Published in final edited form as:

Mech Ageing Dev. 2008 ; 129(1-2): 91–98.

Physiological Assembly and Activity of Human Telomerase Complexes

Kathleen Collins

Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720-3200

Abstract

Telomerase is a specialized reverse transcriptase conserved throughout almost all eukaryotic life. It plays a fundamental role in genome maintenance, adding back the telomeric DNA repeats lost from chromosome ends due to incomplete replication or damage. The protein and RNA subunits of telomerase fold and function in a co-dependent manner to establish a high fidelity of telomeric repeat synthesis. Over the past two decades, studies of telomerase have uncovered previously unanticipated levels of complexity in its assembly, activity and regulation. This review describes the current understanding of telomerase in humans, with particular focus on telomerase biogenesis and regulation in its cellular context.

Detection of human telomerase activity

Studies of human telomerase began with the development of assays that could detect the enzyme in cell extract. HeLa human cervical carcinoma cells provided the first source of telomeric repeat synthesis activity in vertebrates (Morin, 1989). The vast majority of human cancer cell lines examined since then have been found to harbor active telomerase (Shay and Wright, 2005). In the pioneering characterization of human telomerase activity *in vitro*, DNA synthesis was assayed by monitoring the extension of oligonucleotide primers with deoxynucleotide triphosphates required to generate TTAGGG repeats (Morin, 1989; Morin, 1991). Subsequently, a PCR-amplified version of the telomerase product assay was developed, termed the TRAP assay, which has allowed robust detection of telomerase activity in a broad range of cancers and cancer cell extracts (Kim et al., 1994). The direct primer extension or ‘conventional’ assay uses a telomeric repeat primer, while the TRAP assay uses non-telomeric sequence primer with a much lower telomerase binding affinity. Also, the PCR step of the TRAP assay preferentially amplifies the longer, multi-repeat telomerase products. Competition for primer binding by nucleic acid in cell extract and changes in the nucleotide and repeat addition processivity of product synthesis dramatically impact TRAP assay results. For this reason, comparisons of TRAP assay products between whole cell extracts from similar cells are more reliable than cross-comparison across different cell extracts or comparison of crude extract with partially purified enzyme fractions (Keith et al., 2007).

In contrast with extracts from cancer cells, extracts from most normal human somatic cells harbor very low or undetectable telomerase activity. Mechanisms by which telomerase is inhibited in human somatic cells are numerous, including down-regulation of hTERT by transcriptional silencing, alternative splicing and degradation (Collins and Mitchell, 2002; Forsyth et al., 2002; Autexier and Lue, 2006; Collins, 2006). The relatively few human somatic cells that are ‘telomerase-positive,’ as judged by *in vitro* telomerase activity assay, are from

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

fetal tissues or rapidly proliferating compartments of adult hematopoietic, epithelial and germline lineages (Collins and Mitchell, 2002; Forsyth et al., 2002). Conclusions about 'telomerase-positive' versus 'telomerase-negative' cell types have major caveats in the non-specific activity assay inhibitors in cell extract and the potential for disrupted telomerase regulation upon cell lysis. A small percentage of telomerase-positive cells in a mixed tissue could escape detection (false negative) or give the appearance of telomerase-positive majority cell type (false positive). For these reasons, one important direction for future research will be the development of improved methods for measuring telomerase activation using small numbers of cells and using whole tissues in a manner that can detect individual rather than average cell activity.

Telomerase subunit requirements for catalytic activity

Telomerase catalytic activity requires the association of two universal telomerase subunits: telomerase RNA (TER) and telomerase reverse transcriptase (TERT). TER provides the template for repeat synthesis and has numerous other motifs that are important for function in vitro and in vivo (Theimer and Feigon, 2006). Some of the non-template structures contribute to TERT binding and catalytic activity, while others are important for stability and regulation (Figure 1A). TERT provides the active site for magnesium-catalyzed nucleotide addition (Autexier and Lue, 2006). The central TERT polymerase domain has motifs conserved among reverse transcriptases and some unique to TERT. The TERT-specific N- and C-terminal extensions from the polymerase domain contribute interactions with TER and single-stranded DNA (Figure 1B). Heterologous reconstitution studies expressing human TER (hTR) and human TERT (hTERT) in rabbit reticulocyte lysate were the first to reveal that these two telomerase-specific components can reconstitute a telomeric repeat synthesis activity (Weinrich et al., 1997).

TER is constitutively expressed in human cells, and it ubiquitously assembles as a stable ribonucleoprotein complex (RNP). In contrast, most somatic cells repress hTERT production at the transcriptional level (Cong et al., 2002; Cairney and Keith, 2007). Constitutive expression of hTERT in primary fibroblasts is sufficient to induce telomerase catalytic activation and telomere maintenance (Bodnar et al., 1998; Shay and Wright, 2005). Studies using transgenes to express hTERT have provided a wealth of understanding of telomerase structure/function relationships, for example enabling a screen of linker-scan mutations across the hTERT N- and C-terminal extensions from the active site (Armbruster et al., 2001; Banik et al., 2002). The general utility of hTERT expression for extending the proliferative life span of primary cells in culture may have contributed to an over-simplifying perception that hTERT is always limiting for telomere length maintenance. Recent studies show that hTR can limit telomerase function at telomeres in primary cells and in cancer cell lines as well (Wong and Collins, 2006; Cristofari et al., 2006).

