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CHROMOSOME END MAINTENANCE BY TELOMERASE

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Running head: Telomeres and Telomerase
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Telomeres, protein-DNA complexes at the ends of eukaryotic linear chromosomes, are essential for genome stability. The accumulation of chromosomal abnormalities in the absence of proper telomere function is implicated in human aging and cancer. Repetitive telomeric sequences are maintained by telomerase, a ribonucleoprotein (RNP) complex containing a reverse transcriptase subunit, a template RNA, and accessory components. Telomere elongation is regulated at multiple levels, including assembly of the telomerase holoenzyme, recruitment of telomerase to the chromosome terminus, and telomere accessibility. This review provides an overview of telomerase structure, function, regulation, and the role of telomerase in human disease.

The ends of linear eukaryotic chromosomes, telomeres, are highly specialized structures essential for genome stability. The telomere was recognized to be critical for chromosome function more than 70 years ago in independent studies by Herman Muller in *Drosophila melanogaster* and Barbara McClintock in *Zea mays* (1). In 1938, Muller wrote that telomeres "must have a special function, that of sealing the end of the chromosome." (2) We now know that this "seal" is provided by the interaction of numerous proteins with a specialized DNA sequence at chromosome ends. This cap distinguishes normal chromosome termini from broken DNA ends. Erosion of telomeric DNA or disruption of telomeric-binding proteins "uncaps" the end, leading to nucleolytic resection and/or fusion with another telomere or broken DNA end.

Telomere Structure and Replication

Telomeres are composed of double-stranded repeat sequences and a short, single stranded G-rich 3' overhang (the G-tail). Human telomeres contain repeats of the sequence CCCTAA/TTAGGG that vary from 2 to 50 kilo base pairs and a G-tail of 100-250 bases detected throughout the cell cycle. In the budding yeast *Saccharomyces cerevisiae*, telomeres are shorter (250-350 bp) and heterogeneous in sequence ($C_{1\text{-}3}A/TG_{1\text{-}3}$). A 10-15 nucleotide G-tail increases in length to 50-100 bases in late S phase (3).

Proteins that specifically interact with either the double-stranded or single-stranded portions of the telomere recruit additional proteins to the chromosome end, nucleating formation of a specialized higher-order complex (reviewed in ref. (4)). In mammalian cells, the G-tails invade the duplex portion of the telomere to form a t-loop structure (5). A six-subunit complex consisting of TRF1, TRF2, TIN2, Rap1, TPP1 and POT1, termed shelterin, is responsible for formation of the t-loop and protection of telomeres (4,6).

The complete replication of linear DNA molecules is problematic for the eukaryotic DNA replication machinery. This "end replication problem", originally proposed by James Watson and Alexey Olovnikov in the early 1970's (7,8), poses that removal of the terminal RNA primer on the lagging strand leaves an unreplicated gap, resulting in loss of terminal sequences. The discovery that chromosome termini have a 3' overhang requires reexamination of this problem. Since the longer, G-rich strand is the template for discontinuous replication, removal of the RNA primer should recreate a G-tail, although the observation that most C-rich strands end in the same terminal nucleotide suggests that some additional processing does occur (9). On the shorter leading strand, replication to the extreme terminus creates a blunt-ended molecule that must be processed to create a single-stranded overhang structure, resulting in net sequence loss (Fig. 1A; see ref. (10)). Processing of both strands is tightly regulated, but the final overhang length and sequence varies between yeast, ciliates, and humans (9), suggesting mechanistic differences.

Telomerase: A reverse transcriptase with an intrinsic RNA template

Given the loss of essential genetic information predicted by the end-replication problem, eukaryotic cells require a mechanism to maintain telomeres. A series of biochemical and genetic experiments performed in the 1980's identified and characterized this evolutionarily conserved mechanism: the enzyme telomerase.

