Point of VIEW

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Telomerase Unplugged

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ABSTRACT The control of telomerase activity at chromosome ends by telomere-binding proteins is critical for telomere length homeostasis. Two recent papers identify TPP1 as a critical mediator of this control. TPP1 forms part of the telomeric shelterin complex while also associating with telomerase, stimulating its activity and processivity.

The specialized DNA protein complexes at chromosome ends, known as telomeres, behave much differently from chromosome ends generated upon damage, which are readily resealed by ligation. Telomeres instead protect chromosome ends from DNA repair activities and nucleolytic degradation. In addition, telomere length provides a molecular clock that regulates the life span of cells. The clock starts ticking when telomerase shuts off, which occurs in most tissues of the body during the later stages of embryogenesis. Because of the end-replication problem and nucleolytic processing, critically short telomeres occur after a certain number of cell division cycles have taken place in the absence of telomerase. The cells then stop proliferating and enter so-called cellular senescence. Optimal telomere length setting during the developmental stages when telomerase is expressed is therefore crucial for long-term survival. The length reserve must be sufficient to avoid premature cellular senescence, and telomeres should be short enough to induce cellular senescence in cells that have lost normal growth control before they form tumors. But how is this control achieved, and how can telomerase, when expressed during development or in rare stem or stemlike cells, distinguish long and short telomeres to equilibrate their length? It is inferred that the answer must come from telomere-binding proteins that appear to modulate telomere structure in a length-dependent manner to favor elongation of the shortest telomeres (*1*, *2*). Notably, this regulation only functions if telomerase levels are limiting, because high telomerase levels lead to continuous telomere elongation (*3*). Now, two recent papers by Wang *et al*. (*4*) and Xin *et al*. (*5*) identify TPP1 (formerly known as TINT1/PTOP/PIP1) as a central player of this control. TPP1 forms part of the telomere capping complex termed shelterin (*6*), where it bridges *via* TIN2 (TRF1-interacting nuclear protein 2) interactions between the double-stranded telomere-binding proteins TRF1 and TRF2 (telomeric-repeat binding factor 1 and 2) and the telomeric 3′ overhang binding protein POT1 (protection of telomeres 1) (Figure 1). The papers now identify TPP1 as the first shelterin component to physically interact with telomerase. Strikingly, TPP1 also stimulates telomerase processivity and enzymatic activity. In addition, the papers provide evidence for a structural and functional conservation of the POT1–TPP1 complex all the way from ciliated protozoa to humans.

TPP1 was initially identified as a TIN2- and POT1-interacting protein (see Figure 1 and Figure 2, panel a) of the shelterin complex (*7*–*9*). POT1 was discovered through its sequence similarity with the ciliate *Oxytricha nova* single-stranded telomere-end-binding protein α (TEBPα) (*10*). In *Oxytricha*, TEBPα forms a complex with the TEBPβ subunit to tightly bind the telomeric 3′ overhang in this organism. Wang *et al*. and Xin *et al*. now provide compelling evidence that TPP1 is orthologous to TEBPβ. TEBPα and TEBPβ both contain oligonucleotide/oligosaccharide binding (OB) folds, which in TEBPα bind the single-stranded

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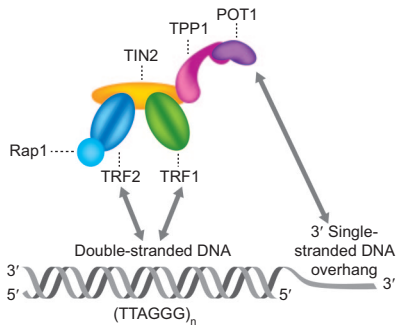

Figure 1. The shelterin complex and its interaction with telomeric DNA. Mammalian telomeres consist of 5′-TTAGGG-3′/5′-CCCTAA-3′ DNA repeats, reaching 5–15 kb in length in human cells. Telomeres end with single-stranded 3′ overhangs of the G-rich strand of several hundred nucleotides. The double-stranded telomeric repeats are directly bound by TRF1 and TRF2 homodimers, myb-domain proteins that show pronounced specificity for the TTAGGG tandem arrays (*23, 24*). The TRF1 and TRF2 dimers are bridged and stabilized by TIN2 and TPP1 (*25*) through protein–protein interactions. TPP1 also recruits the single-stranded telomeric DNA binding factor POT1 (*7, 8*). Human repressor/activator protein 1 (Rap1) binds to the telomeric chromatin through interaction with TRF2 (*26*).

