changes in their structure and function occur during cancer development and aging. Many human cell types display telomere erosion, a process that is thought

to limit the proliferative capacity of transformed cells and has the hallmark of a tumor suppressor system. In most human cancer, the telomere barrier has been bypassed through the activation of a telomere maintenance system, making telomere replication an attractive target for therapeutic intervention. Although the programmed shortening of human telomeres may be effective in limiting the cancer burden early in life, the same program may have detrimental consequences late in life. In the aged, short telomeres are predictive of diminished health and longevity, and at least one human premature aging syndrome is associated with compromised telomere function (12, 13). Diminishing telomere function late in life may even promote genome instability and therefore contribute to the higher incidence of cancer in the aged. The role of telomeres in cancer and aging has been reviewed extensively elsewhere (14, 15).

TELOMERE MAINTENANCE BY TELOMERASE

The most versatile and widely used method of telomere maintenance is based on telomerase (Figure 1) (16, 17). A two-component ribonucleoprotein enzyme, telomerase contains a highly conserved reverse transcriptase [telomerase reverse transcriptase, TERT, (18–20)] and an associated template RNA (telomerase RNA component, TERC, also referred to as TR or TER (21–24). TERT is most closely related to the reverse transcriptases of non-LTR retroposons and group II introns (23), and like these RTs, it extends the 3′ end of a DNA rather than an RNA primer (25). The primer for telomerase is the chromosome terminus, which can be positioned on an alignment site in TERC such that the 3′ end of the telomere is adjacent to the short (often 6 nt) template sequence (Figure 1*A* and *B*). Extension of the telomere terminus results in the addition of one telomeric repeat, and repeated alignment and extension steps can endow chromosome ends with the direct repeat arrays typical of telomeres. Although the sequence and size of telomerase RNAs are highly variable, they share structural motifs (Figure 1*C*) (26, 27), which may mediate the interaction with TERT, or control of the alignment, extension, and translocation steps. After elongation of the 3′ end, C-strand synthesis is presumably required to create double-stranded telomeric DNA, but the details of this step have only been examined in ciliates [(28–30), reviewed in (31)]. In addition, Tetrahymena telomeres have a precisely defined terminal structure that is generated by nucleolytic processing (32, 33), and it will be interesting to learn whether similar terminus transactions are required in other organisms.

In most unicellular organisms, telomerase has a housekeeping function, and its core components are always expressed. In contrast, telomerase is strongly suppressed in the human soma, a phenotype also observed in old world monkeys and new world primates (but not in prosimians, such as lemurs) [(18, 20, 34, 35); reviewed in (36)]. Robust telomerase activity is restricted to ovaries, testes, and highly proliferative tissues. This regulation place exists primarily at the level of

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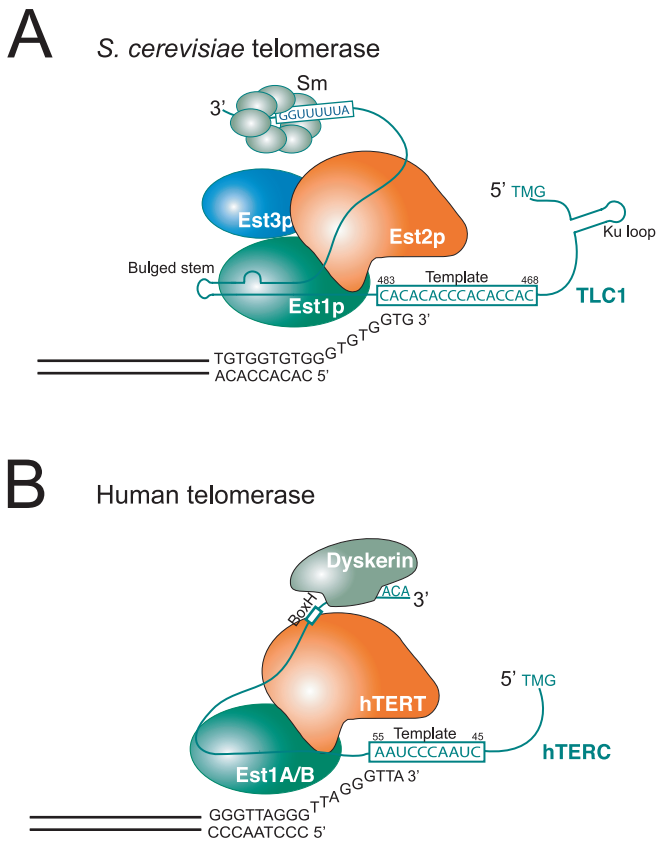


transcription of the *hTERT* gene; hTERC is virtually ubiquitous (24). The repression of *hTERT* transcription involves multiple genes previously implicated in tumorigenesis, which include Menin, the Mad/Myc pathway, and the TGFβ target Sip1 [(37); reviewed in (38)].

Exogenous expression of hTERT in primary human fibroblasts is sufficient to reconstitute telomerase activity and to counteract telomere erosion. The resulting telomere maintenance immortalizes most human cell types (39–41). Like primary cells, tumor cells require a telomere maintenance system for long-term proliferation, and in the majority of cases, this is provided by upregulation of hTERT [reviewed in (36)]. Telomerase activity per se does not induce transformation (42), and although telomerase is necessary for immortalization, *hTERT* is not an oncogene (43, 44). Conversely, oncogenic transformation does not require telomerase activity, and cells with very long telomeres can be fully transformed into a tumorigenic phenotype in vitro without a telomere maintenance system (45). Similarly, certain childhood tumors that originate in young cells with long telomeres can be cancerous and metastatic even though they lack telomerase. However, the extensive proliferation of cells during the prolonged multistep tumorigenesis pathway that leads to most adult human cancers is predicted to exhaust the telomere reserve, necessitating telomerase activation (46).

Once hTERT and hTERC are expressed, they have to be properly assembled and targeted to chromosome ends. Some of the biogenesis of telomerase is likely to take place in the nucleolus because GFP tagged hTERT is localized to nucleoli in G1, from which it moves to the nucleoplasm in S/G2 (47). Similarly, yeast Est2p, the TERT component of telomerase in *Sacharomyces cerevisiae*, is enriched in the nucleolus upon overexpression (48). Attempts to visualize telomerase at telomeres have failed. The only physical evidence for the association of telomerase with chromosome ends comes from chromatin precipitation studies in yeast showing that Est2p is present on telomeres during G1 and S phase (49, 50).

The presence of Est2p at telomeres in S phase is expected based on the finding that telomerase can extend chromosome ends during and immediately after DNA replication (51). Telomerase can even extend a telomere-like substrate in cells arrested in mitosis, and in this setting, its action is dependent on components of lagging-strand synthesis (52). It is not known whether telomerase can act on both newly replicated (sister) telomeres, and it remains to be determined whether telomerase can also act before DNA replication.

Figure 1 Telomerase holoenzyme in yeast and man. *A*. Budding yeast telomerase docked at a telomere 3′ end. *B*. Human telomerase docked at a telomere 3′ end. *C*. Conserved structural motifs in vertebrate telomerase RNAs [after (27)]. The positions of *DKC* mutations in the *hTERC* gene are indicated in red.

TELOMERASE ACCESSORY FACTORS

The telomerase holoenzyme often contains additional proteins that are not required for catalysis per se. In *S. cerevisiae*, telomerase is composed of the usual reverse transcriptase and RNA core components (Est2 and TLC1, respectively) and two accessory factors, Est1, which binds to a bulged stem in TLC1, and Est3 (Figure 1*A*) (3, 53–55). Although Est1 and Est3 are not required for in vitro telomerase activity (56, 57), mutations in these genes lead to progressive telomere shortening, the so-called ever shorter telomeres (est) phenotype (3, 53–55). This est phenotype is also observed for strains lacking the core components of telomerase and points to a complete failure in telomere maintenance (21, 54). In addition, TLC1 RNA, which is generated by RNA polymerase II and contains a trimethylguanosine cap, has an association with Sm proteins (Figure 1*A*), previously implicated in snRNP biogenesis (58).

Accessory factors have also been found for human telomerase. The human genome contains at least three *EST1* orthologs, two of which (*EST1A* and *B*) were recently shown to encode telomerase associated proteins, suggesting a conserved role for Est1 in telomerase regulation [(59, 60); reviewed in (61)]. A confounding issue in the analysis of the *EST1A* gene is its role in nonsense mediated decay (62). Mammalian Est3p orthologs have not been identified to date, and there is no indication that the mammalian telomerase RNA interacts with Sm proteins. Instead, human telomerase has an important interaction with another RNA binding protein, dyskerin (63). Dyskerin is a putative pseudouridine synthase that has been proposed to play a role in ribosomal processing because it binds to many small nucleolar RNAs (snoRNA) (64). Like the snoRNAs, hTERC contains a H/ACA motif that constitutes the dyskerin binding site (63) (Figure 1*B* and *C*). The H/ACA motif is conserved among vertebrate telomerase RNAs (27), but it is absent from yeast and ciliate telomerase RNAs.