Biochemical and genetic approaches to identify telomerase components – Pioneering studies demonstrating that linear DNA molecules capped with *Tetrahymena* telomeres were stable in yeast and acquired the characteristic yeast heterogeneous telomere repeat first suggested the existence of a telomere-maintenance enzyme (1). In 1985, Carol Greider and Elizabeth Blackburn reported the discovery of an activity in *Tetrahymena* nuclear extracts capable of elongating a synthetic telomeric $(TTGGGG)_4$ oligonucleotide (11). They named this enzyme "telomere terminal transferase," later shortened to "telomerase."

Treatment with RNase inactivated telomerase, suggesting that an RNA molecule provided the template for nucleotide addition (12). When the gene encoding the RNA was cloned in 1989, the presence of the sequence CAACCCCAA (complementary to the *Tetrahymena* telomeric repeat) provided evidence of this mechanism (13). The catalytic protein component was identified through a convergence of biochemistry and genetics. Joachim Lingner and Tom Cech identified two proteins (p123 and p43) that copurified with the ciliate telomerase RNA (14). At nearly the same time, a yeast genetic screen performed by Victoria Lundblad's group yielded several genes that caused an Ever-Shorter Telomere (EST) phenotype when mutated (15). Cloning of *EST2* and p123 revealed homologous proteins with motifs similar to known reverse transcriptases (RTs; ref. (16)). A year later, the catalytic subunit was identified in humans (the human TElomerase Reverse Transcriptase or hTERT) by multiple groups (see ref. (17)).

In the wake of these groundbreaking experiments, the telomerase field has expanded rapidly, with the identification of telomerase in many organisms, including plants (18). Here we draw from examples in mammals, ciliates, and yeast, highlighting those organisms in which telomerase function and regulation are best understood.

Mechanism of telomerase action – Even though the catalytic protein had not yet been identified, the general model of telomerase action originally proposed by Greider and Blackburn in 1989 (13) was remarkably accurate. Telomerase utilizes an intrinsic RNA molecule (TER) as the template for nucleotide addition to the chromosome terminus by a catalytic reverse transcriptase (TERT; Fig. 1B). Complementarity between the chromosome terminus and the telomerase RNA facilitates alignment of the DNA terminus adjacent to a short RNA template sequence. After reverse transcription of the template, repeated cycles of alignment and extension endow telomeres with their characteristic G-rich repeat arrays. The ability of telomerase to mediate a single round of synthesis is described as nucleotide-addition processivity, while realignment of the same enzyme for a second round of addition is described as repeat-addition processivity (Fig. 1B; ref. (19)). After telomerase-mediated extension of the 3' end, the lagging-strand synthesis machinery fills in the opposite strand.

The catalytic protein subunit of telomerase - Significant effort has been invested to understand the structure and function of TERT, homologs of which have been identified from a large number of organisms. With few exceptions, these homologs share a common domain structure (Fig. 1C; see ref. (19)). The central region of TERT contains homology with other RTs, including seven canonical motifs that contribute to catalytic activity (17). Like other polymerases, TERT contains a triad of absolutely conserved aspartic acid residues thought to be essential for metal binding and enzyme chemistry, mutation of which destroys TERT catalytic activity (16). A recently published crystal structure of TERT from the beetle *Tribolium castaneum* reveals the right-handed "fingers, palm, and thumb" domain structure characteristic of all nucleic acid polymerases (20). The fingers and palm are contributed by the highly conserved RT motifs, while the less conserved region C-terminal to the RT domain (the C-terminal extension or CTE) forms the thumb. Yeast TERT from which the CTE has been deleted maintains short but stable

telomeres *in vivo* (though enzyme processivity is reduced *in vitro*) and *C. elegans* TERT lacks this domain entirely (19). In contrast, some mutations in the hTERT CTE impair telomere maintenance while retaining catalytic activity, suggesting that this domain may have a telomere-maintenance role distinct from enzymatic function (19).