Biogenesis of mature telomerase RNA and telomerase RNP

TER biosynthesis, processing and structure differ between vertebrates, ciliates and budding yeasts, the three broad phylogenetic groups in which TER has been most extensively characterized (Collins, 2006). Vertebrate TERs are synthesized as 3'-extended precursors by RNA Polymerase II, the same enzyme that synthesizes mRNA. Many steps of processing are required to convert an incompletely defined TER precursor into the mature, 451-nucleotide hTR (Figure 2A). These events must distinguish a nascent TER transcript from other transcripts such that the TER precursor acquires features of hTR rather than a processed mRNA. For example, mature hTR lacks the poly-adenosine tail of a mRNA and bears a 5' tri-methyl rather than mono-methyl guanosine cap (Feng et al., 1995; Fu and Collins, 2006).

Cellular accumulation of mature hTR requires a region within the RNA that folds to form a so-called stem-Hinge-stem-ACA (H/ACA) motif (Figure 1A). This motif is shared by a large family of small nucleolar (sno) RNAs and related small Cajal body (sca) RNAs (Matera et al., 2007). Most H/ACA-motif snoRNAs and scaRNAs guide ribosomal and spliceosomal RNA modification, determining the site-specificity of uridine conversion to pseudouridine. Because the fundamental building block of the H/ACA motif is evolutionary ancient, vertebrate TER would have co-opted it from a pre-existing RNA. Curiously, although H/ACA-motif snoRNAs are independently transcribed in yeast, human H/ACA-motif snoRNAs and scaRNAs are processed from spliced introns of host mRNA transcripts. Thus, the hTR biogenesis pathway is unique among the human H/ACA-motif RNAs (Figure 2A). Notably, if mature hTR is transcribed by RNA Polymerase II within an intron context or if it is transcribed by RNA Polymerase III, only the 3' half of the molecule harboring the H/ACA domain accumulates to detectable level (Theimer et al., 2007; Mitchell et al., 1999a). In addition to its H/ACA motif, hTR also requires a motif termed the biogenesis box (BIO box) for *in vivo* accumulation (Fu and Collins, 2003). The BIO box is in the loop of the hTR 3' stem in a region not conserved among H/ACA-motif snoRNAs (Figure 1A).

The H/ACA motifs of vertebrate TERs, snoRNAs and scaRNAs all assemble with the same set of shared proteins, termed dyskerin, NHP2, NOP10 and GAR1 (Figure 2B). Dyskerin contains the active site that catalyzes pseudouridine formation. Each of the four H/ACA-motif binding proteins has direct RNA binding activity, but none of them assembles independently with a H/ACA-motif RNA *in vivo*. Dyskerin, NHP2 and NOP10 are deposited co-transcriptionally with the help of the H/ACA RNP assembly chaperone NAF1, which is subsequently exchanged for GAR1 (Darzacq et al., 2006). Dyskerin, NHP2 and NOP10 have been identified by mass spectrometry in affinity purified human telomerase (Fu and Collins, 2007; Cohen et al., 2007) and are all essential for the stability of hTR and the other human H/ACA-motif snoRNAs *in vivo* (Hoareau-Aveilla et al., 2006; Fu and Collins, 2007; Walne et al., 2007). GAR1 is important for localization of H/ACA-motif snoRNAs to the nucleolus in yeast, and it is important for the dyskerin-mediated catalysis of pseudouridine formation within a H/ACA-motif snoRNP (Ye, 2007). Although GAR1 is a stoichiometric binding partner of H/ACA-motif snoRNAs, it is not essential for their accumulation. In the case of telomerase RNP in particular, GAR1 may be substoichiometric or less tightly bound than dyskerin, NHP2 and NOP10 (Fu and Collins, 2007).

Human telomerase deficiency

Patients with the X-linked form of dyskeratosis congenita (DC) harbor small changes in the gene encoding dyskerin (Vulliamy et al., 2006). Young males who inherit a mutant dyskerin gene on their X-chromosome suffer progressive, fatal bone marrow failure. Numerous disease-linked single amino acid changes in dyskerin have been reported, which cluster in regions of the protein separate from the surfaces crucial for H/ACA RNP subunit interactions or catalytic activity (Rashid et al., 2006). Primary cells from X-linked DC patients have about one-fifth of the wild-type level of hTR, but they show no obvious change in the accumulation levels of other H/ACA-motif RNAs or their processing targets (Mitchell et al., 1999b; Wong et al., 2004; Wong and Collins, 2006). X-linked DC patient cells that express hTERT fail to elongate telomeres, unlike family-matched control cells (Wong and Collins, 2006). This defect in telomere maintenance can be rescued by expression of additional hTR precursor, confirming that it is the reduced accumulation of hTR that compromises telomerase function. Telomere-rescued X-linked DC patient cells grow indefinitely in culture without any defect in the ribosomal RNA processing and modification reactions dependent on H/ACA-motif snoRNAs (Wong and Collins, 2006). This molecular separation in dyskerin requirements for biogenesis of telomerase versus other H/ACA-motif RNAs is not surprising, given the unique features of hTR biogenesis. For example, the hTR precursor must be processed as an independent rather

than an intron-encoded transcript, retain its 5' extension from the H/ACA motif and gain a 5' tri-methylguanosine cap (Figure 2A).