Evidence in favor of the functional significance of the binding of dyskerin to hTERC comes from the genetics of a rare human disease, dyskeratosis congenita (DKC) (65). The X-linked form of DKC is due to a mutation in dyskerin, whereas the autosomal dominant form is due to mutations in the hTERC gene (63). DKC is classically described as a triad of muco-cutaneous changes that include abnormal skin pigmentation, nail dystrophy, and mucosal leukoplakia (65). The most profound defect in DKC and the leading cause of death is bone marrow failure. Additional symptoms include developmental delay, short stature, extensive dental caries/loss, hair loss/gray hair, pulmonary disease, and increased incidence of cancer. Patients with dyskerin mutations have fivefold less hTERC than unaffected siblings, implicating dyskerin in processing or stability of the telomerase RNA (63). Their telomerase activity is diminished, and these defects correlate with shorter telomeres and chromosome end fusions, which are pathognomonic for telomere dysfunction (63, 66).

Because dyskerin deficiency affects both telomerase RNA and ribosomal RNA, it is difficult to establish the contribution of telomere dysfunction to the

DKC (67). However, the autosomal form of DKC is due to mutations in the *hTERC* gene (12) (Figure 1*C*), showing conclusively that DKC can be induced by a telomere defect. In each case of autosomal DKC, the expression of *hTERC* is diminished, and affected individuals have very short telomeres. The phenotype of these heterozygous patients is probably due to haploinsufficiency of the human telomerase RNA; a similar situation is seen in mice lacking mTerc (68). In addition to DKC, mutations in the *hTERC* gene can cause aplastic anemia, further strengthening the link between telomerase function and bone marrow maintenance (69, 70).

Two- and three-hybrid screens as well as coimmunoprecipitation experiments have suggested that human telomerase has potential interactions with a large number of additional factors. Although some of these interacting proteins may play a role in biogenesis, stability, and localization of telomerase, the functional significance of most of these interactions has not been established [reviewed in (36, 71, 72)].

TELOMERASE-INDEPENDENT TELOMERE LENGTH CHANGES

Telomerase is not the only activity that affects the length of telomeres. In human cells and in fungi, telomeres can be maintained by a recombination-based mechanisms, referred to as ALT in human cells and as the survivor pathway in yeast [reviewed in (73, 74)]. Furthermore, telomeres can be shortened by exonucleolytic attack, and they can undergo large sudden deletions. The latter, termed telomere rapid deletion (TRD), has been proposed to constitute a second sizing mechanism for telomeres in *S. cerevisiae* [reviewed in (75)], and it is anticipated that similar deletions could affect telomere length in mammals. Although this review is focused on the telomerase pathway, it is possible that some of the regulatory events discussed below do not act directly on telomerase but affect one or more of these other telomere lengthening or shortening events.

REGULATION OF TELOMERASE AT THE TELOMERE TERMINUS: THE ROLE OF CDC13

A priori, the telomere terminus is expected to be a prime site for telomerase regulation. By analogy to the control of RNA polymerases, regulation of telomerase could take place at the level of the recruitment to the telomere terminus, at the initiation of elongation, or at the rate and processivity of the elongation cycles. Indeed, telomere maintenance in *S. cerevisiae* is primarily regulated by a telomere terminus specific factor, Cdc13 (Figure 2) [reviewed by (76)]. Its initial identification as a cell division cycle mutant reflects the essential role of *CDC13* in the protection of telomeres (77). Cells lacking *CDC13* function accumulate single-stranded DNA at

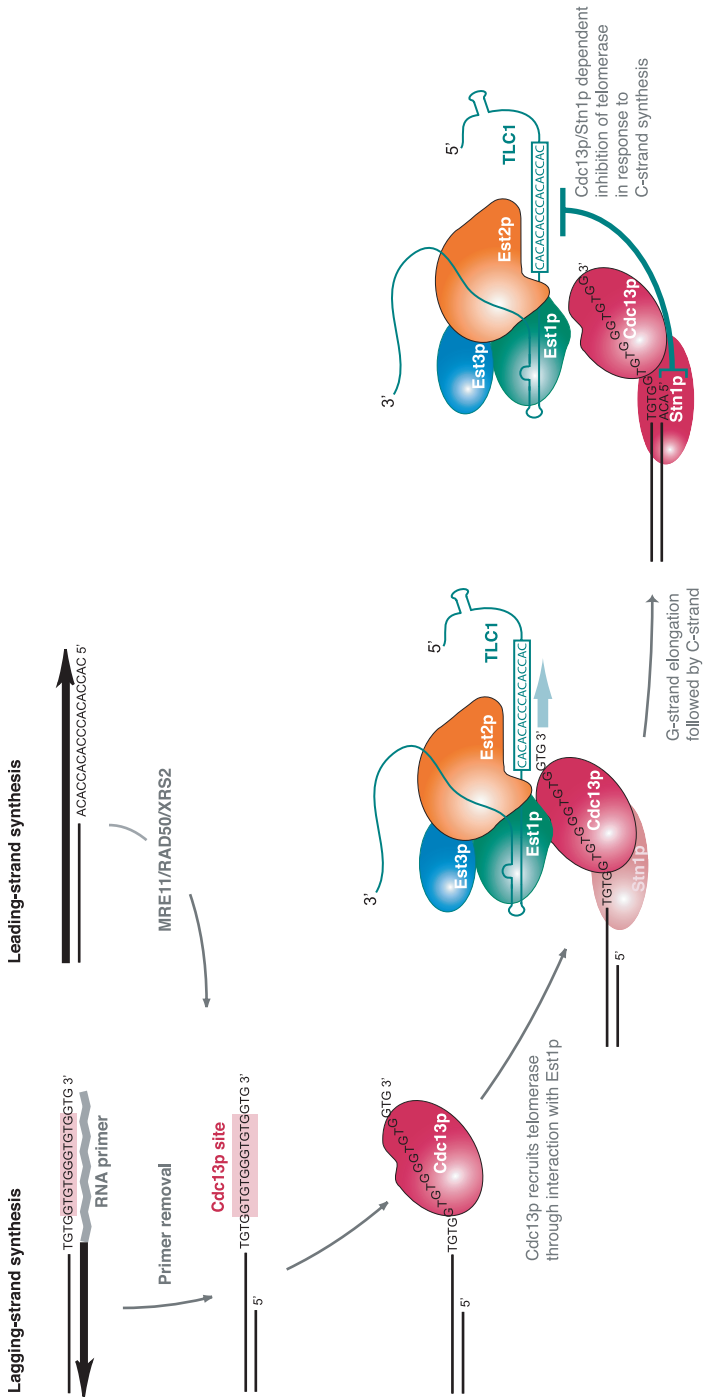


Figure 2 Recruitment of yeast telomerase. Speculative model of how the interaction of Cdc13 with the telomerase holoenzyme regulates recruitment of telomerase to the telomere.

chromosome ends, which induces a *RAD9*-dependent cell cycle arrest (77). However, *CDC13* was independently identified in a screen for *est* genes as EST4 [a mutation referred to *cdc13–2*^{est} (54)], pointing to a crucial role for Cdc13 in telomerase-mediated telomere maintenance.

Cdc13 is a single-stranded DNA-binding protein with a preference for the G-rich strand of yeast telomeric DNA (78, 79) (Figure 2). The current model for its function proposes that Cdc13 interacts with Est1, thereby recruiting telomerase to the telomere terminus. In support of this model, the telomere maintenance defect of the *cdc13–2*^{est} mutant can be suppressed by a specific mutation in EST1 (*est1–60*), which has an est phenotype on its own. Such allele specific suppression is most easily interpreted as a restoration of a physical interaction. In agreement, the mutations represent a charge swap in which the phenotype of a Glu->Lys mutation in *cdc13–2*^{est} is suppressed by the reverse (Lys->Glu) change in *est1–60* (80). Thus, the est phenotype of *cdc13* mutants could be explained if the Cdc13-Est1 interaction is necessary to recruit telomerase to the telomere terminus.

The Cdc13-Est1 telomerase recruitment model is consistent with a number of gene fusion experiments in which Cdc13 or its DNA-binding domain (DBD) were fused to protein components of the telomerase complex, i.e., Est2, Est1, and Est3 The resulting fusions rescue the telomere maintenance defects of *cdc13–2*^{est} and *est1*Δ strains (80, 81). For example, a fusion of the Cdc13 DBD to Est2 suppresses the requirement for Est1 in telomere maintenance. Collectively, these experiments suggest that Cdc13 interacts with Est1 to recruit telomerase to the very end of the telomere and that this recruitment step is essential for telomere maintenance (Figure 2).

