Switching Human Telomerase On and Off with hPOT1 Protein * in Vitro

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POT1 (protein of telomeres 1) protein binds the G-rich single-stranded telomeric DNA at the ends of chromosomes. In human cells hPOT1 is involved in telomere length regulation, but the mechanism of this regulation remains unknown. Examination of the high-resolution crystal structure of the hPOT1-TTAGGGTTAG complex suggested that it would not be extended by telomerase, a hypothesis that we confirm by in vitro assays with recombinant telomerase. On the other hand, when hPOT1 is bound at a position one telomeric repeat before the 3'-end, leaving an 8-nucleotide 3'-tail, the complex is extended with improved activity and processivity. Thus, depending on its location relative to the DNA 3'-end, hPOT1 can either inhibit telomerase action or form a preferred substrate for telomerase. We propose that another factor catalyzes the interconversion of these states in vivo.

Telomerase, the ribonucleoprotein enzyme that replicates the ends of eukaryotic chromosomes, is regulated at multiple levels. The final level of regulation, the one most proximal to the site of enzyme action, appears to involve proteins that bind to the single-stranded DNA 3'-extensions or "tails" at the ends of chromosomes, which are the substrates for telomerase extension. These proteins include Cdc13p from Saccharomyces cerevisiae (1–3) and the distantly related POT1 proteins found in fission yeast, plants, and animals including humans (4–6). POT1 was discovered only recently (4), identified by its modest degree of sequence similarity with the subunits of telomere end-binding proteins from various ciliated protozoa (7–9). It is essential for chromosome end protection in the fission yeast Schizosaccharomyces pombe; deletion of the pot1+ gene leads to telomere loss and chromosome end fusion, and rare survivors live with all circular chromosomes (4). Human POT1 (hPOT1)1 protein localizes to chromosome telomeres (4, 5, 10) and binds specifically to single-stranded telomeric DNA repeats ending with the sequence GTTAG (6, 11–13). Its N-terminal half is necessary and sufficient for specific DNA binding, whereas its C-terminal half interacts with another protein called PIP1, PTIP, or TINT1 (12, 14, 15). PIP1 bridges POT1 to the double-stranded telomeric DNA-binding protein TRF1. hPOT1 can either facilitate or repress telomerase extension when overexpressed in tissue culture cells (10, 16, 17).

X-ray crystal structures of the N-terminal region of S. pombe POT1 bound to telomeric DNA revealed a single oligonucleotide/oligosaccharide-binding fold bound to a telomeric hexanucleotide (18). The crystal structure explained the enormous specificity of the protein for the telomeric sequence and for DNA over RNA, the latter being vastly more abundant than ssDNA in the nucleus. Human POT1 requires at least 10 nucleotides for tight binding, so it was at first unclear how this longer sequence would be recognized. This question was answered by the crystal structure of the N-terminal half of hPOT1 bound to a telomeric decanucleotide, which revealed two oligonucleotide/oligosaccharide-binding folds; the first (which resembles the S. pombe structure) binds 6 nucleotides and the second forms a pocket that buries the 3'-terminal guanine base (see Fig. 1 (13). Thus, hPOT1 appears to be "designed" to be a chromosome end-capping protein. Consistent with this hypothesis, the preferred hPOT1-binding sequence (ending in GTTAG) is the same sequence produced by each round of telomerase extension (19) and is the most common 3'-end sequence of telomeres in telomerase-positive human cells.2

A different model for human chromosome end protection is the T-loop model. The 3'-ssDNA tail of the chromosome could invade the double-stranded telomeric DNA, forming a local displacement loop structure that sequesters the 3'-tail by base pairing to an internal complementary strand (20, 21). This model is based on impressive electron micrographs from several laboratories (20, 21). At this point, the state of the 3'-tail in a T-loop has not been analyzed, and there is no biochemical evidence that the structure is resistant to telomerase extension. Furthermore, a DNA structure, unlike a protein, cannot be subjected to a genetic knock-out to test its importance in vivo. In any case, T-loops and hPOT1 capping of chromosome ends are not mutually exclusive models but could represent alternative states of a dynamic telomere.