Disease phenotypes arising from mutations in telomerase subunit genes are a reverse genetic approach to understanding the requirements for human telomerase function in the organism. The scope of disease known to result from telomerase deficiency extends beyond DC to include aplastic anemia, pulmonary fibrosis, Hoyeraal-Hreidarsson syndrome and cancer (Vulliamy et al., 2006). These diverse clinical presentations are just an inkling of the much greater phenotypic diversity that may be linked to telomere maintenance deficiency in the future. The spectrum of phenotypes can be much broader than currently anticipated, because mutations that impose a different extent and/or mechanism of premature telomere attrition will impact different subsets of cell types (Collins and Mitchell, 2002; Wong and Collins, 2003).

Defining the somatic cell types that support telomerase function *in vivo* is complicated by the fact that telomere length rather than telomerase itself is the primary factor acting as a barrier for cellular renewal. Reduced telomere maintenance in telomerase-positive cells of the fetus, infant and adult can all contribute to disease in a phenotypically silent manner, if the telomerase-positive cells retain sufficiently long telomeres to avoid activating a short-telomere growth checkpoint. Inversely, telomerase-negative or telomerase-low cells descendant from telomerase-positive lineage progenitors will be the cells that manifest disease phenotypes if the cumulative nature of telomere erosion brings them to critically short telomere length. Finally, disease onset can be accelerated by inheritance of shorter-than-normal telomeres along with the gene mutation (Vulliamy et al., 2004). These and other factors complicate the prediction of genotype-phenotype relationships.

Telomerase RNP assembly with TERT

Telomerase RNP assembles with newly expressed hTERT to produce functional enzyme *in vivo*. Likewise, cell extracts with inactive telomerase RNP can be combined with cell extracts harboring only hTERT to produce active enzyme *in vitro* (Tesmer et al., 1999). The specificity of endogenous hTERT assembly with telomerase RNP has not been characterized in detail, despite many studies of recombinant TERT-TER interaction. Precedent from studies of ciliate telomerase suggests that the initial RNP assembly of TER induces a TER conformational change that promotes TERT binding (Prathapam et al., 2005; O'Connor and Collins, 2006; Stone et al., 2007). A similar hierarchical RNP assembly mechanism would be logical to expect for human telomerase as well (Figure 2B), because hTR assembly with the H/ACA-motif binding proteins would constrain the relative orientation of the two hTR regions that bind hTERT. Proteomic and molecular genetic studies do not reveal comparable enrichment of RNA binding proteins other than the H/ACA RNP proteins in endogenously assembled catalytically active RNP (Fu and Collins, 2007; Cohen et al., 2007). However, other proteins could bind to assembly intermediates or bind transiently to the active RNP to govern hTR-hTERT interaction.

Several lines of evidence suggest that hTERT assembly with telomerase RNP involves the protein folding chaperone HSP90. In rabbit reticulocyte lysate, reconstitution of hTERT and hTR as active RNP depends on HSP90 and its co-chaperones (Holt et al., 1999). Product turnover by recombinant human telomerase is also influenced by HSP90 (Keppler et al., 2006). Treatment of human cells with the HSP90 inhibitor geldanamycin reduces the level of telomerase catalytic activity in extract, consistent with blocking an active RNP assembly process (Holt et al., 1999; Forsythe et al., 2001). In another study, treatment of human cells with geldanamycin induced hTERT ubiquitination and proteosomal degradation (Kim et al., 2005). These findings suggest the hypothesis that catalytically active telomerase complexes exist in a HSP90-influenced equilibrium of assembly, disassembly and subunit degradation.

The physiological significance of these findings remains to be understood, and other factors involved in the dynamic balance of active RNP assembly remain to be defined.

In addition to HSP90, hTERT interactions have been detected with the RNP assembly chaperone SMN (Bachand et al., 2002) and the RNP transport chaperone PHAX (Boulon et al., 2004). Two other hTERT-interacting proteins, the nucleolar acetyltransferase NAT10 and the nucleolar GTPase GNL3L, are also candidate regulators of the hTERT-hTR interaction (Fu and Collins, 2007). Over-expression of either protein reduces telomere length without change in the level of hTR or hTERT mRNA. In addition, the nucleolar protein Pin X1 has been proposed to either block TERT-TER assembly (Lin and Blackburn, 2004) or inhibit RNP activity (Zhou and Lu, 2001; Banik and Counter, 2004), apparently acting by divergent mechanisms in yeast and human cells, respectively. Although no chaperone activity has been described for human KIP (a putative calcium binding protein isolated by interaction with DNA-dependent protein kinase), it is a candidate regulator of hTERT-hTR interaction: over-expression of KIP increases telomerase activity in extract and lengthens telomeres in vivo without changing the level of hTERT mRNA or hTR (Lee et al., 2004).

Regulation of telomere elongation

Telomerase function requires its engagement with an extreme chromosome 3' terminus. Telomerase association with a telomere could involve recruitment of a pre-formed catalytically active complex or separate recruitment of hTERT and telomerase RNP for assembly on replicating telomeres (Holt et al., 1997; Jady et al., 2006; Tomlinson et al., 2006). Unfortunately, assays of telomerase catalytic activity in vitro do not provide sufficient opportunity to define factors that link telomerase complexes with telomere substrates. Instead, candidate approaches have been taken to identify these factors. Because some of the heterogeneous nuclear RNP (hnRNP) proteins that package mRNA can also bind to hTR and/or telomeric repeat DNA oligonucleotides, they have been hypothesized as factors that bridge human telomerase to telomeres (Ford et al., 2002). At least in the case of hnRNP C, however, protein-hTR interaction can be inhibited without compromising telomere elongation (Fu and Collins, 2007).