The region of TERT located N-terminal to the RT domain contributes to properties unique to telomerase including association with the intrinsic RNA template, binding of additional protein components, and modulation of processivity. Sequence alignments and mutagenesis have identified several functionally important N-terminal regions termed GQ, CP, QFP and T (Fig. 1c; see ref. (19) for alternate nomenclature). The CP, QFP and T regions contribute to high-affinity interactions with the telomerase RNA. The most N-terminal region (GQ; RID1 in hTERT) displays low-affinity RNA interactions, but may also mediate association with other telomerase components (*e.g*. Est3 in *S. cerevisiae*) and provide an "anchor site" at which telomerase associates with the DNA primer (19). Such protein/DNA contact may facilitate repeat addition processivity by allowing telomerase to remain associated with the telomere while repositioning the 3' DNA terminus within the active site. A crystal structure of the most N-terminal structural domain (TEN) containing the GQ motif of *T. thermophila* revealed a putative primer-binding surface and identified residues contributing to DNA interaction (21). This domain is lacking in *T. castaneum* TERT (20), so the structural relationship of TEN to the catalytic domain is unknown.

The telomerase RNA component - The RNA component of telomerase (TER) has been cloned from many different organisms and shows great variability in length, sequence, and structure (22). In common between all TERs is a short template sequence located on an unpaired region of the RNA and complementary to the telomeric repeat. In contrast to other RTs, reverse transcription is constrained to this short template. In both human and yeast TER, a stem-loop structure prevents telomerase extension past the end of the template (23,24), while 5' boundary definition in *Tetrahymena* may require interaction between TERT and an unpaired sequence located immediately 5' of the template (25).

Those TERs that have been extensively studied bind proteins involved in RNP biogenesis. The human telomerase RNA (hTR) is transcribed by RNA polymerase II and stabilized by association with a group of proteins (dyskerin, GAR1, NOP10, and NHP2) involved in the accumulation of small nucleolar (sno) and small Cajal body (sca) RNAs (26). Two ATPases, pontin and reptin, contribute to RNA stability and telomerase assembly (27). The *S. cerevisiae* telomerase RNA (TeLomerase Component 1; *TLC1*) is stabilized by association with the Sm proteins, a complex of seven polypeptides that contributes to the assembly of RNPs necessary for pre-mRNA splicing (28). The detailed mechanism of telomerase assembly and intracellular trafficking has been recently reviewed (26) and remains an area of active research.

Additional Protein Components Contribute to the Activity of Telomerase - Telomerase activity from several different organisms can be reconstituted in rabbit reticulocyte lysate by expression of TERT and TER, indicating that these subunits comprise the catalytic core of the enzyme (19). Numerous proteins associate with telomerase, many of which, as discussed above, contribute to RNP biogenesis. However, some factors contribute directly to telomere replication *in vivo*.

The role of such proteins has been most extensively studied in *S. cerevisiae*. The Est1 and Est3 proteins are essential for telomere elongation *in vivo* (15,29), but extract prepared from cells lacking either protein retains telomerase activity, suggesting a regulatory role (30). Est1 binds the telomerase RNA via a bulged stem structure (31) and associates directly with Cdc13 (32), a protein that binds the single-strand telomeric overhang. Direct fusion of Est2 to Cdc13 bypasses the requirement for Est1 in telomere maintenance (3), suggesting that the Est1-Cdc13 interaction recruits telomerase to telomeres (see below). Two human homologs, hEST1A and B, also associate with telomerase (33,34). Although hEST1A uncaps chromosomes when overexpressed (34), a role in telomerase recruitment has not been established. EST1A may promote telomere integrity by modulating the accumulation of RNA transcripts at telomeres (35).

Like Est1, the Est3 protein coimmunoprecipitates with the catalytic core of

telomerase (36), but its function is poorly understood. Structural modeling suggests that Est3 is homologous to mammalian TPP1 (37,38), a component of the shelterin complex. This evolutionary relationship raises questions about the function of this accessory protein and its differential association with the telomerase complex (in yeast) or the telomere-binding complex (in higher organisms).