Here we use purified hPOT1 and recombinant human telomerase to describe the molecular details by which hPOT1 can by itself repress or permit telomerase action in vitro. This work demonstrates that a telomeric DNA-bound protein can actually enhance telomerase activity and processivity.

MATERIALS AND METHODS

Protein Expression and Purification—hPOT1V1 and V2 were expressed in a baculovirus-insect cell system and purified as described previously (13).

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1 The abbreviations used are: hPOT1, human POT1; ssDNA, single-stranded DNA; HA, hemagglutinin; SVP1, snake venom phosphodiesterase I.

2 Steir, A. J., Chai, W., Shay, J. W., and Wright, W. E. (2005) Mol. Cell 18, 131–138.
In Vitro Reconstitution of Human Telomerase—C-terminal HA-tagged human TERT was expressed from pHET-HA2 and hTER from phTERT-HA by using the TNT quick-coupled transcription/translation system (Promega). Each 500-μl reaction contained 400 μl of TNT quick mix, 40 μl of PCR enhancer (Promega), 20 μl of 1 mM methionine, 20 μl of water, and 1.05 μg of each plasmid DNA. After incubation at 30 °C for 2 h, the reconstituted telomerase complex was affinity-purified on anti-HA F7-agarose beads (Santa Cruz Biotechnology). Anti-HA F7-agarose beads (150 μl), washed with TMG-100, were added for immunoprecipitation at 4 °C overnight. The beads were washed with 1× telomerase assay buffer (see below) four times and then resuspended in 1× telomerase assay buffer. The quantity of 35S-hTERT was determined.

Telomerase Activity Assay—Activity of the immunopurified human telomerase complex reconstituted in vitro was determined by a direct assay protocol modified from a published protocol (22). Briefly, the reaction mixture (20 μl) contained 1× telomerase assay buffer (50 mM Tris-HCl, pH 8.0, 50 mM KCl, 1 mM MgCl2, 5 mM 2-mercaptoethanol, and 1 mM spermidine), 100 nM telomeric DNA primer, 0.5 mM dATP, 0.5 mM dGTP, and 1.25 mM [32P]dGTP (800 Ci/mmol) with 6 μl of immunopurified complex.

The reaction was incubated at 30 °C for 1 h, and the products were precipitated with the addition of 100 μl of 3.6 mM NH4OAc, 20 μg of glycogen, and 450 μl of ethanol. After incubation at −80 °C for 1 h, samples were centrifuged at 4 °C for 20 min, and the pellets were washed with 70% ethanol and resuspended in 1× gel-loading buffer (40% formamide, 10 mM Tris-HCl, pH 8.0, 10 mM EDTA, and 0.05% xylene cyanol). The heat-denatured samples were loaded onto a 10% polyacrylamide, 1× TBE (Tris borate-EDTA), 7 M urea denaturing gel for electrophoresis. After electrophoresis, the gel was dried and quantified using a PhosphorImager.

Snake Venom Phosphodiesterase I (SVPI) Digestion—The hPOT1-primer complex was performed before the addition of SVPI. The complex mixture (9 μl) contained 1.1× telomerase assay buffer (see above), 0.11× protein buffer, 370 nM hPOT1V2, and 117 nM 5′-labeled [32P]DNA at room temperature for 30 min. Then 0.3 μg of SVPI in 1 μl of stock solution (100 μM Tris-Cl, pH 8.9, 100 mM NaCl, and 15 mM MgCl2) was added to start the digestion. The reaction was incubated at 30 °C for 5 min and then stopped by the addition of 1 μl of 100 mM EDTA. After heat inactivation at 95 °C for 2 min, 10 μl of 94% formamide, 1× TBE, and loading dye were added to the sample. 10 μl of the final mixture was loaded onto a 10% polyacrylamide, 1× TBE, 7 M urea denaturing gel for electrophoresis. After electrophoresis, the gel was dried and quantified using a PhosphorImager.