Precedents for mechanisms of telomerase recruitment to telomeres have been established in budding yeast. The DNA end-binding heterodimer Ku simultaneously binds to yeast telomeres and to a yeast-specific motif within TER (Fisher and Zakian, 2005). Distinct specificities of human Ku interaction with hTR and hTERT have been reported (Chai et al., 2002; Ting et al., 2005), but the significance of these putative direct Telomerase interactions has not been tested in physiological context. The budding yeast telomerase protein Est1p binds to both the single-stranded telomeric repeat binding protein Cdc13p to telomerase RNP, but the human proteins hEST1A/hSMG6 and hEST1B/hSMG5 harboring limited similarity to Est1p have non-conserved roles in telomere biology (Lundblad, 2003; Azzalin and Lingner, 2006). Instead of using a mechanism similar to yeast telomerase, human telomerase recruitment to telomeres may be accomplished by interaction with the single-stranded telomeric repeat binding proteins TPP1 and POT1 (Wang et al., 2007; Xin et al., 2007). The higher-order properties of a short telomere could affect the single-stranded telomeric DNA binding proteins in a manner that promotes telomerase association.

In situ hybridization studies suggest that for most of the cell cycle, the highest concentration of hTR is in Cajal bodies (Jady et al., 2004; Zhu et al., 2004). These intranuclear structures are sites of small nuclear RNP biogenesis and function (Handwerger and Gall, 2006). The numerous scaRNAs enriched in Cajal bodies have two copies of a tetranucleotide motif for Cajal body localization, the CAB box, while hTR has a single copy (Figure 1A) required for its steady-state Cajal body accumulation (Jady et al., 2004). The CAB box directly or indirectly

determines hTR and scaRNA association with a subset of Sm proteins (Fu and Collins, 2006; Fu and Collins, 2007). In human primary fibroblasts, mutation of the hTR CAB box does not inhibit hTR accumulation or telomerase function in telomere elongation, suggesting that Cajal body localization plays a regulatory rather than essential role (Fu and Collins, 2007). In cancer cell lines, however, mutation of the hTR CAB box was described to partially inhibit hTR function in telomere elongation (Cristofari et al., 2007).

These contrasting conclusions could reflect the difference in assay conditions between primary cells, where the assay was rescue of hTR deficiency and short telomeres, and cancer cells, where the assay was hTR over-expression to detect telomere over-elongation. A more interesting possibility is that hTR CAB box mutations reduced hTR function in cancer but not normal cells by having a cell-type specific impact on hTR accumulation. Even wild-type hTR biological stability varies substantially across cultured human cell lines (Yi et al., 1999). In one model for hTR CAB box function (Fu and Collins, 2007), Cajal body association promotes hTR recovery or re-import into the nucleus after each cycle of mitosis. Telomerase RNP stranded in the cytoplasm may be subject to more rapid degradation, with the consequence that the biological half-life of CAB-box-mutant hTR decreases only when cells have a wild-type hTR half-life of more than several cell division cycles. It is possible that even a small decrease in CAB-box-mutant hTR accounts for inhibition of telomerase function in cancer cells, because telomerase in vivo function is not directly proportional to levels of subunit accumulation. Alternately, the hTR CAB box may promote telomerase RNP localization to Cajal bodies and/or the nucleoplasm in a manner that is not essential for telomere elongation but has a modest stimulatory influence.

Telomerase negative regulation

Rarely, human somatic cells show a starkly divergent relationship between telomerase catalytic activation in cell extract and telomerase function on chromosome ends in vivo. Lymphocytes stimulated to proliferate in culture induce sustained telomerase catalytic activation but only transient telomere maintenance (Bodnar et al., 1996). Also, although forced hTERT expression in cultured hematopoietic progenitor CD34+ cells does increase telomerase catalytic activity detected by in vitro assay, it does not prevent telomere erosion (Wang et al., 2005). Understanding how cells restrain telomerase from telomere elongation has obvious clinical significance, but only limited characterizations of negative regulatory mechanisms beyond the transcriptional level have been described (Bickenbach et al., 1998; Liu et al., 2001; Wong et al., 2002; Akiyama et al., 2003; Haendeler et al., 2003).

At least some mechanisms for telomerase negative regulation are likely to involve relocalization of telomerase RNP, hTERT and/or active RNP. For cell types in which hTR can be detected by in situ hybridization, the highest intranuclear concentration of hTR is in Cajal bodies (Jády et al., 2004; Zhu et al., 2004). A large fraction of hTR may be nucleoplasmic, perhaps too diffuse to detect by in situ hybridization. Also, minor fractions of hTR are associated with nucleoli or with a subset of telomeres in S-phase (Mitchell et al., 1999a; Jády et al., 2006; Tomlinson et al., 2006). Although hTERT localizes to the same general compartments as hTR, its relative distribution changes across the cell cycle in an independent manner (Tomlinson et al., 2006). Typically hTERT is observed in the nucleoplasm and nucleoli, each with diffuse and/or punctate pattern. Independent physical sequestration of hTR and hTERT could have evolved to increase the specificity of telomerase activation, with the possible consequence that only a subset of the hTR or hTERT pool becomes assembled into active RNP in any given cell cycle. This restraint on active telomerase assembly would account for why telomere maintenance in human cells requires an atypically high level of TER and TERT compared to telomere maintenance in single-celled eukaryotes (Wong and Collins, 2006; Cristofari and Lingner, 2006; Mozdy and Cech, 2006).