Regulation of Telomerase Activity

The extent and timing of telomere replication are regulated by several mechanisms including transcriptional and posttranscriptional regulation of TERT, recruitment of telomerase to the chromosome terminus, and control of telomerase accessibility by telomeric binding factors.

Transcriptional and posttranscriptional regulation of TERT - Transcriptional regulation of hTERT is a major factor limiting telomerase activity in human cells. hTERT expression is low or absent in most somatic cells, but is activated during embryonic development, in some stem cells, and in most tumors (Fig. 2; ref. (39)). Expression of hTR also limits telomerase function in some cell types (40). Post-transcriptional events including alternative splicing of TERT, phosphorylation and ubiquitination of the enzyme, and intracellular trafficking of telomerase components add additional layers of complexity to the regulation of telomerase (40,41). The relative contributions of these activities to telomerase function remain incompletely understood.

Recruitment of telomerase to telomeres – The activity of telomerase at the telomere is modulated, at least in part, by regulating its recruitment to the extreme chromosome terminus. This event is separable from telomerase association with telomeric chromatin. These mechanisms are best characterized in *S. cerevisiae*. Est2 (yeast TERT) is constitutively associated with telomeric DNA, though telomeres are only extended during S phase. Telomeric enrichment is high in G1 phase, decreases in early S phase and rises again in late S phase (42). G1 association depends upon interaction of a 48nt stem-loop of the *TLC1* RNA with Ku, a heterodimeric complex that binds at the junction of single-stranded and double-stranded telomeric DNA, while the peak of Est2p enrichment in late S phase requires Cdc13p (3).

In contrast to Est2, the telomerase accessory factor Est1 is only telomere-associated in late S through G2 phases (42). During G1 phase, Est2 may be sequestered from its substrate by the interaction of *TLC1* RNA with the Ku complex. As the cell cycle progresses, several events facilitate the productive recruitment of telomerase to its site of action. First, Est1 expression increases as cells enter S phase. Second, progression to late S phase results in telomerase-independent lengthening of the single-stranded telomeric overhang (43), allowing increased association by Cdc13 (42). Third, phosphorylation of Cdc13 by the cyclin-dependent kinase (CDK1) facilitates interaction with Est1 (44). Mutations that prevent phosphorylation of Cdc13 by CDK1 reduce but do not abolish telomerase recruitment and activity (44) suggesting that additional mechanisms contribute to this regulation (see below).

The association of mammalian TERT with the chromosome terminus is also cell cycle regulated (detected only in S phase; ref. (45)) and some mutations in TERT can be rescued by fusion to telomere binding proteins (46). Although the mechanism of telomerase recruitment to human telomeres is an area of further research, this evidence suggests that recruitment of the enzyme is an important regulatory step.

Control of telomerase accessibility to the chromosome terminus – The extent of telomerase activity in cells is significantly regulated through the modulation of telomerase access to telomeres by telomere-binding proteins. In *S. cerevisiae*, sequence-specific binding of Rap1 protein to the double-stranded telomeric repeats "measures" telomere length through a mechanism in which the number of Rap1 molecules bound to a telomere inversely correlates with the action of telomerase at that telomere (4,47). In *S. pombe* and human cells, Rap1 homologs contribute to negative telomere length regulation, although in both cases Rap1 associates indirectly with telomeric DNA (through Taz1 or Trf2, respectively). Like Trf2 and Rap1, other components of the telomere-binding shelterin complex act as negative regulators of telomerase in mammals (4).

In *S. cerevisiae*, the pattern of telomere addition has been analyzed during a single S phase (48). Intriguingly, telomerase does not act on every telomere in each cell cycle, but

preferentially extends the shortest telomeres. At wild-type length, only 6-8% of telomeres are extended, while the frequency increases to 50% upon shortening of telomeres to 100 nucleotides (48). These data suggest that the telomere switches between telomerase-extendible and non-extendible states in a manner influenced by telomere length. The frequency of telomere elongation increases when Rap1p function is compromised, implying that Rap1p facilitates formation of a state that discourages telomerase action (48).