DNA and in TEBPβ mediate protein–protein interaction with α. Similarly, POT1 binds single-stranded telomeric repeats through its two N-terminal OB folds (*11*), whereas its C-terminal half is responsible for interaction with TPP1 (*7*). Analysis of truncation mutants led to identification of a POT1 binding region in the central part of TPP1 (recruitment domain (RD) in Figure 2, panel a) and a TIN2-interaction domain (TID) at the C-terminus (*7, 9*) (TID in Figure 2, panel a). The N-terminus of TPP1 contains a short domain of unknown function as well as a predicted OB fold. Intriguingly, the crystal structure of the TPP1 OB fold closely resembles that of the TEBPβ OB fold (*4*). In addition to the structural similarity, the POT1–TPP1 complex also resembles TEBPα–β in the way it binds to the telomeric tracts. TPP1 lacks detectable DNA binding activity, and it is POT1 that positions the complex on the DNA, exerting a preference for the very end of the overhang (*4, 12*). Band-shift experiments in both papers show that TPP1 increases the affinity of POT1 to various telomeric oligonucleotides and stabilizes the ternary complex.

The six-component shelterin complex has been known for some time to be involved in telomere length control (*1, 2*). For example, inhibition of TRF1 leads to telomere elongation, whereas overexpression of TRF1 causes telomere shortening in human telomerase-positive cells without affecting telomerase activity. Reduction of TIN2 protein levels or the overexpression of mutant alleles that disrupt TIN2 interaction with TPP1 leads to telomere elongation (*13, 14*). Suppression of TPP1 by RNA interference or the disruption of the TPP1–POT1 interaction, which is accompanied by the loss of the POT1 signal at telomeres, also results in telomere lengthening (*7, 8*). Thus, reinforcement of the shelterin complex seems to inhibit telomerase. Longer telomeres load more shelterin complexes, and this may provide a length-sensing mechanism (*1*). Furthermore, shelterin, particularly TRF2, promotes formation of t-loops in which the telomeric 3′ overhang is tucked into the double-stranded part of the telomere (*15*). This may sequester the 3′ overhang from DNA repair factors as well as from telomerase. In addition, shelterin may promote binding of POT1 to the telomeric 3′ end, which also inhibits telomerase (*16*). Indeed, *in vitro* studies demonstrate that POT1, when bound to the telomeric 3′ end, prohibits binding and extension by telomerase (Figure 2, panel b, state i) (*12, 17*). Thus, the shelterin complex so far has been mostly linked to telomerase inhibition. However, telomeres must switch from nonextendible to extendible states, at least in telomerase-positive cells (*3, 18*).

Now, both new papers strengthen the view that shelterin components also have a telomerase-activating function. A physical interaction between TPP1 and human telomerase is demonstrated by coimmunoprecipitation of TPP1 and telomerase expressed in rabbit reticulocyte lysate and in cell extracts (*5*). TPP1 OB fold is necessary and sufficient for this interaction, an indication that TPP1 recruits telomerase to telomeres through its OB fold (*5*). Because TPP1 does not bind telomeric DNA directly, it could exert this function when bound either to the double-stranded telomeric tract *via* TIN2/TRF1/TRF2 or to the 3′ overhang *via* POT1 (*5*) (Figure 2, panel b, state ii). Wang *et al.* (*4*) performed detailed *in vitro* telomerase activity assays in the presence of POT1 and TPP1 and discovered activating functions, apart from a possible recruitment function. POT1 has been known to inhibit telomerase activity when bound to the telomeric 3′ end, and this inhibition is not overcome upon association with TPP1. Therefore, Wang *et al.* forced binding of POT1 with a primer DNA point mutation to a more upstream register, leaving a telomerase-extendible 3′ tail (Figure 2, panel b, state iii). Indeed, under this experimental condition, POT1 and TPP1 not only improved total telomerase activity but also increased telomerase processivity, an effect requiring the TPP1–POT1 interaction. Moreover, the two proteins together were able to rescue telomerase processivity on a G-quadruplex-forming oligonucleotide, probably because of the ability of POT1 to trap an open form of this structure (*19*).