Nucleoplasmic hTERT relocates to nucleoli and/or cytoplasm upon cell treatment with ionizing radiation or oxidative stress (Haendeler et al., 2003; Wong et al., 2002). Stress-induced relocation of hTR has not been examined, but it is noteworthy that Cajal bodies are rapidly dispersed by UV irradiation (Cioce et al., 2006). Is hTERT relocation a mechanism to down-regulate telomerase function? Although there is currently no answer for this question, studies examining hTERT localization in normal human somatic cells provide suggestive supporting evidence. Quiescent lymphocytes stained with an antibody against hTERT have cross-reacting protein only in the cytoplasm, but this signal moves to the nucleus when cells are stimulated to proliferate (Liu et al., 2001). Similar observations of TERT nuclear translocation have been reported for vascular smooth muscle cells (Minamino and Kourembanas, 2001). Several kinase activities have been implicated in hTERT relocation, but to date no impact of hTERT post-translational modification on telomere maintenance has been established. Clearly, much remains to be learned about the mechanisms and biological significance of the dynamics of telomerase subunit localization.

Telomerase negative regulation by physical separation of TERT and TER may have created an opportunity for multi-cellular eukaryotes to evolve additional independent functions for these subunits. Complexes of hTERT lacking telomerase activity have been implicated in DNA damage response and cell survival (Masutomi et al., 2005; Sung et al., 2005), while hTR has been implicated in regulation of the damage-activated kinase ATR and recovery from UV irradiation (Kedde et al., 2006). Mice lacking either telomerase subunit are viable and fertile for many generations, suggesting that telomere-independent function(s) of these subunits are non-essential in mice. However, if telomerase subunit complexes lacking repeat synthesis activity do possess independent biological roles, then some of the highly elaborate cellular regulation of hTR and hTERT may be linked to their alternative functions rather than to their function in telomere maintenance.

Acknowledgements

Funding for human telomerase research in the Collins lab is provided by N.I.H. and the American Federation for Aging Research. I thank past and present lab members for their many contributions and Tim Errington and Emily Egan for manuscript comments.

References

- Akiyama M, Hideshima T, Hayashi T, Tai YT, Mitsiades CS, Mitsiades N, Chauhan D, Richardson P, Munshi NC, Anderson KC. Nuclear factor-kappaB p65 mediates tumor necrosis factor alpha-induced nuclear translocation of telomerase reverse transcriptase protein. *Cancer Res* 2003;63:18–21. [PubMed: 12517770]
- Armbruster BN, Banik SSR, Guo C, Smith AC, Counter CM. N-terminal domains of the human telomerase catalytic subunit required for enzyme activity in vivo. *Mol Cell Biol* 2001;22:7775–7786. [PubMed: 11604512]
- Autexier C, Lue NF. The structure and function of telomerase reverse transcriptase. *Annu Rev Biochem* 2006;75:493–517. [PubMed: 16756500]
- Azzalin CM, Lingner J. The double life of UPF1 in RNA and DNA stability pathways. *Cell Cycle* 2006;5:1496–1498. [PubMed: 16861888]
- Bachand F, Boisvert FM, Cote J, Richard S, Autexier C. The product of the survival of motor neuron (SMN) gene is a human telomerase-associated protein. *Mol Biol Cell* 2002;13:3192–3202. [PubMed: 12221125]
- Banik SS, Counter CM. Characterization of interactions between PinX1 and human telomerase subunits hTERT and hTR. *J Biol Chem* 2004;279:51745–51748. [PubMed: 15381700]
- Banik SSR, Guo C, Smith AC, Margolis SS, Richardson DA, Tirado CA, Counter CM. C-terminal regions of the human telomerase catalytic subunit essential for in vivo enzyme activity. *Mol Cell Biol* 2002;22:6234–6246. [PubMed: 12167716]