A series of recent publications point to a central role of the yeast homolog of the ataxia-telangectasia-mutated (ATM) kinase, Tel1, in promoting telomerase activity at short telomeres (reviewed in (49)). Tel1 preferentially associates with short telomeres *in vivo* and is required for telomerase association with, and activity on, these short telomeres. Cdc13 is a substrate of Tel1 (50), raising the possibility that Tel1-dependent phosphorylation of Cdc13 contributes to the regulation of telomerase recruitment. Telomerase also preferentially elongates critically short telomeres in mouse cells (51), suggesting that similar regulatory mechanisms may protect mammalian genome stability in the face of net telomere shortening.

Role of Telomerase in Disease

Since telomerase activity is low or absent in most human cells, telomeres shorten with each division. When telomeres reach a threshold length, checkpoint-mediated cellular senescence is initiated, halting cellular division (Fig. 2A). As a result, telomere length serves as a cellular "clock" to limit proliferation. This limitation may contribute to the aging process in both normal and disease states, while inappropriate activation of telomerase allows unregulated cell growth [Fig. 2; ref. (52)].

Role of telomerase in degenerative diseases – Much of our understanding of the role of telomerase in human health comes from studies of diseases of telomerase deficiency. Dyskeratosis congenita (DKC), a disease associated with progressive bone marrow failure and defects in other highly proliferative tissues, results from mutations affecting the stability or activity of telomerase [Fig. 2A; ref. (53)]. Autosomal dominant DKC is associated with haploinsufficiency of hTR or hTERT, while some recessive forms are linked to genes with roles in hTR maturation and stability (dyskerin, *NHP2*, and *NOP10*; refs. (53-55)). The recent demonstration that aplastic anemia and idiopathic pulmonary fibrosis can be caused by telomerase dysfunction provides further evidence of the critical role of telomerase in cell proliferation (53). Many of these diseases involve phenotypes typically associated with aging, suggesting that limitations on tissue renewal imposed by telomere shortening may contribute to the normal aging process (53).

Telomerase and Cancer - In humans, the endogenous limit imposed on proliferation may serve an important tumor-suppressive role (Fig. 2B). Rarely, a checkpoint-deficient cell continues dividing, causing telomeres to "uncap." This phase (crisis) is characterized by frequent chromosomal fusions and rearrangements. The massive genome instability that results from these processes is usually lethal. Occasionally, telomerase can be re-activated to restore telomeres, thereby stabilizing the genome and allowing cancer cells to escape normal limits on cellular proliferation (Fig. 2B; ref. (39)). Telomerase re-activation occurs in at least 85% of human tumors. Telomeres in the remaining tumors are maintained by an alternate, recombination-based mechanism termed ALT (56).

Transcriptional regulation of hTERT is a major determinant of telomerase activity in cancer cells, but multiple other mechanisms for the tumor-specific up-regulation of telomerase have been proposed, such as gene amplification, alternative splicing, and changes in subcellular localization and phosphorylation of the enzyme (41). The relative importance of each of these mechanisms is incompletely understood.

Because of the near-ubiquitous activation of telomerase during oncogenesis, telomerase is an attractive target for anti-cancer drugs and gene therapy. Telomerase inhibitors can trigger apoptotic cell death when telomeres reach a critical length. In addition, hTER and hTERT regulatory sequences can be used to drive selective expression of cytotoxic agents in tumor cells. Telomerase is a candidate antigen for cancer vaccines aimed at harnessing the immune system to selectively target TERT-positive cancer cells. Clinical trials testing telomerase inhibitors and

vaccine-type approaches are currently underway (57).

Summary

By distinguishing normal and broken chromosome ends and providing a mechanism for complete replication, telomeres play an essential role in safeguarding genomic integrity. The enzyme telomerase, a unique reverse transcriptase with an intrinsic RNA template, is responsible for synthesizing telomeric DNA. A myriad of regulatory events ensure that the shortest telomeres are specifically targeted for replication by telomerase and that telomere length is appropriately modulated. Future work will continue to address the full complement of proteins that comprise the telomerase complex and the mechanisms that regulate telomerase access to telomeres. Such understanding is critical to our ability to manipulate telomerase function in disease where both inappropriate up-regulation and down-regulation of telomerase have detrimental consequences.