Est1 may have a second role in addition to bridging the interaction between telomerase and Cdc13. In cells that express a Cdc13-Est2 fusion, the presence of Est1 results in much longer telomeres, suggesting a positive regulatory role that is independent of recruitment (80, 81). Furthermore, certain mutant Est1 alleles lack this positive regulatory function, whereas others are specifically defective in recruitment but still can stimulate telomere elongation in the Cdc13-Est2 fusion context (82). These separation-of-function mutations argue that Est1 plays multiple roles in telomere maintenance. Because Est1 does not affect the catalytic activity of telomerase as measured in cell lysates (56, 57), new assays may be required to reveal how Est1 affects telomerase in vivo.

Indirect evidence suggests that recruitment of telomerase involves multiple steps. ChIP experiments have shown that Est2 can bind to telomeres in G1 and that this association is not dependent on Cdc13 (49). One possibility is that Est2 first binds to telomeric chromatin in G1 and subsequently becomes positioned at the telomere terminus by Cdc13. Indeed, Cdc13 deficiency has a substantial effect on the presence of Est2 at telomeres in S phase (49). Potentially, the G1 recruitment of Est2 could be mediated by an interaction between TLC1 and one or more proteins in the telomeric complex. Evidence indicating such an interaction came from overexpression of TLC1, which was found to interfere with

telomeric silencing (21). Later studies indicated that this attribute of TLC1 is dependent on a genetic interaction between a stem-loop structure in TLC1 and the NHEJ protein, Ku (83). Ku is a component of the telomeric chromatin in yeast, it may therefore facilitate recruitment of the Est2/TLC1 complex to telomeres.

Because Cdc13 binds to single-stranded DNA, it is pertinent to ask when its binding site is available at telomeres and how single-stranded telomeric DNA is generated (Figure 2). Although long (>50 nt), single-stranded 3′ tails are only observed in late S phase (84); G1 telomeres have shorter 3′ overhangs that are still sufficient to recruit Cdc13 (R.J. Wellinger, personal communication). How are these overhangs created? One candidate is the Mre11/Rad50/Xrs2 complex, which is known to act as a nuclease in certain settings [reviewed in (85, 86)]. An indirect assay performed on nocodazole blocked (G2/M) cells implicated the Mre11 complex in the loading of Cdc13 (87). However, the in vitro nuclease activity of Mre11 complex has the wrong (3′->5′) polarity (88), and mutations in the nuclease domain of *MRE11* do not have a telomere maintenance defect (89, 90). Furthermore, most *rad50*Δ strains do not have an est phenotype, and their telomere shortening rates are moderate compared to est strains (91), indicating that other pathways for Cdc13 loading must be available. Cdc13 binding sites could simply be created passively by DNA replication (Figure 2) when the last RNA primer of lagging strand DNA synthesis is removed. Extension of the lagging end should be sufficient to counteract all telomere attrition. In addition, genetic and physical assays suggest an interaction of Cdc13 or one of its protein partners with the machinery executing lagging strand DNA synthesis (52, 92–94). Perhaps this provides Cdc13 with an alternative way to arrive at telomeres while they are in the process of DNA replication.

In addition to its main role as a positive regulator of telomere maintenance, Cdc13 also limits telomere elongation. This is deduced from the telomere elongation phenotype of certain mutations in *CDC13* or the gene for its interacting partner Stn1 (92, 93). For instance, in strains carrying the *cdc13–5* mutation, telomerase elongates telomeres to four times their usual length. The telomeres also have excessive G overhangs in late S phase that become duplex with delayed kinetics, suggesting a defect in the coordination of lagging-strand synthesis with telomere elongation. Overexpression of Stn1 suppresses both the telomere elongation and G-strand overhang phenotypes, pointing to Stn1 as a critical factor in this aspect of telomere replication (Figure 2). Consistent with the idea that Stn1 controls C-strand synthesis, overexpression of Stn1 also suppressed the inappropriate telomere elongation in DNA polymerase α mutants (92, 93). Lundblad and colleagues (92) proposed a two-step model in which Cdc13 would first recruit telomerase to the telomere, allowing extension of the G strand. Subsequently, Cdc13 together with Stn1 would promote C-strand synthesis with this event and limit further elongation of the telomere by telomerase (Figure 2).

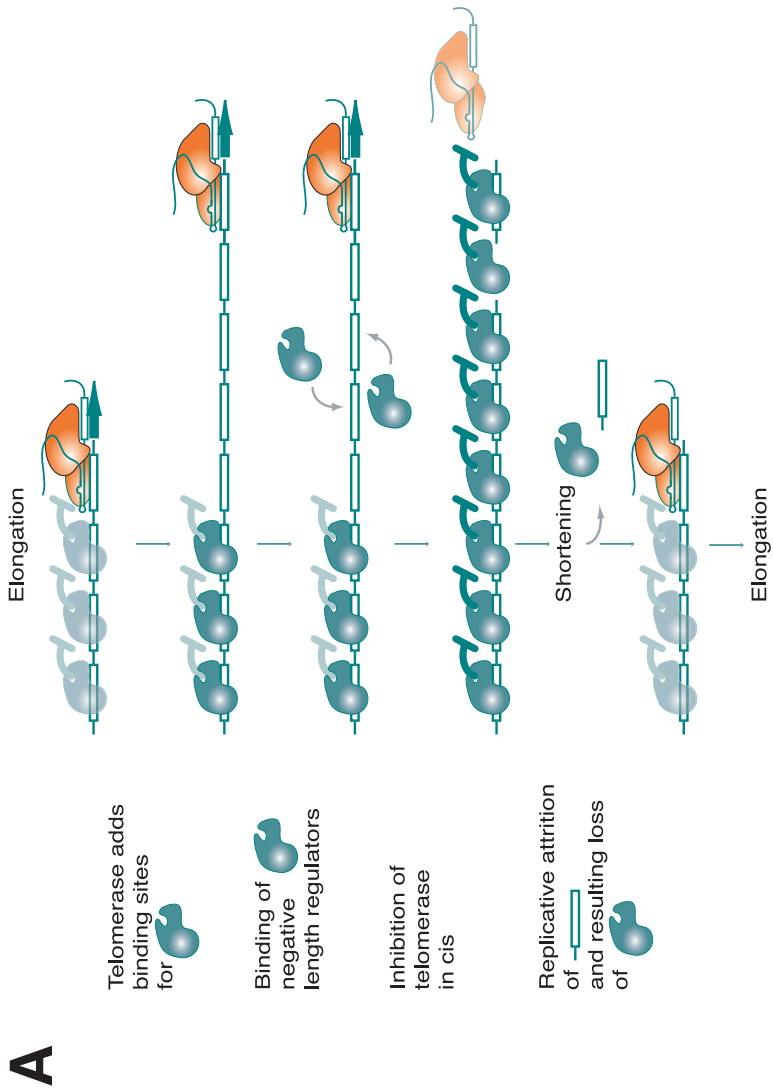
TELOMERE LENGTH HOMEOSTASIS: *CIS*-ACTING CONTROL BY FACTORS BINDING TO DUPLEX TELOMERIC REPEATS

When cells use telomerase-independent methods of telomere maintenance, the length of the individual telomeres is highly variable from chromosome end to chromosome end. By contrast, when telomerase is available, for instance in wild-type yeast or in mammalian tumor cells, telomeres are stably maintained within a relatively narrow size distribution. In these settings, there is a balance between the replicative attrition of telomeres and their elongation by telomerase (95–97). Crosses between closely related species of mice showed that this stable length setting is under genetic control (98, 99).

Telomere length is influenced by the level of telomerase expression but also depends on a control pathway that acts in cis at each individual telomere (Figure 3). The earliest observations on *cis*-acting telomere length control were made by Blackburn and colleagues (96, 100), who introduced an exogenous linear plasmid into budding yeast and found that cells added new telomeres with the same length as the endogenous telomeres. Similarly, a new telomere can be formed after transfection of a telomere seed into mammalian cells; in this process the new telomere undergoes gradual lengthening until it matches the host cell telomeres (101–103). Similar growth of newly formed telomeres was noted in ES cells that had healed a *I-SceI*-cut chromosome with the addition of a new telomere (104). During these telomere healing events, the other telomeres in the cell remain stable, indicating that telomere length control acts in cis at each individual chromosome end. To achieve such control, the length of each individual telomere has to be monitored and regulated independently. Obviously, *cis*-acting length control can not be exerted through changes in the expression of telomerase. Rather, telomeres engage factors that modulate how telomerase acts at the telomere terminus. Thus, like other chromosomal elements, such as enhancers and replication start sites, telomeres recruit a polymerase and locally control its action.