RESULTS

hPOT1 Inhibits the Action of Telomerase—Two forms of hPOT1 were studied: version 1, the full-length protein, and version 2, one of the five splicing variants identified previously by mRNA analysis that represents the entire DNA-binding domain of the protein (5, 13). Full-length hPOT1 version 1 and splicing variant 2 were expressed and purified as described (13). In cases where both hPOT1 versions 1 and 2 were tested and found to have equivalent activity, we use hPOT1 to represent both proteins.

The crystal structure of the DNA-binding domain of hPOT1 bound to TTAGGGTTAG suggests that the complex would not be extended by telomerase. The 3′-terminal guanine (G10) tightly associates with hPOT1 so that it could not align with the template region of telomerase RNA (Fig. 1). To investigate the ability of telomerase to extend hPOT1-DNA complexes, human telomerase was reconstituted from in vitro transcribed telomerase RNA (hTER) and in vitro translated hemagglutinin-tagged human telomerase catalytic subunit (HA-hTERT) in a rabbit reticulocyte lysate. The reconstituted telomerase complex was then subjected to affinity immunopurification via the HA tag. Use of a direct primer extension assay rather than a PCR-based assay allowed quantitation of both activity and processivity.

In the absence of hPOT1, the purified reconstituted telomerase extended two telomeric primers, a and b, to give the hexanucleotide repeat ladder characteristic of human telomerase activity (Fig. 2A, lanes 3 and 4). Comparisons with Primer b extended by 2 or 4 nucleotides confirmed that the darkest bands in the telomerase ladders represented extension products ending in the sequence TTAG (Fig. 2, a versus 2b, and 3 versus 4). These results support the conclusion that the inhibition of telomerase activity was independent of the order of addition of hPOT1 and telomerase (Fig. 2A, lanes 6 and 12). Thus, under these conditions the off rate of the primer from telomerase is high enough, and the off rate of the primer from hPOT1 is low enough that telomerase cannot compete with hPOT1 for primer binding.

To examine whether the inhibition of telomerase by hPOT1 was specific to this protein, a parallel telomerase activity assay was carried out using SpPot1pN, the ssDNA-binding domain of the fission yeast (S. pombe) P1 protein. Previous biochemical and structural studies have demonstrated that SpPot1pN binds with high sequence specificity to its cognate telomeric ssDNA, whose sequence is very similar to that of human telomere (GGTTAC versus GGTTAG) (18, 23). However, unlike hPOT1, SpPot1pN had no effect on telomerase activity (Fig. 2, lanes 1, 2, 7, and 8). These results support the conclusion that hPOT1 is a telomerase activity modulator specific for the human telomeric sequence.
**Regulation of Human Telomerase by hPOT1**

**Fig. 2.** hPOT1 inhibits telomere elongation by telomerase. A, the direct telomerase activity assay was performed with Primers *a* and *b* in the presence of protein buffer (–), hPOT1, or *S. pombe* (S.p.) Pot1pN (which does not bind the human telomeric DNA in the binding assay). DNA primers were either premixed with hPOT1 and nucleotides (lanes 7–12) or premixed with telomerase followed by hPOT1 and nucleotides (lanes 1–6). A control telomerase activity assay was performed with 5'-labeled DNA Primers *a* and *b* without hPOT1. hPOT1 was then added (lanes 2 and 4) after the reaction of primer extension.

4), indicating that hPOT1 does not interfere with the recovery of elongation products.

**hPOT1 Inhibits Telomerase Activity by Controlling the Accessibility of the Telomeric ssDNA Substrate to Telomerase—**The inhibition of telomerase activity by hPOT1 could take place at the level of the sequestration of the telomeric DNA substrate or by direct interaction between hPOT1 and telomerase. To address this issue, we made use of the observation that an oligonucleotide with three guanines at the 3'-end has a lower affinity for hPOT1 than an oligonucleotide with one guanine (13). Therefore (TTAGGG)$_3$ (Fig. 2, Primer *a*) was employed in the telomerase activity assays. hPOT1 gave less inhibition of the extension of Primer *a* than Primer *b* (Fig. 2A, lanes 5, 6, 11, and 12) under the same experimental condition. These data indicate that the inhibition of telomerase activity by hPOT1 is primer-specific. Thus, we propose that hPOT1 is able to prevent the action of telomerase by controlling the accessibility of the telomeric ssDNA substrate to the telomerase.