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Figure legends

Fig. 1. The end-replication problem and telomerase. *A.* End-replication problem. Replication of a telomere containing a 3' overhang on the lagging-strand template (dark blue) and a recessed 5' terminus on the leading-strand template (red) is diagrammed. RNA primer is depicted as a dotted line. *B.* General model of telomerase function. The template of the telomerase RNA (CA-rich sequence; shown from *Tetrahymena*) is complementary to the 3' terminal overhang. TERT protein (blue oval) adds nucleotides (red) until the template boundary is reached. Translocation and repositioning of the 3' terminus allows addition of a second repeat (green). *C.* Generalized domain structure of TERT. Conserved aspartic acid residues (D) necessary for catalysis are indicated. The N-terminus contains regions that confer high-affinity (green) and low-affinity (blue) RNA binding. For details see ref. (19).

Fig. 2. Telomere dynamics during normal and abnormal cell growth. *A.* In general, germ cells express telomerase and maintain long telomeres (light gray) while absent or low expression of telomerase in somatic cells and adult stem cells is insufficient to prevent telomere shortening (medium gray). Cell proliferation slows or ceases when cell cycle checkpoints are activated by short telomeres (dotted line), perhaps contributing to normal aging. Individuals with reduced telomerase activity exhaust telomere reserves more rapidly (dark gray), leading to premature aging, particularly in highly proliferative tissues. *B.* Model of telomerase activation during tumorigenesis. When cell cycle checkpoints fail, cells divide in the face of severe telomere loss. Telomere upcapping causes genome instability and lethality (crisis). Reactivation of telomerase (or initiation of ALT) allows cells to exit crisis and proliferate with short but stable telomeres.