Negative Feedback Control by the Yeast Rap1/Rif1/Rif2 Complex

As telomeres become longer, their further extension by telomerase is progressively inhibited (105). This is an imprecise and stochastic process that keeps telomeres within a broad size range. Telomere length control involves a negative feedback loop in which the addition of new telomeric repeats by telomerase creates binding sites for a telomerase regulator (Figure 3*A*). In budding yeasts, the main *cis*-acting regulator of telomere length is the repressor/activator protein 1, Rap1 (106), reviewed in (107). *S.cerevisiae* Rap1 has a central Myb-type DNA-binding domain, which binds to a loosely defined recognition site present ~20 times within the heterogeneous TG1–3 tract of yeast telomeres (108–110).



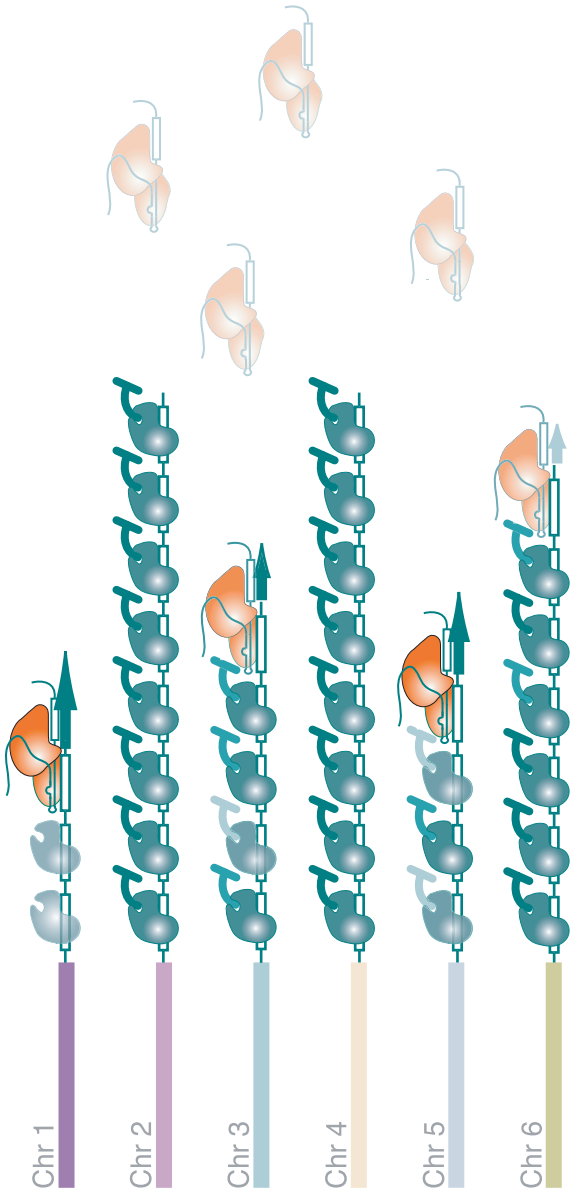


Figure 3 Telomere length homeostasis. The figure displays the principles of *cis*-acting telomere length regulation by telomere binding factors. *A*. Depiction of the negative feedback regulation of telomerase at individual telomeres. Examples of the *cis*-acting negative regulators are TRF1, Taz1, and Rap1. *B*. Schematic representation of different telomere lengthening states within one nucleus. Short telomeres (chromosomes 1 and 5, in this example) are a good substrate for telomerase, whereas within the same nucleus, long telomeres (chromosomes 2 and 4) are not elongated by the enzyme.

Its C terminus is a protein interaction domain that is crucial for telomere length regulation and gene silencing (111–122). Two telomere length regulators, the Rap1 interacting factors Rif1 and Rif2, bind to the Rap1 C terminus, and the same domain recruits the silencing proteins Sir3 and Sir4. Rap1 also contains a BRCT protein interaction domain in its N terminus and a *trans*-activation domain that is important for transcriptional regulation (107, 123).

Although *S.cerevisiae RAP1* is essential, barring assessment of its null phenotype, overexpression studies and several *rap1* temperature sensitive mutants reveal phenotypes consistent with its role as a negative regulator of telomere length (111, 112, 114). Furthermore, deletion of the nonessential *RIF1* and *RIF2* genes, results in extensive telomere elongation (113, 121). Tethering experiments showed that the number of Rap1 molecules bound at each individual chromosome end serves as a gauge for the length of the telomeric repeat array (120). In these studies, the C terminus of Rap1, which includes the region where Rif1 and Rif2 interact, was fused to the DNA-binding domain of Gal4 and tethered to an engineered telomere with subtelomeric Gal4-binding sites. A negative correlation was seen between the number of Gal4 sites and the stable length setting of that telomere, suggesting that as more Rap1 C termini were tethered to the telomere, the final telomere length was shorter. Experiments of this type established the *cis*-acting nature of the feedback control and demonstrated that the number of telomeric repeats at individual chromosome ends is sensed through the number of bound Rap1 molecules (105, 120, 124, 125). Elegant studies in *Kluyveromyces lactis* further confirmed this model and also illuminated the particular importance of the most terminal telomeric repeats in the Rap1p counting mechanism (126–129).

The current challenge is to determine how Rap1 exerts its control. The Rap1 pathway may be connected to a second pathway for telomere length control (discussed below) that involves the DNA damage response kinases Tel1 and Mec1. The effects of Rap1, Rif1, and Rif2 on telomere length are greatly decreased in cells lacking the DNA damage response kinase Tel1 (130, 131). For instance, in *tel1*Δ cells, the counting of Rap1 at telomeres is diminished, and the *rap1–17* mutation, which normally generates extremely long telomeres, no longer has this effect on telomere length (130, 131). One interpretation is that Rap1/Rif1/Rif2 act on Tel1 and Mec1, but the epistasis relationships are not completely straightforward, and other interpretations have been offered (132).

Negative Feedback Control by the Mammalian TRF1 Complex

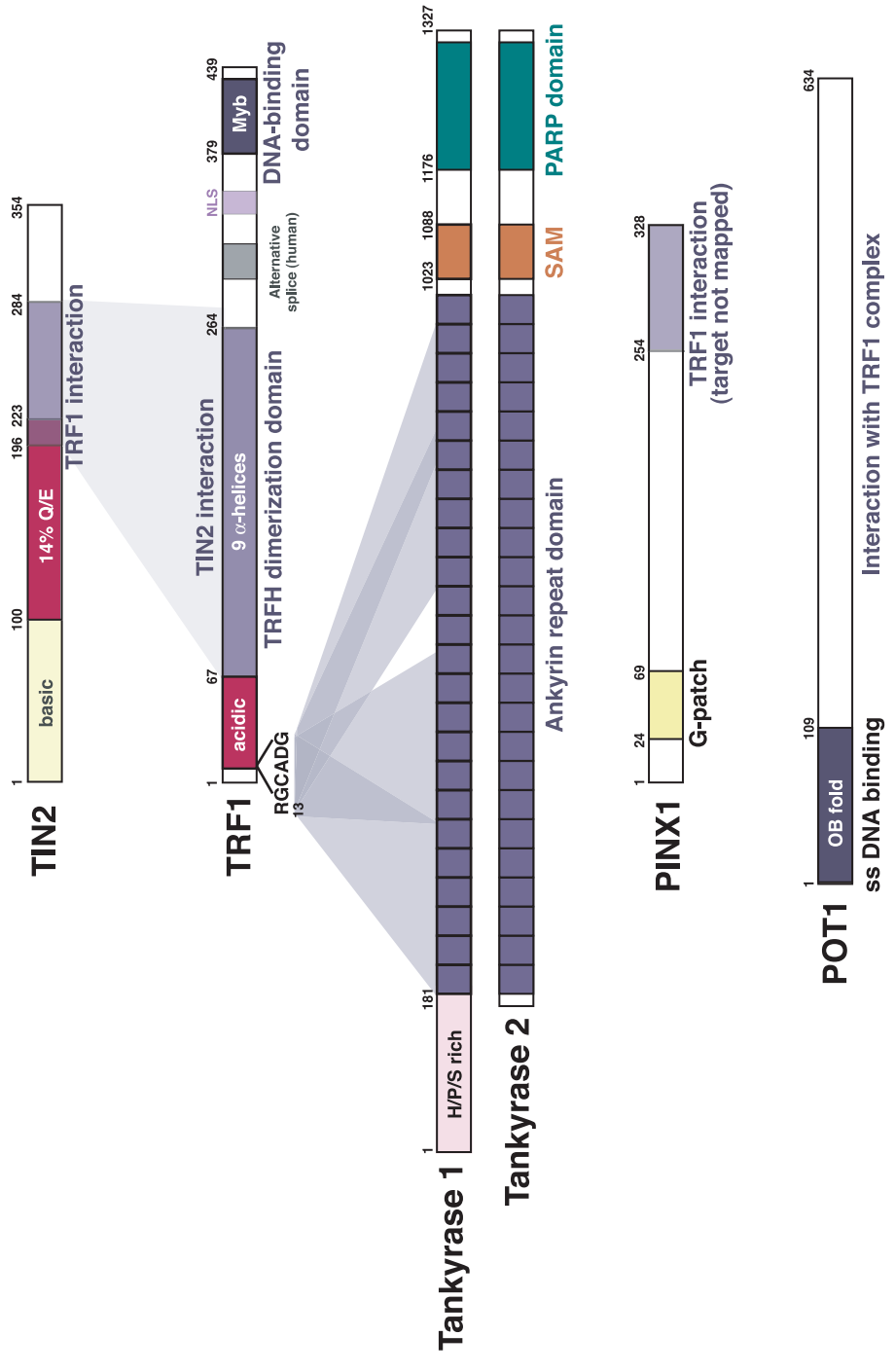
Telomere length control in human cells has been studied in tissue culture systems using immortalized cell lines, which usually maintain their telomeres at a stable length setting. The design of these experiments has to take into account the extensive variation in telomere length. Although tumor telomeres are usually stably maintained, their length can range from ~2 to greater than 20 kb probably due to genetic changes incurred during tumorigenesis [reviewed in (133)]. This