**DNA-binding Domain of hPOT1 Is Sufficient to Alter Telomerase Activity—**When the telomerase activity assay (Fig. 2) was carried out with the full-length protein, instead of the DNA-binding domain of hPOT1, the differential activity of Primers *a* and *b* persisted (data not shown). The increased processivity of extension (see below) was also conferred by both versions of hPOT1. Thus, although the C-terminal domain of hPOT1 is required to bring additional proteins into the complex (10, 14, 15), it is not involved in the effects on telomerase action described in this paper.

**One hPOT1-primer Complex Is a Telomerase Substrate—**To further study the different effects of hPOT1 on telomere extension of Primers *a* and *b*, we performed a telomerase activity assay with increasing amounts of hPOT1 and 100 nM primer. This primer concentration was chosen because it is well above the $K_d$ for hPOT1 binding (13), so equimolar hPOT1 should give complete binding. For Primer *b*, when the concentration of hPOT1 was lower than that of the primer, telomerase was able to extend the primer (Fig. 3, *A*, lanes 2 and 4, and *B*). Increasing the concentration of hPOT1 to 100 nM and above completely eliminated telomerase activity in a stepwise manner (Fig. 3, *A*, lanes 6, 8, 10, 12, and 14, and *B*). On the contrary, for Primer *a*, although the overall activity was greatly reduced in the presence of hPOT1, the activity reached a plateau (12% of the maximum activity) and did not disappear even at protein concentration as high as 667 nM (Fig. 3, *A* and *B*). These results suggested that the hPOT1-Primer *a* complex still could serve as a substrate for telomerase, although with reduced overall activity.

**hPOT1-bound Primers Give a Modest Increase in Telomerase Processivity—**hPOT1 not only modulated the activity of telomerase, but it also increased the product size distribution. For Primer *a*, the formation of shorter extension products was affected more strongly than the formation of the longer ones when the concentration of hPOT1 was greater than 100 nM, the concentration of the primer (Fig. 3A). The radioactivity of the bottom band, which corresponds to the first cycle of template copying (Fig. 3A, +4), was greatly reduced, whereas the formation of the longer products (+22 and beyond), corresponding to four and more cycles of template copying, was only weakly inhibited. Under these experimental conditions of high primer concentration, longer extension products result from processive extension, not from rebinding of previously extended products (24–27). Thus, the processivity of telomerase was increased when hPOT1 was bound. To quantitatively measure telomerase processivity, the intensity of each prominent band was measured, normalized, and plotted as described previously (27, 28). The processivity of telomerase reaction is inversely related to the slope of the line. The processivity increases ~1.4-fold in the presence of hPOT1 (Fig. 3, *C* and *D*).

It seemed possible that the observation of longer reaction products might result from the sequestration of “free” primers from telomerase by hPOT1. In other words, the reaction might have changed from a processive to a distributive mode of elongation. To test this possibility, we carried out a telomere extension assay with decreasing amounts of Primer *a* in the absence of hPOT1. If the only function of hPOT1 is to reduce the concentration of free primer available for telomerase activity, the presence of hPOT1 would be equivalent to low primer concentration. However, for a primer concentration ranging from 10 μM to 5 nM, the processivity remained unchanged (Fig. 4). In an independent test, we added increasing concen-
trations of various antisense oligonucleotides to the primers prior to extension; we achieved decreased activity but never observed the increased processivity seen with hPOT1 (data not shown). Thus, the increase of the processivity observed in Fig. 3 was the result of a property of hPOT1 other than just sequestering primers from telomerase.

