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Review

Physiological assembly and activity of human telomerase complexes

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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.

Keywords: Human telomerase; Telomerase RNA; Telomerase reverse transcriptase; Ribonucleoprotein; Biogenesis; Dyskeratosis congenita

1. 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 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.

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2. 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 (Fig. 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 (Fig. 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 and Lingner, 2006).

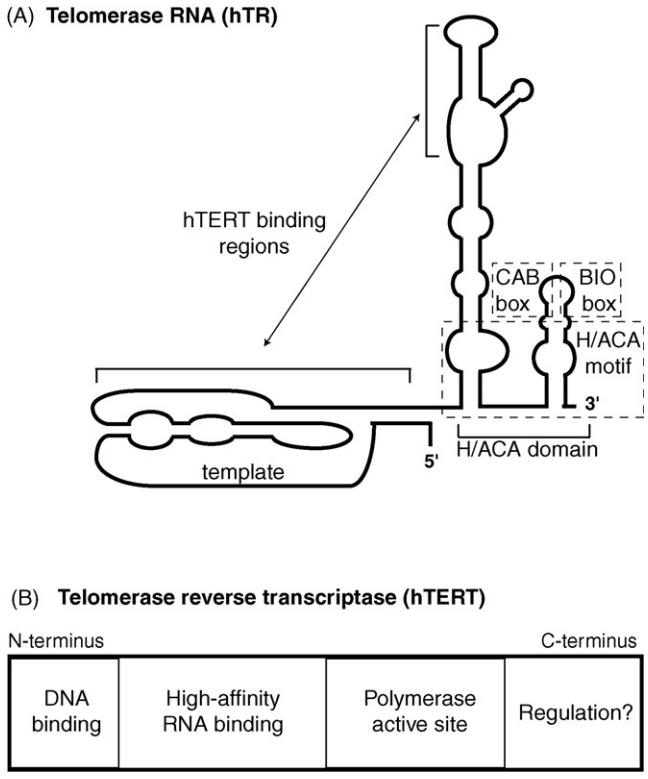

Fig. 1. Motifs and domains of human telomerase RNA and telomerase reverse transcriptase. (A) The secondary structure of the 451-nucleotide mature hTR molecule is depicted. Boxes or brackets indicate the locations of motifs involved in telomerase RNP biogenesis (the H/ACA motif and BIO box), hTERT binding 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.

3. Biogenesis of mature telomerase RNA and telomerase RNP

TER biosynthesis, processing and structure differ among 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 (Fig. 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 (Fig. 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 (Fig. 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′