variability bars direct comparison between different tumor cell lines. Furthermore, within each tumor cell line, there can be extensive variation in telomere length between subclones, probably due to epigenetic changes. For instance, subclones of workhorse tumor cell lines, such as HeLa, 293, and HT1080 cells, can vary widely with regard to telomere length setting, telomerase levels, and telomere dynamics (initial growth or shortening after subcloning) (134; B. van Steensel and T. de Lange, unpublished information). Because of this variation, the effect of exogenously expressed genes can not be evaluated in a small number of transfected clones. This problem can be circumvented by studying a large number of individually altered cells simultaneously [for instance, by retroviral infection (135)] or by using cell lines in which inducible gene expression is used to control for clonal variation (97).

Using such inducible gene expression systems, a feedback loop that controls human telomere length was identified (97, 136). The main control is exerted by the TTAGGG repeat binding factor 1 (TRF1), a small dimeric protein with a C-terminal Myb type DNA-binding domain that has exquisite specificity for the sequence TTAGGGTTAG (137–141) (Figure 4). TRF1 binds to the duplex telomeric TTAGGG repeat array, and the total number of TRF1 molecules per chromosome end is correlated with the length of the telomeric tract. ChIP experiments on different cell lines showed that TRF1 immunoprecipitates 20% to 30% of the telomeric DNA, regardless of whether the telomeres were 4 kb or 25 kb, indicating that longer telomeres contain much more TRF1 (142). Furthermore, immunofluorescence studies showed that the TRF1 signal increases with telomere length (136). Thus, TRF1 behaves analogous to Rap1 in that the amount of TRF1 present at telomeres reflects their lengths.

The role of TRF1 in telomere length control was revealed by changing its expression level in the Tet-inducible HTC75 line, a subclone of the human fibrosarcoma cell line HT1080 (97, 136). Overexpression of TRF1 caused telomeres to gradually shorten until a new length setting was achieved. Inversely, partial inhibition of TRF1 through expression of a dominant negative allele resulted in progressive elongation of the telomeres to a new equilibrium length. These telomere length changes occurred even though the telomerase activity was not altered, consistent with a *cis*-acting regulatory pathway. Furthermore, TRF1 levels do not affect the rate of telomere shortening in telomerase negative cells, indicating that TRF1 alters telomere length through an effect on telomere elongation (143). Hence, it was proposed that TRF1 controls the action of telomerase at each individual telomere (97). By tethering a lacI-TRF1 fusion to a subtelomeric array of lacO sites, Gilson and colleagues were able to provide direct proof for the idea that TRF1 can limit telomere elongation in cis (144).

According to the model (Figure 3*B*), a long telomere recruits a large number of TRF1 molecules, which block telomerase from adding more repeats. Conversely, a telomere that is short contains less TRF1 and has a greater chance of being elongated. As a consequence, all telomeres in a given cell line will eventually converge to a similar median telomere length setting. At this equilib-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1
basic
100
14% Q/E
196
223
284
354
TRF1 interaction
TRF1
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acidic
67
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13
TIN2 interaction
9 α-helices
264
TRFH dimerization domain
NLS
Alternative splice (human)
379
Myb
439
DNA-binding domain
Tankyrase 1
1
H/P/S rich
181
Ankyrin repeat domain
1023
1088
1176
SAM
Tankyrase 2
1327
PARP domain
PINX1
1
24
G-patch
69
254
328
TRF1 interaction (target not mapped)
POT1
1
OB fold
109
ss DNA binding
634
Interaction with TRF1 complex

rium length, the amount of telomere-bound TRF1 is sufficient to prevent inappropriate elongation by telomerase but low enough to allow the enzyme to counteract telomere shortening. The final stable length setting in a cell population is determined by the telomerase activity, the rate of telomere shortening, and the levels of telomere length control factors such as TRF1. Changes in each of these parameters can reset telomere length to a new equilibrium. Under extreme circumstances, for instance when one of these factors is absent, no new equilibrium is reached, and telomeres are ultimately lost (e.g., in cells lacking telomerase) or display a run-away elongation phenotype in which telomere continue to lengthen [so far only observed in *K. lactis* (128)].

TRF1 Partners: Tankyrase 1 and 2, TIN2, and PINX1

The binding of TRF1 to telomeres can be inhibited by two related enzymes, tankyrase 1 and 2 (145–151) (Figure 4). The tankyrases are poly(ADP-ribose) polymerases (PARPs), that were originally identified as TRF1 interacting proteins (Figure 4). The two enzymes are nearly identical in amino acid sequence and form both homo- and heterodimers, suggesting that they are functionally similar (or identical) (148, 149, 152). It is likely that the tankyrases also have a multitude of nontelomeric functions, because they are present in the Golgi, nuclear pore complexes, and centrosomes, where they have additional interacting partners (147, 149–151, 153, 154). Tankyrases can ADP-ribosylate TRF1 in vitro, and this modification diminishes the ability of TRF1 to bind to telomeric DNA in vitro (146). Forced overexpression of tankyrase 1 in the nucleus results in removal of TRF1 from telomeres in vivo as determined by IF and ChIP (142, 145, 148). Consistent with these findings, overexpression of tankyrase 1 leads to telomere elongation, the phenotype seen upon TRF1 inhibition.

A second interacting partner of TRF1, TIN2 (Figure 4), can also affect telomere length (135). TIN2 is a small protein with no known domains apart from its C-terminal TRF1 binding domain. It can form a ternary protein complex with both TRF1 and tankyrase, and the presence of TIN2 appears to stabilize the TRF1-tankyrase interaction (J. Ye & T. de Lange, submitted). Conversely, tankyrase promotes the interaction between TRF1 and TIN2. The formation of this ternary complex may be important for the nuclear import of tankyrase. Tankyrase lacks a nuclear localization signal and is predominantly cytoplasmic. But it can be brought into the nucleus through interaction with TRF1 (154), and TIN2 may facilitate this process. In vitro, TIN2 protects TRF1 from being modified by the tankyrases, explaining the stabilizing effect of TIN2 on the TRF1-tankyrase interaction (J. Ye & T. de Lange, submitted). Furthermore, RNAi mediated inhibition of TIN2 results in loss of TRF1 from telomeres, and

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Figure 4 The TRF1 telomere length regulation complex. Domain structure and features of TRF1 and its partners are shown.

this effect is reversed by 3AB, a tankyrase inhibitor. Thus, TIN2 appears to protect TRF1 from being modified by tankyrase. The modulation of tankyrase by TIN2 can explain how tankyrase can accumulate on telomeres even though the enzyme has the ability to dislodge its telomere tethering partner, TRF1.

A fourth TRF1 interacting protein, PINX1 has been proposed to affect telomere length control (155). PINX1 can inhibit telomerase in vitro, and it has been suggested that PINX1 affects telomere length by altering the telomerase activity throughout the nucleus. Such *trans*-acting control of telomerase is not consistent with the proposed role for other components of the TRF1 complex, which are thought to act in cis at individual chromosome ends. It will be interesting to see how these various mechanisms of length control are integrated. Strikingly, PINX1 is the only component of the TRF1 complex that is conserved in budding yeast. Deletion of the budding yeast ortholog of PINX1 (Gno1p) affects rRNA maturation but has no effect on telomere length (156). Human PINX1 is concentrated in the nucleolus (155), the site of both rRNA and, possibly, telomerase maturation.