**Telomeric Sequences Have Overlapped hPOT1-binding Sites—Primer a** has two overlapped and therefore mutually exclusive hPOT1-binding sites, highlighted by boxes in Fig. 5A. Hereafter we refer to them as the 5'- and 3'-binding sites. Once hPOT1 binds to the 5'-binding site, it leaves an eight-nucleotide 3'-overhang (GGTTAGGG), which is not able to bind another hPOT1 molecule (13). Similarly, the binding of hPOT1 to the 3'-site will generate two single-stranded overhangs. One is at the 5'-end (TTAGGG) and the other at the 3'-end (GG), and neither of them can bind a second hPOT1 molecule. To study the two hPOT1-binding properties in solution, SVPI was used to cleave the unbound nucleotides from the 3'-end. SVPI digests single-stranded DNA unidirectionally from the 3'- to the 5'-end. The mutant primers were preincubated with a 5-fold excess of hPOT1 to ensure that every primer DNA was bound by an hPOT1 molecule before the addition of SVPI. As shown in Fig. 5B, SVPI cleaved the last 7 or 8 nucleotides from Primer a5 in the presence of hPOT1, indicating that hPOT1 binds only the 5'- but not the 3'-site. On the contrary, SVPI only cleaved the last two guanines from the hPOT1-Primer a3 complex, indicating that hPOT1 protects the 3'-end hPOT1-binding site from SVPI digestion. As a control, an oligonucleotide with 17 non-telomeric nucleotides was completely digested by SVPI in the presence of hPOT1. Thus, we concluded that there are two mutually exclusive hPOT1-binding sites in Primer a, and the mutant primers, a5 and a3, have only the 5'- or 3'-end hPOT1-binding site, respectively.

**One hPOT1-primer Complex Is a Telomerase Substrate with Improved Activity and Processivity—**We have shown that hPOT1 bound to Primer a5 generates an 8-nucleotide 3'-overhang, which is subject to SVPI digestion. This overhang was predicted to be long enough to align with the RNA template and potentially could serve as a substrate for telomerase. To test this hypothesis, we performed a telomerase activity assay using both Primers a3 and a5. In the absence of hPOT1, both primers were extended by telomerase perfectly well (Fig. 5C, lanes 1 and 3). However, the presence of hPOT1 almost completely inhibited telomerase activity of Primer a3 (Fig. 5C, lanes 1 and 2). In sharp contrast, Primer a5 bound to hPOT1 was a substrate for telomerase and even showed a modest increase in activity (1.2-fold) compared with that without hPOT1 (Fig. 5C, lanes 3 and 4). Moreover, the telomerase processivity for Primer a5 showed a stepwise increase (~1.4-fold) at equimolar hPOT1, which is comparable with that observed for Primer a (Fig. 5D). Thus, we conclude that hPOT1-Primer a5 complex is not a substrate for telomerase extension, whereas hPOT1-Primer a5 is a substrate with improved activity and processivity.

**Telomerase Requires a 3'-Overhang ≥8 Nucleotides on an hPOT1-ssDNA Complex—**Not only does Primer a have two...
overlapped hPOT1-binding sites, but Primer b does as well. However, there was no residual activity for Primer b in the presence of hPOT1. To address this issue, we designed two mutant primers (c5 and b5) based on Primer a5 (Fig. 6). They both have the same point mutation as Primer a5, which only allows hPOT1 to bind their 5'-binding sites, but they have a 1- or 2-nucleotide shorter 3'-tail, respectively. Although hPOT1-Primer b5 complex has a 6-nucleotide single-stranded overhang and theoretically still can align with the template region of hTER, the telomerase activity was almost undetectable (Fig. 6). For complex of hPOT1-Primer c5, there was some activity, but it was greatly reduced (18%) (Fig. 6). Primer b has the same 3'-sequence as Primer b5, explaining why the hPOT1-Primer b complex was not extended by telomerase (Figs. 2 and 3). Thus, we conclude that a 3'-overhang of 6 nucleotides is not enough, but an overhang of 8 nucleotides enables an hPOT1-ssDNA complex to serve as a substrate for telomerase.