References

1. Blackburn, E. H., Greider, C. W., and Szostak, J. W. (2006) *Nat Med* **12**, 1133-1138
2. Muller, H. J. (1938) *Collecting Net* **8**, 182-198
3. Bianchi, A., and Shore, D. (2008) *Molecular Cell* **31**, 153-165
4. Smogorzewska, A., and de Lange, T. (2004) *Annual Review of Biochemistry* **73**, 177-208
5. Wei, C., and Price, M. (2003) *Cellular and Molecular Life Sciences (CMLS)* **60**, 2283-2294
6. de Lange, T. (2005) *Genes & Development* **19**, 2100-2110
7. Watson, J. (1972) *Nature New Biol* **239**, 197-201
8. Olovnikov, A. M. (1973) *J.Theor.Biol.* **41**, 181-190
9. Sfeir, A., Shay, J., and Wright, W. (2005) *Cell Cycle* **4**, 1467-1470
10. Lingner, J., Cooper, J. P., and Cech, T. R. (1995) *Science* **269**, 1533-1534
11. Greider, C. W., and Blackburn, E. H. (1985) *Cell* **43**, 405-413
12. Greider, C. W., and Blackburn, E. H. (1987) *Cell* **51**, 887-898
13. Greider, C. W., and Blackburn, E. H. (1989) *Nature* **337**, 331-337
14. Lingner, J., and Cech, T. R. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 10712-10717
15. Lendvay, T. S., Morris, D. K., Sah, J., Balasubramanian, B., and Lundblad, V. (1996) *Genetics* **144**, 1399-1412
16. Lingner, J., Hughes, T. R., Shevchenko, A., Mann, M., Lundblad, V., and Cech, T. R. (1997a) *Science* **276**, 561-567
17. Nakamura, T. M., Morin, G. B., Chapman, K. B., Weinrich, S. L., Andrews, W. H., Lingner, J., Harley, C. B., and Cech, T. R. (1997) *Science* **277**, 955-959
18. Riha, K., and Shippen, D. E. (2003) *Chromosome Research* **11**, 263-275
19. Autexier, C., and Lue, N. F. (2006) *Annual Review of Biochemistry* **75**, 493
20. Gillis, A. J., Schuller, A. P., and Skordalakes, E. (2008) *Nature* **455**, 633-637
21. Jacobs, S. A., Podell, E. R., and Cech, T. R. (2006) *Nat Struct Mol Biol* **13**, 218-225
22. Theimer, C. A., and Feigon, J. (2006) *Current Opinion in Structural Biology* **16**, 307-318
23. Tzfati, Y., Fulton, T. B., Roy, J., and Blackburn, E. H. (2000) *Science* **288**, 863-867.
24. Chen, J.-L., and Greider, C. W. (2003) *Genes & Development* **17**, 2747-2752
25. Lai, C. K., Miller, M. C., and Collins, K. (2002) *Genes & Development* **16**, 415-420
26. Collins, K. (2008) *Mechanisms of Ageing and Development* **129**, 91-98
27. Venteicher, A. S., Meng, Z., Mason, P. J., Veenstra, T. D., and Artandi, S. E. (2008) *Cell* **132**, 945-957
28. Seto, A. G., Zaug, A. J., Sabel, S. G., Wolin, S. L., and Cech, T. R. (1999) *Nature* **401**, 177-180
29. Lundblad, V., and Szostak, J. W. (1989) *Cell* **57**, 633-643
30. Lingner, J., Cech, T. R., Hughes, T. R., and Lundblad, V. (1997b) *PNAS* **94**, 11190-11195
31. Seto, A. G., Livengood, A. J., Tzfati, Y., Blackburn, E. H., and Cech, T. R. (2002) *Genes Dev.* **16**, 2800-2812
32. Pennock, E., Buckley, K., and Lundblad, V. (2001) *Cell* **104**, 387-396.
33. Snow, B. E., Erdmann, N., Cruickshank, J., Goldman, H., Gill, R. M., Robinson, M. O., and Harrington, L. (2003) *Curr Biol* **13**, 698-704
34. Reichenbach, P., Hoss, M., Azzalin, C. M., Nabholz, M., Bucher, P., and Lingner, J. (2003) *Curr Biol* **13**, 568-574

35. Azzalin, C. M., and Lingner, J. (2008) *Cell Cycle* **7**, 1161-1165
36. Hughes, T. R., Evans, S. K., Weilbaecher, R. G., and Lundblad, V. (2000) *Curr. Biol.* **10**, 809-812.
37. Lee, J., Mandell, E. K., Tucey, T. M., Morris, D. K., and Lundblad, V. (2008) *Nat Struct Mol Biol* **15**, 990-997
38. Young Yu, E., Wang, F., Lei, M., and Lue, N. F. (2008) *Nat Struct Mol Biol* **15**, 985-989
39. Shay, J. W., and Wright, W. E. (2006) *Nat Rev Drug Discov* **5**, 577-584
40. Cairney, C. J., and Keith, W. N. (2008) *Biochimie* **90**, 13-23
41. Aisner, D. L., Wright, W. E., and Shay, J. W. (2002) *Current Opinion in Genetics & Development* **12**, 80-85
42. Taggart, A. K. P., Teng, S.-C., and Zakian, V. A. (2002) *Science* **297**, 1023-1026
43. Wellinger, R. J., Wolf, A. J., and Zakian, V. A. (1993) *Cell* **72**, 51-60
44. Li, S., Makovets, S., Matsuguchi, T., Blethrow, J. D., Shokat, K. M., and Blackburn, E. H. (2009) *Cell* **136**, 50-61
45. Tomlinson, R. L., Ziegler, T. D., Supakorndej, T., Terns, R. M., and Terns, M. P. (2006) *Mol Biol Cell* **17**, 955-965
46. Armbruster, B. N., Linardic, C. M., Veldman, T., Bansal, N. P., Downie, D. L., and Counter, C. M. (2004) *Mol. Cell. Biol.* **24**, 3552-3561
47. Marcand, S., Gilson, E., and Shore, D. (1997) *Science* **275**, 986-990
48. Teixeira, M. T., M, A., P, S., and Lingner, J. (2004) *Cell* **117**, 323-335
49. Sabourin, M., and Zakian, V. (2008) *Trends in Cell Biol* **18**, 337-346
50. Tseng, S.-F., Lin, J.-J., and Teng, S.-C. (2006) *Nucl. Acids Res.* **34**, 6327-6336
51. Hemann, M. T., Strong, M. A., Hao, L.-Y., and Greider, C. W. (2001) *Cell* **107**, 67-77
52. Finkel, T., Serrano, M., and Blasco, M. A. (2007) *Nature* **448**, 767-774
53. Aubert, G., and Lansdorp, P. M. (2008) *Physiol. Rev.* **88**, 557-579
54. Vulliamy, T., Beswick, R., Kirwan, M., Marrone, A., Digweed, M., Walne, A., and Dokal, I. (2008) *Proceedings of the National Academy of Sciences* **105**, 8073-8078
55. Walne, A. J., Vulliamy, T., Marrone, A., Beswick, R., Kirwan, M., Masunari, Y., Al-Qurashi, F.-h., Aljurf, M., and Dokal, I. (2007) *Hum. Mol. Genet.* **16**, 1619-1629
56. Cesare, A., and Reddel, R. (2008) *129* **1-2**
57. Shay, J. W., and Keith, W. N. (2008) *Br J Cancer* **98**, 677-683