So far, there is no model that integrates the effects of TRF1, TIN2, tankyrase, and PINX1 on telomere length control. To a great extent, this is due to the fact that these studies have involved overexpression strategies with the associated concern of whether certain phenotypes reflect the real function of the protein or an effect of overexpression (e.g., through titration of other factors). Only in the case of TRF1 has its role as a negative regulator of telomere length been confirmed by the opposing phenotypes of overexpression of the full length protein and a dominant negative allele. Therefore, it will be important to analyze the inhibition phenotype of the TRF1 interacting factors with alternative strategies. The fact that deletion of Trf1 from the mouse genome leads to early embryonic death (157) does not bode well for using mouse genetics to address these questions. Instead, the use of RNAi approaches may provide a more viable alternative.

Telomere Length Control by POT1: Connecting the TRF1 Complex to the Telomere Terminus

One of the main challenges in the dissection of telomere length control is to determine how proteins bound to the duplex telomeric DNA regulate telomerase. The dilemma is that telomerase acts at the 3′ overhang at a considerable distance from most of the regulatory factors, such as TRF1 or Rap1. A recent analysis of human POT1 has shed light on this question.

POT1 was identified based on its sequence similarity to proteins that bind to single-stranded telomeric DNA in ciliates (158). The human version of POT1 has a single-stranded DNA-binding domain in its N terminus, which allows the proteins to bind to arrays of the sequence TAGGGTTAG with great sequence specificity (158; D. Loayza, H. Parsons, K. Hoke, J. Donigian, and T. de Lange, submitted) (Figure 4). In vivo, POT1 associates with telomeres, and this binding is diminished when TRF2 is inhibited, a situation that leads to degradation of the

telomeric overhang (142). These findings show that POT1 is a single-strand telomeric DNA-binding factor.

However, an N-terminal truncation form of POT1 ($POT1^{\Delta OB}$), which lacks the DNA-binding domain, can still associate with telomeres, indicating that binding to single-stranded DNA is not necessary for the association of POT1 with telomeres (142). This second mechanism for telomere association depends on an interaction of POT1 with the TRF1 complex and is proposed to be crucial for telomere length control (142). Endogenous POT1 can be removed from telomeres through inhibition of TRF1, and as was shown for the TRF1 complex, longer telomeres contain more POT1. These data are consistent with POT1 being recruited to the telomeric chromatin by the TRF1 complex and indicate that POT1, like the TRF1 complex, could function as a protein-counting devise to measure telomere length.

The role of POT1 in telomere length homeostasis is apparent from the telomere elongation phenotype of $POT1^{\Delta OB}$. When this mutant is expressed, the endogenous POT1 is repressed (through an unknown mechanism) so that the only version of POT1 at telomeres is the $POT1^{\Delta OB}$ protein. Telomerase positive cells expressing $POT1^{\Delta OB}$ show immediate and extensive telomere elongation. Their telomeres grow from a median of ~6 kb to 20 kb in the course of 40 PD, which is an unusually high rate of telomere elongation and suggests a complete lack of telomerase inhibition. This elongation occurs even though the telomeres contain large amounts of TRF1 and its interacting proteins. Apparently, the displacement of full length POT1 by $POT1^{\Delta OB}$ has abrogated the ability of the TRF1 complex to control telomerase. It was therefore proposed that POT1 functions downstream of the TRF1 complex to relay the negative regulation to the telomere terminus (142).

The model for POT1-mediated telomere length control proposes that the loading of POT1 on the single-stranded telomeric DNA inhibits telomerase from elongating the telomere (Figure 5*A*). As telomeres get longer, more TRF1 complex is present at the chromosome end, increasing the chance of POT1 being present on the single-stranded telomeric DNA where it would block telomerase. The model is based on the finding that the binding of POT1 to telomeres is greatly improved by its association with the TRF1 complex present on the double-stranded telomeric repeat array. For instance, using ChIP, it was found that removal of the TRF1 complex also diminished the association of POT1 with telomeres, even though the length of the single-stranded telomeric DNA was unaffected (142). Thus, through TRF1-mediated loading, POT1 could function to transduce information about the length of the telomere to the telomere terminus.

How does POT1 inhibit telomerase? It could be as simple as blocking access to the 3′ end (Figure 5*A*). POT1 has some preference to bind to its recognition site at a 3′ end (158, 159; D. Loayza, H. Parsons, K. Hoke, J. Donigian, and T. de Lange, submitted), and its physical presence there may simply preclude telomerase from accessing the end. A second model is based on the unusual architecture of telomeres (Figure 5*B*). Mammalian telomeres have been observed

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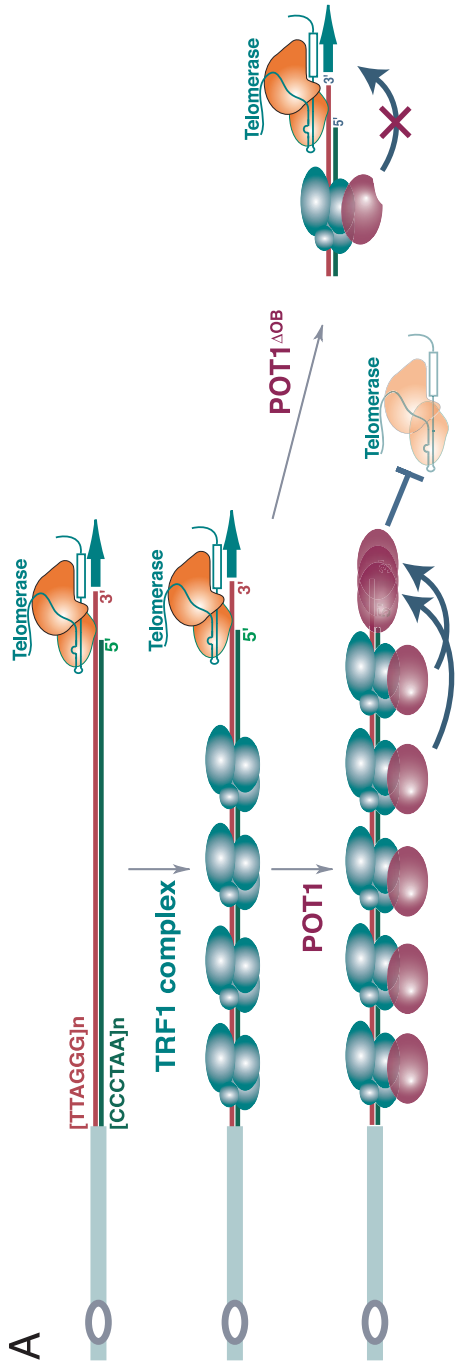

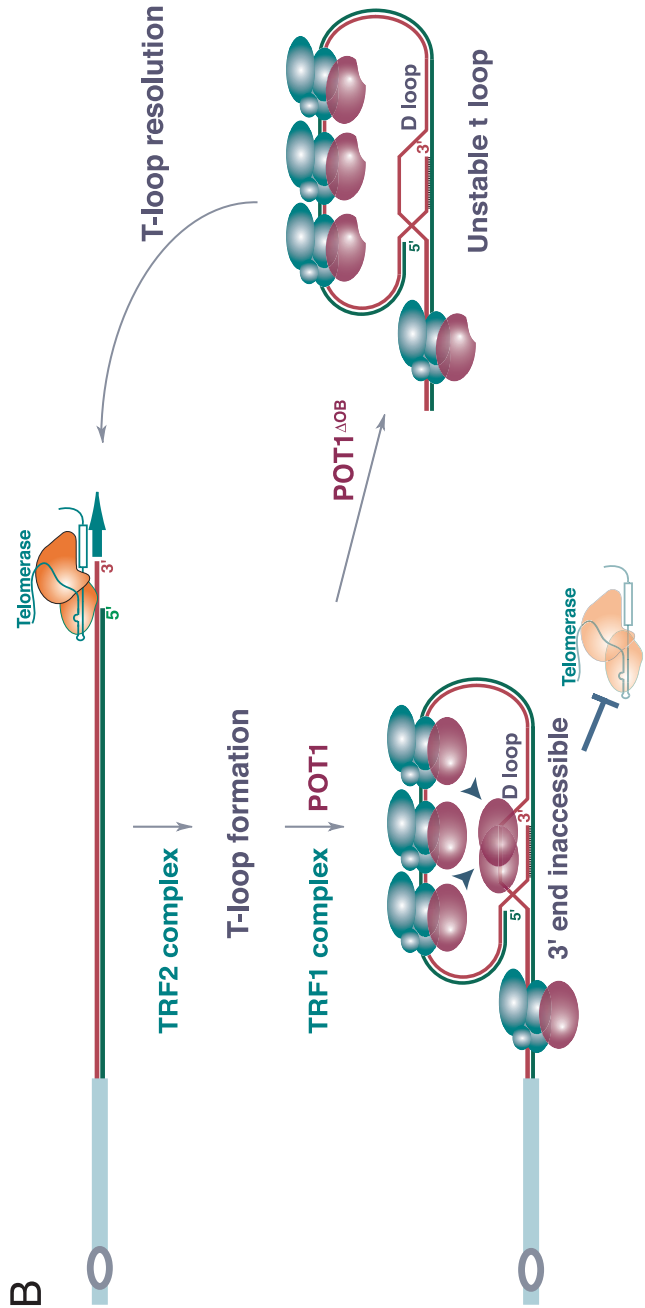

Figure 5 Proposed role for POT1 in telomere length regulation by the TRF1 complex.