- Bickenbach JR, Vormwald-Dogan V, Bachor C, Bleuel K, Schnapp G, Boukamp P. Telomerase is not an epidermal stem cell marker and is downregulated by calcium. *J Invest Dermatol* 1998;111:1045–1052. [PubMed: 9856815]
- Bodnar AG, Kim NW, Effros RB, Chiu CP. Mechanism of telomerase induction during T cell activation. *Exp Cell Res* 1996;228:58–64. [PubMed: 8892971]
- Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu C, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998;279:349–352. [PubMed: 9454332]
- Boulon S, Verheggen C, Jady BE, Girard C, Pescia C, Paul C, Ospina JK, Kiss T, Matera AG, Bordonne R, Bertrand E. PHAX and CRM1 are required sequentially to transport U3 snoRNA to nucleoli. *Mol Cell* 2004;16:777–787. [PubMed: 15574332]
- Cairney CJ, Keith WN. Telomerase redefined: Integrated regulation of hTR and hTERT for telomere maintenance and telomerase activity. *Biochimie*. 2007in press
- Chai W, Ford LP, Lenertz L, Wright WE, Shay JW. Human Ku70/80 associates physically with telomerase through interaction with hTERT. *J Biol Chem* 2002;277:47242–47247. [PubMed: 12377759]
- Cioce M, Boulon S, Matera AG, Lamond A. UV-induced fragmentation of Cajal bodies. *J Cell Biol* 2006;175:401–413. [PubMed: 17088425]
- Cohen SB, Graham ME, Lovrecz GO, Bache N, Robinson PJ, Reddel RR. Protein composition of catalytically active human telomerase from immortal cells. *Science* 2007;315:1850–1853. [PubMed: 17395830]
- Cong YS, Wright WE, Shay JW. Human telomerase and its regulation. *Microbiol Mol Biol Rev* 2002;66:407–425. [PubMed: 12208997]
- Collins K, Mitchell JR. Telomerase in the human organism. *Oncogene* 2002;21:564–579. [PubMed: 11850781]
- Collins K. The biogenesis and regulation of telomerase holoenzymes. *Nat Rev Mol Cell Biol* 2006;7:484–494. [PubMed: 16829980]
- Cristofari G, Lingner J. Telomere length homeostasis requires that telomerase levels are limiting. *EMBO J* 2006;25:565–574. [PubMed: 16424902]
- Cristofari G, Adolf E, Reichenbach P, Sikora K, Terns RM, Terns MP, Lingner J. Human telomerase RNA accumulation in cajal bodies facilitates telomerase recruitment to telomeres and telomere elongation. *Mol Cell* 2007;27:882–829. [PubMed: 17889662]
- Darzacq X, Kittur N, Roy S, Shav-Tal Y, Singer RH, Meier UT. Stepwise RNP assembly at the site of H/ACA RNA transcription in human cells. *J Cell Biol* 2006;173:207–218. [PubMed: 16618814]
- Feng J, Funk WD, Wang S, Weinrich SL, Ailion AA, Chiu C, Adams RR, Chang E, Allsopp RC, Yu J, Le S, West M, Harley CB, Andrews WH, Greider CW, Villeponteau B. The RNA component of human telomerase. *Science* 1995;269:1236–1241. [PubMed: 7544491]
- Fisher TS, Zakian VA. Ku: a multifunctional protein involved in telomere maintenance. *DNA Repair* 2005;4:1215–1226. [PubMed: 15979949]
- Ford LP, Wright WE, Shay JW. A model for heterogeneous nuclear ribonucleoproteins in telomere and telomerase regulation. *Oncogene* 2002;21:580–583. [PubMed: 11850782]
- Forsythe HL, Jarvis JL, Turner JW, Elmore LW, Holt SE. Stable association of hsp90 and p23, but not hsp70, with active human telomerase. *J Biol Chem* 2001;276:15571–15574. [PubMed: 11274138]
- Forsyth NR, Wright WE, Shay JW. Telomerase and differentiation in multicellular organisms: turn it off, turn it on, and turn it off again. *Differentiation* 2002;69:188–197. [PubMed: 11841477]
- Fu D, Collins K. Distinct biogenesis pathways for human telomerase RNA and H/ACA small nucleolar RNAs. *Mol Cell* 2003;11:1361–1372. [PubMed: 12769858]
- Fu D, Collins K. Human telomerase and Cajal body ribonucleoproteins share a unique specificity of Sm protein association. *Genes Dev* 2006;20:531–536. [PubMed: 16481465]
- Fu D, Collins K. Purification of endogenous human telomerase complexes identifies factors involved in telomerase biogenesis and telomere length regulation. *Mol Cell*. 2007in press

- Haendeler J, Hoffmann J, Brandes RP, Zeiher AM, Dimmeler S. Hydrogen peroxide triggers nuclear export of telomerase reverse transcriptase via Src kinase family-dependent phosphorylation of tyrosine 707. *Mol Cell Biol* 2003;23:4598–610. [PubMed: 12808100]
- Handwerger KE, Gall JG. Subnuclear organelles: new insights into form and function. *Trends Cell Biol* 2006;16:19–26. [PubMed: 16325406]
- Hoareau-Aveilla C, Bonoli M, Caizergues-Ferrer M, Henry Y. hNaf1 is required for accumulation of human box H/ACA snoRNPs, scaRNPs, and telomerase. *RNA* 2006;12:832–40. [PubMed: 16601202]
- Holt SE, Aisner DL, Shay JW, Wright WE. Lack of cell cycle regulation of telomerase activity in human cells. *Proc Natl Acad Sci USA* 1997;94:10687–10692. [PubMed: 9380696]
- Holt SE, Aisner DL, Baur J, Tesmer VM, Dy M, Ouellette M, Trager JB, Morin GB, Toft DO, Shay JW, Wright WE, White MA. Functional requirement of p23 and Hsp90 in telomerase complexes. *Genes Dev* 1999;13:817–826. [PubMed: 10197982]
- Jády BE, Bertrand E, Kiss T. Human telomerase RNA and box H/ACA scaRNAs share a common Cajal body-specific localization signal. *J Cell Biol* 2004;164:647–652. [PubMed: 14981093]
- Jády BE, Richard P, Bertrand E, Kiss T. Cell cycle-dependent recruitment of telomerase RNA and Cajal bodies to human telomeres. *Mol Biol Cell* 2006;17:944–954. [PubMed: 16319170]
- Kedde M, le Sage C, Duursma A, Zlotorynski E, van Leeuwen B, Nijkamp W, Beijersbergen R, Agami R. Telomerase-independent regulation of ATR by human telomerase RNA. *J Biol Chem* 2006;281:40503–40514. [PubMed: 17098743]
- Keith WN, Thomson CM, Howcroft J, Maitland NJ, Shay JW. Seeding drug discovery: integrating telomerase cancer biology and cellular senescence to uncover new therapeutic opportunities in targeting cancer stem cells. *Drug Disc Today* 2007;121:611–621.
- Keppeler BR, Grady AT, Jarstfer MB. The biochemical role of the heat shock protein 90 chaperone complex in establishing human telomerase activity. *J Biol Chem* 2006;281:19840–19848. [PubMed: 16714764]
- Kim JH, Park SM, Kang MR, Oh SY, Lee TH, Muller MT, Chung IK. Ubiquitin ligase MKRN1 modulates telomere length homeostasis through a proteolysis of hTERT. *Genes Dev* 2005;19:776–781. [PubMed: 15805468]
- Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994;266:2011–2015. [PubMed: 7605428]
- Lee GE, Yu EY, Cho CH, Lee J, Muller MT, Chung IK. DNA-protein kinase catalytic subunit-interacting protein KIP binds telomerase by interacting with human telomerase reverse transcriptase. *J Biol Chem* 2004;279:34750–34755. [PubMed: 15190070]
- Lin J, Blackburn EH. Nucleolar protein PinX1p regulates telomerase by sequestering its protein catalytic subunit in an inactive complex lacking telomerase RNA. *Genes Dev* 2004;18:387–396. [PubMed: 14977919]
- Liu K, Hodes RJ, Weng N. Cutting edge: telomerase activation in human T lymphocytes does not require increase in telomerase reverse transcriptase (hTERT) protein but is associated with hTERT phosphorylation and nuclear translocation. *J Immunol* 2001;166:4826–4830. [PubMed: 11290757]
- Lundblad V. Telomere replication: an Est fest. *Curr Biol* 2003;13:R439–441. [PubMed: 12781153]
- Masutomi K, Possemato R, Wong JM, Currier JL, Tothova Z, Manola JB, Ganesan S, Lansdorp PM, Collins K, Hahn WC. The telomerase reverse transcriptase regulates chromatin state and DNA damage responses. *Proc Natl Acad Sci USA* 2005;102:8222–8227. [PubMed: 15928077]
- Matera AG, Terns RM, Terns MP. Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. *Nat Rev Mol Cell Biol* 2007;8:209–20. [PubMed: 17318225]
- Minamino T, Kourembanas S. Mechanisms of telomerase induction during vascular smooth muscle cell proliferation. *Circ Res* 2001;89:237–243. [PubMed: 11485973]
- Mitchell JR, Cheng J, Collins K. A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3' end. *Mol Cell Biol* 1999a;19:567–576. [PubMed: 9858580]
- Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* 1999b;402:551–555. [PubMed: 10591218]

- Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 1989;59:521–529. [PubMed: 2805070]
- Morin GB. Recognition of a chromosome truncation site associated with alpha-thalassaemia by human telomerase. *Nature* 1991;353:454–456. [PubMed: 1896089]
- Mozdy AD, Cech TR. Low abundance of telomerase in yeast: implications for telomerase haploinsufficiency. *RNA* 2006;12:1721–1737. [PubMed: 16894218]
- O'Connor CM, Collins K. A novel RNA binding domain in *Tetrahymena* telomerase p65 initiates hierarchical assembly of telomerase holoenzyme. *Mol Cell Biol* 2006;26:2029–2036. [PubMed: 16507983]
- Prathapam R, Witkin KL, O'Connor CM, Collins K. A telomerase holoenzyme protein enhances telomerase RNA assembly with telomerase reverse transcriptase. *Nat Struct Mol Biol* 2005;12:252–257. [PubMed: 15696174]
- Rashid R, Liang B, Baker DL, Youssef OA, He Y, Phipps K, Terns RM, Terns MP, Li H. Crystal structure of a Cbf5-Nop10-Gar1 complex and implications in RNA-guided pseudouridylation and dyskeratosis congenita. *Mol Cell* 2006;21:249–260. [PubMed: 16427014]
- Shay JW, Wright WE. Senescence and immortalization: role of telomeres and telomerase. *Carcinogenesis* 2005;26:867–874. [PubMed: 15471900]
- Stone MS, Mihalusova M, O'Connor CM, Prathapam R, Collins K, Zhuang X. Stepwise protein-mediated RNA folding directs assembly of telomerase ribonucleoprotein. *Nature* 2007;446:458–461. [PubMed: 17322903]
- Sung YH, Choi YS, Cheong C, Lee HW. The pleiotropy of telomerase against cell death. *Mol Cells* 2005;19:303–309. [PubMed: 15995345]
- Tesmer VM, Ford LP, Holt SE, Frank BC, Yi X, Aisner DL, Ouellette M, Shay JW, Wright WE. Two inactive fragments of the integral RNA cooperate to assemble active telomerase with the human protein catalytic subunit (hTERT) in vitro. *Mol Cell Biol* 1999;19:6207–6216. [PubMed: 10454567]
- Theimer CA, Feigon J. Structure and function of telomerase RNA. *Curr Opin Struct Biol* 2006;16:307–318. [PubMed: 16713250]
- Theimer CA, Jady BE, Chim N, Richard P, Breece KE, Kiss T, Feigon J. Structural and functional characterization of human telomerase RNA processing and cajal body localization signals. *Mol Cell* 2007;27:869–881. [PubMed: 17889661]
- Ting NS, Yu Y, Pohorelic B, Lees-Miller SP, Beattie TL. Human Ku70/80 interacts directly with hTR, the RNA component of human telomerase. *Nucl Acids Res* 2005;33:2090–2098. [PubMed: 15824061]
- Tomlinson RL, Ziegler TD, Supakorndej T, Terns RM, Terns MP. Cell cycle-regulated trafficking of human telomerase to telomeres. *Mol Biol Cell* 2006;17:955–65. [PubMed: 16339074]
- Vulliamy T, Marrone A, Szydlo R, Walne A, Mason PJ, Dokal I. Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. *Nat Genet* 2004;36:447–449. [PubMed: 15098033]
- Vulliamy TJ, Marrone A, Knight SW, Walne A, Mason PJ, Dokal I. Mutations in dyskeratosis congenita: their impact on telomere length and the diversity of clinical presentation. *Blood* 2006;107:2680–2685. [PubMed: 16332973]
- Walne AJ, Vulliamy T, Marrone A, Beswick R, Kirwan M, Masunari Y, Al-Qurashi FH, Aljurf M, Dokal I. Genetic heterogeneity in autosomal recessive dyskeratosis congenita with one subtype due to mutations in the telomerase-associated protein NOP10. *Hum Mol Genet* 2007;16:1619–1629. [PubMed: 17507419]
- Wang F, Podell ER, Zaug AJ, Yang Y, Baciú P, Cech TR, Lei M. The POT1-TPP1 telomere complex is a telomerase processivity factor. *Nature* 2007;445:506–510. [PubMed: 17237768]
- Wang JCY, Warner JK, Erdmann N, Lansdorp PM, Harrington L, Dick JE. Dissociation of telomerase activity and telomere length maintenance in primitive human hematopoietic cells. *Proc Natl Acad Sci USA* 2005;102:14398–14403. [PubMed: 16172394]
- Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB, Morin GB. Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. *Nat Genet* 1997;17:498–502. [PubMed: 9398860]