Figure 1

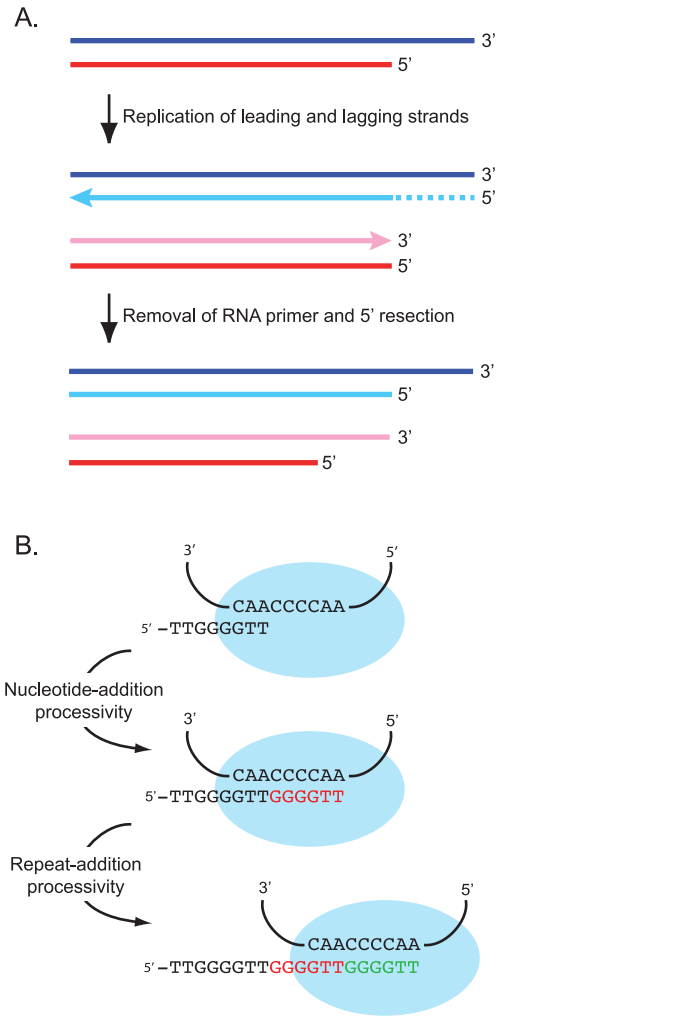

Figure 2