in an altered conformation, called t-loops (160), which are large duplex loops formed through the strand-invasion of the G-strand overhang into the duplex part of the telomere. How t loops are created in vivo is not yet known, but in vitro, TRF2 can remodel telomeric DNA into t-loop like structures (161). In the t-loop configuration, the single-stranded 3′ overhang of the telomere terminus is thought to be base-paired to the C-strand sequences. Because telomerase requires an unpaired 3′ end (17, 162), the telomere terminus is unlikely to be accessible to telomerase when telomeres are in t loops. Based on its biochemical features, POT1 should have the ability to bind to the displaced TTAGGG repeats at the base of the t loop (the D loop) (D. Loayza, H. Parsons, K. Hoke, J. Donigian, and T. de Lange, submitted). Potentially the binding of POT1 to the D loop could stabilize t loops (e.g., by preventing branch-migration) and thereby block telomerase from gaining access to the 3′ telomere terminus. Both models explain why $POT1^{\Delta OB}$ abrogates the ability of the TRF1 complex to control telomere length. Although the TRF1 complex can still recruit $POT1^{\Delta OB}$ to the telomeric chromatin, this form of POT1 can not inhibit telomerase because it lacks single-stranded DNA-binding activity (Figure 5*A* and *B*).

POT1 is similar to Cdc13 in *S. cerevisiae* in that they both use an OB-fold to bind to single-stranded telomeric DNA (158, 163). This is a protein motif that is used to recognize single-stranded nucleic acids in numerous settings, which include DNA replication (e.g., replication protein A). As discussed above, the main Cdc13 functions are to protect chromosome ends from degradation and to recruit telomerase. POT1 may be functionally similar because deficiency in pot1 in *Schizosaccharomyces pombe* results in rapid telomere loss (158). Furthermore, human POT1 has been proposed to play a role in telomerase recruitment on the basis of studies in which transfected POT1 induced telomere elongation (164). For both the protective role of human POT1 and to establish whether POT1 is necessary to recruit telomerase, it will be imperative to execute POT1 inhibition studies, for instance using RNAi. Conversely, it will be of interest to establish whether Cdc13 can act as a transducer for the Rap1-dependent telomere length control pathway in *S. cerevisiae*. Given that the *cdc13–5* mutant results in telomere elongation (92), this is a possibility worth pursuing.

FROM YEAST TO MAN: DRASTIC CHANGES IN THE TELOMERE LENGTH CONTROL COMPLEX

Although mammalian TRF1 and Rap1 of *S. cerevisiae* both bind to duplex telomeric DNA and function to control telomere length, these proteins are not orthologs. In fact, the budding yeast protein most closely related to TRF1, Tbf1 (165–167), has not been implicated in telomere biology, and genes for TIN2 and tankyrase are absent from the budding yeast genome. By contrast, mammalian cells do contain a Rap1 ortholog, hRap1, which binds to telomeres and affects their length (168, 169). Overexpression of hRap1 can result in telomere short-

ening, suggesting that hRap1, like scRap1, is a negative regulator of telomere maintenance. Several hRap1 truncation mutants have a telomere elongation phenotype, suggesting that they act as dominant negative alleles (169). Remarkably, hRap1 is not a DNA-binding protein, and its association with telomeres depends on interaction with TRF2, a TRF1 paralog (168) (Figure 6). TRF2 is essential for the protection of chromosome ends [reviewed in (10)] and also contributes to the length regulation of telomeres (136), probably in part through its interaction with hRap1. For human Rap1, the C terminus functions to recruit the protein to telomeres, and telomere length regulation is dependent on the Myb domain and the N-terminal BRCT domain (169). By contrast, in *S.cerevisiae* Rap1, the Myb domain tethers Rap1 to telomeres, and the C terminus recruits the telomere length regulators Rif1 and Rif2. A human ortholog of the yeast Rap1 interacting factor, Rif1, was recently identified, but there is as yet no indication that this protein is a Rap1 interacting factor or associates with telomeres. Rather, Rif1 plays an important role in the ATM-dependent DNA damage response (J. Silverman and T. de Lange, submitted).

TRF1 does have an ortholog in *S. pombe*, the telomere binding protein Taz1 (170) (Figure 6). Like TRF1 and scRap1, Taz1p is a negative regulator of telomere length with taz1 strains showing dramatic telomere elongation (170). Interestingly, Taz1 interacts with the fission yeast ortholog of Rap1 as well as with an ortholog of Rif1p, which is not a Rap1 interacting factor in this organism (171, 172). Both spRap1 and spRif1 behave as negative regulators of telomere length, indicating functional conservation.

Thus, both in mammals and in *S. pombe*, the telomeric complex is built upon a TRF-like factor that interacts with Rap1, whereas in *S. cerevisiae*, Rap1 binds directly to telomeric DNA, and there is no TRF-like protein at telomeres (Figure 6). *S. cerevisiae* Rap1 must have evolved the ability to function as a protein-counting device for telomere length measurements, perhaps co-opting Rif1 and Rif2 in the task of controlling telomerase. With regard to this remarkable rearrangement in the telomeric complex, it will be of interest to learn about the fate (or origin) of Rif2, but so far no orthologs have been found outside the budding yeasts.

This drastic change in architecture of the telomeric complex may relate to the altered sequence of *S. cerevisiae* telomeres (Figure 6). Most eukaryotes have TTAGGG repeats as telomeric DNA or carry a closely related sequence (for instance, TTAGGC in worms, TTTAGGG in plants, and TTACAGG in *S. pombe*), indicating a high degree of sequence conservation in the telomerase template region and a coupled conservation of the telomeric DNA recognition factors. However, the budding yeasts have noncanonical telomeric repeats that are often highly irregular in sequence and diverge very rapidly. We have proposed that the budding yeasts experienced a telomerase catastrophy in which the template region was altered leading to telomeres with a different sequence that could not bind the cognate TRF-like factor (168). The model suggests that these altered telomeric sequences bound budding yeast Rap1 and other essential

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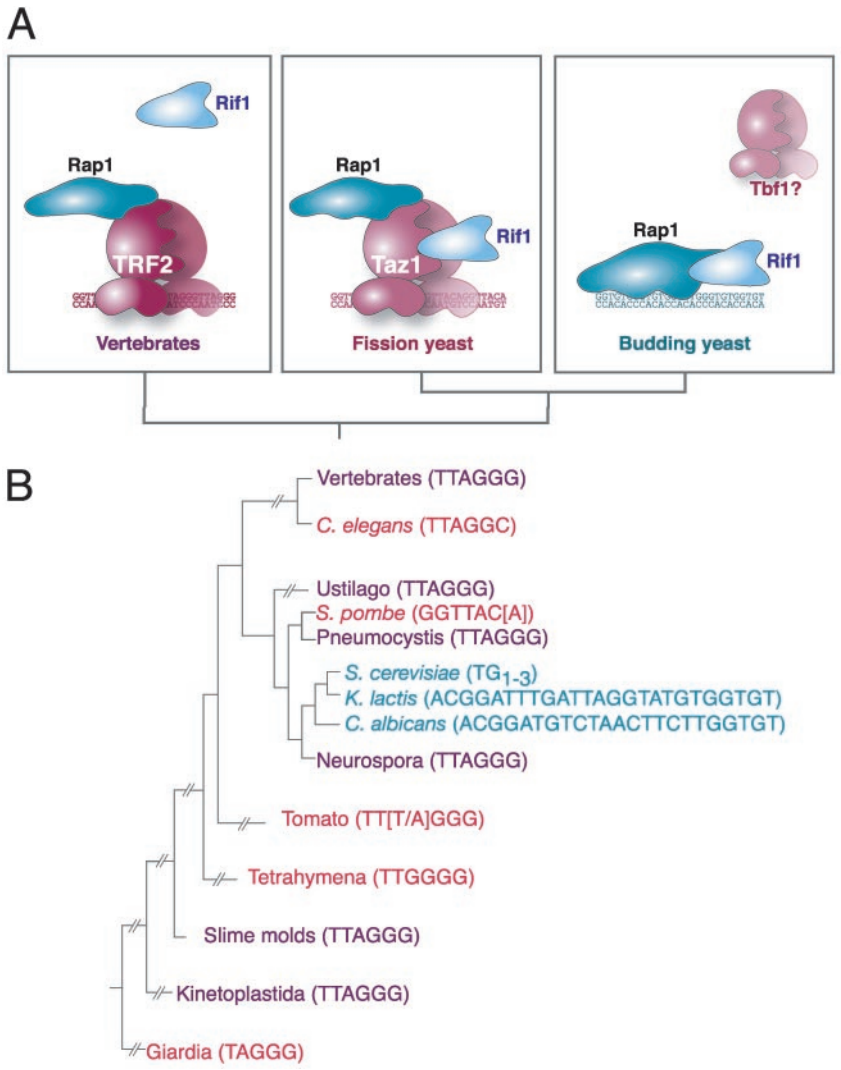