- Wong JM, Kusdra L, Collins K. Subnuclear shuttling of human telomerase induced by transformation and DNA damage. *Nat Cell Biol* 2002;4:731–736. [PubMed: 12198499]
- Wong JM, Kyasa MJ, Hutchins L, Collins K. Telomerase RNA deficiency in peripheral blood mononuclear cells in X-linked dyskeratosis congenita. *Hum Genet* 2004;115:448–455. [PubMed: 15349768]
- Wong JMY, Collins K. Telomere maintenance and disease. *Lancet* 2003;362:983–988. [PubMed: 14511933]
- Wong JMY, Collins K. Telomerase RNA level limits telomere maintenance in X-linked dyskeratosis congenita. *Genes Dev* 2006;20:2848–2858. [PubMed: 17015423]
- Xin H, Liu D, Wan M, Safari A, Kim H, Sun W, O'Connor MS, Songyang Z. TPP1 is a homologue of ciliate TEBP-beta and interacts with POT1 to recruit telomerase. *Nature* 2007;445:559–562. [PubMed: 17237767]
- Ye K. H/ACA guide RNAs, proteins and complexes. *Curr Opin Struct Biol* 2007;17:287–292. [PubMed: 17574834]
- Yi X, Tesmer VM, Savre-Train I, Shay JW, Wright WE. Both transcriptional and posttranscriptional mechanisms regulate human telomerase template RNA levels. *Mol Cell Biol* 1999;19:3989–3997. [PubMed: 10330139]
- Zhou XZ, Lu KP. The Pin2/TRF1-interacting protein PinX1 is a potent telomerase inhibitor. *Cell* 2001;107:347–359. [PubMed: 11701125]
- Zhu Y, Tomlinson RL, Lukowiak AA, Terns RM, Terns MP. Telomerase RNA accumulates in Cajal bodies in human cancer cells. *Mol Biol Cell* 2004;15:81–90. [PubMed: 14528011]

A Telomerase RNA (hTR)

B Telomerase reverse transcriptase (hTERT)

N-terminus		C-terminus	
DNA binding	High-affinity RNA binding	Polymerase active site	Regulation?

Figure 1. Motifs and domains of human telomerase RNA and telomerase reverse transcriptase
A. The secondary structure of the 451-nucleotide mature hTR molecule is depicted. Boxes or brackets indicate the locations of motifs involved in telomerase RNP biogenesis (the H/ACA motif and BIO box), hTERT binding or intranuclear localization (the CAB box). Note that the second half of the molecule, the independently stable H/ACA domain, resembles a snoRNA.
B. A putative architecture of hTERT is depicted with the suggested function of each domain.

A Vertebrate TER biogenesis

B Vertebrate telomerase RNP assembly

Figure 2. Biogenesis pathway for human telomerase RNA and telomerase RNP

A. An ordered series of RNA processing steps is required for production of mature hTR from the precursor synthesized by RNA Polymerase II. Precedents from studies of other nuclear RNPs suggest that the biochemical steps listed are likely coupled with steps of RNP transport between cellular compartments. Which biogenesis steps involve the hTR BIO box or the dyskerin surface defined by X-linked DC mutations are not yet known.

B. An ordered series of RNP assembly steps is required for production of functional telomerase RNP. Assembly of hTR with the H/ACA-motif binding proteins (in green) dyskerin, NHP2 and NOP10 is essential for its accumulation *in vivo*. These proteins recruit the fourth shared H/ACA-motif binding protein GAR1. RNP assembly on the hTR H/ACA motif would be predicted to influence the relative orientation of the hTR binding surfaces for hTERT (in blue), potentially promoting hTR-hTERT interaction. The numerous proteins that are proposed to regulate human telomerase function *in vivo* (in yellow) may interact with the catalytically active hTERT RNP and/or with telomerase RNP or hTERT assembly intermediates. This schematic is intended as a visual aid, not an accurate three-dimensional representation of a model for human telomerase RNP architecture.