In summary, the two papers identify TPP1 as an intimate and direct regulator of human telomerase. TPP1 mediates communication between shelterin and telomerase. It also may provide an ideal target to regulate telomerase activity at individual chromosome ends to trigger preferential recruitment and/or activation at short telomeres. The new data provide a molecular snapshot of the stimulatory effect of the POT1–TPP1 telomere binding protein complex during telomerase-mediated extension. However, the molecular nature of this and other telomeric states and the mechanism of their

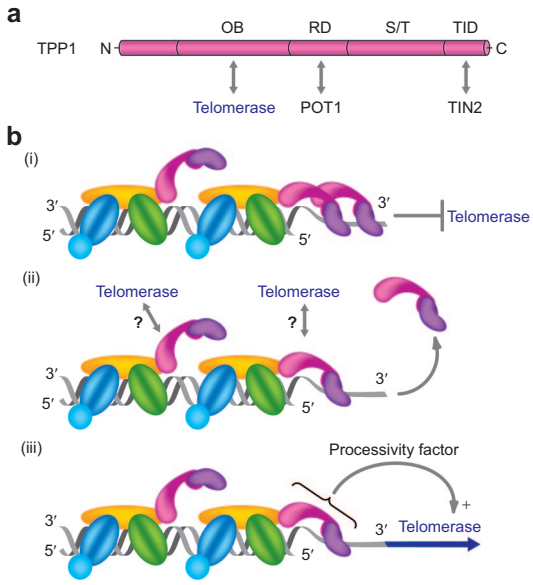

Figure 2. TPP1 and different telomeric states. a) TPP1 mediates interactions between POT1, TIN2, and telomerase (*4*). OB = oligonucleotide/oligosaccharide binding fold; RD = recruitment domain, also called PBD (*4*); S/T = serine/threonine-rich region; TID = TIN2-interaction domain. Nomenclature is from ref 5. b) Telomeric states. (i) Nonextendible state. POT1 is bound to the telomeric 3′ end, preventing elongation by telomerase. The telomere is shown here in a linear form. The t-loop should also correspond to a nonextendible state (see text). (ii) Extendible state. Unknown mechanisms may dissociate or displace the telomeric-end-bound POT1, or prevent it from binding, to allow telomerase access. Telomerase may be enriched at telomeres *via* TPP1, which binds indirectly to the double-stranded telomeric tract. (iii) Extending state. During extension, the activity and processivity of telomerase are stimulated by TPP1 bound to more internal POT1 molecules.

transition remain to be elucidated and must be studied in further detail. A possible model is that shelterin delivers POT1 to telomeric 3′ ends to prevent their elongation by telomerase, resulting in a nonextendible state (Figure 2, panel b, state i) (*12, 16, 17*). Unknown mechanisms may dissociate or displace the telomeric-end-bound POT1 or prevent it from binding, thus allowing telomerase access and producing an extendible state (Figure 2, panel b, state ii). This would permit the formation of an extending state in which telomerase is stimulated by TPP1 bound to more internal POT1 molecules (Figure 2, panel b, state iii). This duality of POT1 is reminiscent of studies in budding yeast, where the single-stranded overhang is bound by Cdc13p (cell division cycle 13), another OB-fold-containing protein that bears weak structural similarity with POT1. Cdc13p protein recruits telomerase holoenzyme in the S-phase *via* a telomerase subunit called Est1p (ever shorter telomere 1) (*20*). This interaction is critical for telomere maintenance, because *est1* yeast strains undergo cellular senescence. However, Cdc13p can also negatively regulate telomere elongation (*21*). Nonetheless, budding yeast Est1p is not homologous to TPP1, and therefore it may not fulfill an analogous function. In *Saccharomyces cerevisiae*, the phosphatidyl inositol-3-like protein kinases Tel1 and mitosis entry checkpoint 1 (Mec1) (ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related in humans)) also seem to play a critical role in preferentially activating telomerase at short telomeres. Interestingly, these kinases also associate with human telomeres in a cell-cycle-dependent manner (*22*), and it will be fascinating to uncover whether similar mechanisms regulate TPP1 function and telomerase activity at human telomeres.

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