Figure 6 Evolution of the telomeric complex [modified from (168)]. *A*. Relationship between telomeric proteins in vertebrates, fission yeast, and budding yeast. TRF2, Taz1, and Tbf1 are structurally related. In budding yeast, Rap1 binds telomeric DNA, whereas human and fission yeast Rap1 bind to telomeres via TRF2 and Taz1, respectively. Budding yeast Rif1 binds to Rap1, whereas fission yeast Rif1 binds to Taz1. The role of human Rif1 at telomeres has not been established. *B*. Exceptional telomeric DNA in the budding yeasts. Representations of a selection of eukaryotes, their approximate evoluationary relationship, and their telomeric sequences are shown. Most eukaryotes have telomeres with TTAGGG repeats or closely related sequences. The budding yeasts (*blue*) stand out as having noncanonical telomeres that rapidly diverge.

factors mediating telomere function. The now dispensable TRF-like binding module was eventually lost or altered. Budding yeast Tbf1 may well be a vestigial TRF that has lost its place in the telomeric complex. In agreement with this scenario, present day budding yeast telomeres can be converted to TTAGGG repeats without loss of viability or length regulation (173, 174).

DNA DAMAGE RESPONSE PATHWAYS AND THE CONTROL OF TELOMERE MAINTENANCE

Several lines of evidence indicate that telomere maintenance is influenced by components of the DNA damage response pathway. The simplest interpretation is that telomeres resemble damaged DNA (perhaps during or immediately after their replication) and that the associated activation of the DNA damage response pathway regulates telomere maintenance by telomerase. In budding and fission yeast, telomere maintenance is strictly dependent on the presence of one of the two DNA damage response kinases, *TEL1* and *MEC1* in *S. cerevisiae* (175) and *tel1+* and *rad3+* in *S. pombe* (176). When one of the two kinases is missing, telomeres are short (more so for *tel1*Δ) but stable (177, 178). When both are absent, an est phenotype is seen (175, 176). Epistasis analysis showed that *TEL1* acts together with *MRE11/RAD50/XRS2* in telomere maintenance (91, 179), a relationship that is reminiscent of the TM (Tel1/Mre11 complex) checkpoint for double-stranded breaks (180, 181). Perhaps the TM checkpoint needs to detect unprocessed telomere ends in order for telomerase to become activated. If this checkpoint is not available, the Mec1p checkpoint can compensate.

What are the targets of Tel1 and Mec1 in the telomere maintenance pathway? It is unlikely that telomerase itself is regulated because *tel1*Δ *mec1*Δ cells have wild-type levels of the enzymatic activity (132). The DNA repair function of the Mre11 complex can be enhanced by a Tel1-dependent DSB signal (180), so a more likely candidate is the Mre11 complex. Perhaps phosphorylation stimulates the ability of the Mre11 complex to facilitate Cdc13p loading. As discussed above, genetic evidence indicates that Tel1 and Mec1 are affected by (or have an effect on) the Rap1 complex, but the details of this interaction are yet to be resolved.

It is also possible that Rif1 and Rif2 are targets of Tel1 and Mec1 signaling. If Rif1 and Rif2 are absent, yeast can maintain telomeric DNA without the help of Tel1 and Mec1 (132), raising the possibility that these kinases counteract inhibitory effects of Rif1 and Rif2. The recent finding that human Rif1 is regulated by the Tel1 ortholog *ATM* makes this scenario particularly attractive (J. Silverman and T. de Lange, submitted). However, other studies place Rif1 and Rif2 upstream of Tel1 in the telomere length control pathway (131).

The connection between telomeres and the DNA damage response is not limited to *TEL1/MEC1* signaling. Telomere maintenance and length control in budding yeast is also influenced by a number other DNA damage response

factors, which include Rad17, Rad53, Mec3, and Ddc1, and a similar set of genes affects telomere length in fission yeast (182–184). Furthermore, both in budding and fission yeast, deletion of Ku leads to very short but stable telomeres (91, 185–189). This phenotype is not related to the deficiency in NHEJ, because loss of DNA ligase IV does not affect telomere length (185, 187).

As many aspects of the DNA damage response pathways are highly conserved, and much of what is learned in *S. cerevisiae* and *S. pombe* will be a guide for studies of other eukaryotes, which include mammals. However, some critical aspects of both the DNA response pathway and the telomeric complex have changed over the course of evolution, so it is important to verify all regulatory pathways in each species. Several lines of evidence implicate the DNA damage response pathway in telomere length control in mammals. Peripheral blood lymphocytes from adenine-thymine (A-T) patients show significant telomere shortening compared to age-matched normal donors (190) and *ATM* deficient mice have slightly shortened telomeres as well as extrachromosomal telomeric DNA (191; C. Greider, personal communication). However, because no telomere maintenance defect was found in an extensive survey of telomerase-positive A-T cell lines (192), the ATM kinase may not play a role in the telomerase pathway per se but may affect other parameters that result in telomere length changes (e.g., telomere shortening rates).

The case is also not clear for the role of Ku70/80, DNA-PKcs, and PARP-1 in telomere length control. Although *DNA-PKcs* null mice have normal telomere length (193, 194), mice with the *DNA-PKcs* SCID mutation have been reported to have longer telomeres (193, 195). Perhaps the SCID mutation, though deficient in NHEJ, may be a gain of function mutation for the role of DNA-PKcs in telomere length control. It is much harder to explain discrepancies in reports on Ku80. Even though two groups measured telomere lengths in the same *Ku86*$^{-/-}$ mouse strain, one report shows shorter telomeres (196), and the other found no change (197). Finally, mice lacking *PARP-1* have shorter telomeres, but this phenotype is only seen when *p53* is also absent (198, 199).

Clearly, dissection of these pathways in mammals is at an early stage. Similarly, the role of DNA damage response genes in telomere length maintenance in organisms, as diverged as trypanosomes (Ku) (200), worms (Mrt2 and Hus1) (201–203), and plants (Ku and Mre11) (204–206), is now beginning to be addressed.

A final gene that merits discussion in the context of the DNA damage response is *TEL2*. This gene was among the first telomere length regulators identified in *S. cerevisiae* (178), yet its role in telomere maintenance has remained somewhat of a mystery. Budding yeast *TEL2* is an essential gene that encodes a protein that can bind double-stranded and single-stranded TG1–3 sequences (207–209). A mutant allele of *TEL2* (*tel2–1*) gives rise to moderately shortened telomeres (178). Tel2 appears to act in the Tel1 pathway because *tel2–1 tel1–1* double mutant cells have a telomere length defect typical of *TEL1* deficiency (178). Furthermore, consistent with Tel2 functioning in the Tel1 pathway, perturbations in the Rap1p telomere length control do not reset telomere length in *tel2–1* cells (207).

The *Caenorhabditis elegans* ortholog of *TEL2* is *rad-5*, a gene required for the DNA damage checkpoints in this organism (202). Like other checkpoint mutants, *rad-5* mutants do not undergo apoptosis after irradiation, and their germline cells fail to arrest in response to DNA damage (202). This phenotype is also seen with *mrt-2* worms, mutant for the *C. elegans* ortholog of *RAD17* and with worms lacking normal *hus-1* function (op241) (201–203, 210). *Rad-5* is allelic with *clk-2*, a gene that affects biological rhythms and life-span (202, 211, 212). By contrast to what is observed in the *S. cerevisiae tel2–1* mutant, worms with either the *rad-5* or *clk-2* mutation have normal telomere length (202). Conversely, *tel2–1* has no checkpoint defect (202). Because each of these mutations map to different parts of the Tel2 protein, further mutational dissection will be required to determine whether *TEL2/rad-5* is a conserved telomere length regulator and DNA damage checkpoint gene. The link between DNA damage response and telomere maintenance does exist in *C. elegans*; both *hus-1* and *mrt-2* have an est-like progressive telomere loss phenotype (201, 203). A human ortholog of *TEL2* has been identified, but its function at telomeres has not been established.

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