To provide an independent test, we synthesized a series of primers in which the hPOT1 binding site was separated from a 3'-terminal GGTTAG sequence by one, two, three, or four A residues. SVPI digestion of the hPOT1-primer complexes showed 3'-tail lengths of 6, 7, 8, and 9 nucleotides, respectively. Telomerase assays showed little or no extension with the first two hPOT1-primer complexes, whereas the ones with the 8- and 9-nucleotide tails were fully active (extended for two repeats at levels similar to the free primer; data not shown). The lack of extension beyond two repeats is explained by the creation of a second hPOT1-binding site. This experiment confirmed that an overhang of 8 nucleotides or longer enables an hPOT1-ssDNA complex to serve as a telomerase substrate.

For comparison, we tested a panel of partially double-stranded DNA molecules with incremental lengths of the single-stranded 3'-overhang (supplemental Fig. 1). A region of non-telomeric sequence in both the telomeric primer and the antisense ssDNA functioned to ensure the correct register of annealing (boxed in supplemental Fig. 1B). Our data indicated that a 5-nucleotide overhang is required for full telomerase activity (supplemental Fig. 1A). This result is consistent with the recently published data of Ref. 29. However, the overhang length requirement is different from the case of an hPOT1-ssDNA complex. Moreover, sequestration of the 5'-portion of a ssDNA primer by converting it to double-stranded DNA does not alter the processivity of telomere extension (supplemental Fig. 1C). Thus, the improved processivity observed in Figs. 3 and 5 is hPOT1 protein-specific.

There remained the possibility that telomerase might prefer very short primers and that the improved processivity and activity of the hPOT1-a5 complex just resulted from it leaving a short 3'-overhang (GGTTAGGG). To test this possibility, we performed telomerase activity assays with a group of short primers in the absence of hPOT1. The 8-mer GGTTAGGG as well as the telomeric 7-mer and 9-mer (GGTTAGGG and GGTTAGGG) were all substrates for telomerase, but their activities were clearly lower than that of the hPOT1-a5 complex (data not shown). Thus, hPOT1 is not merely “hiding” the 5'-end of the telomeric primer but is specifically enhancing telomerase activity.

**DISCUSSION**

Many components involved in telomere length regulation have now been identified. In yeast, elegant genetic and molecular biology studies have provided evidence that the ssDNA-binding protein, Cdc13p, interacts with Stn1p and Ten1p to both positively and negatively regulate telomere length (1, 30–32) and that Cdc13p directly interacts with the telomerase regulatory subunit Est1p (33, 34). The corresponding components in the human system appear to be POT1, its protein partners in the TITTRP complex, and hEST1A (see below). Yet our understanding of how these proteins regulate telomerase is very far from the level at which we understand, for example, how signaling molecules are regulated by phosphorylation or how transcription is regulated by histone acetylation; these latter events have been described with atomic resolution structures and detailed enzymology.

The current work provides a starting point for structural and mechanistic analysis of one level of regulation of human telomerase. We describe two states of the telomeric DNA-protein
complex, one that sequesters the ssDNA from telomerase and therefore represents an “off” state, and another that presents the ssDNA tail in a manner that facilitates telomerase activity and processivity, an “on” state (Fig. 7). We propose that other components of the telomere and of telomerase will provide the switch between these two states in vivo.

hPOT1 Can Negatively and Positively Regulate Telomere Extension—Whether an ssDNA-binding protein will compete with telomerase for the telomeric DNA substrate depends on whether it leaves the 3'-end nucleotides free and whether this 3'-end overhang is able to serve as the substrate for telomerase. hPOT1 is a strong inhibitor when it binds the 3'-terminal sequence GGTTAG, the direct product of telomerase action, for two reasons; hPOT1 buries the base of the 3'-terminal guanine in the protein, making it inaccessible to telomerase (13), and it has a slow enough off rate that the primer is not redistributed to telomerase (this work) (Fig. 7A). Moreover, even when hPOT1 does not bind the last nucleotide of the telomere, but leaves a 3'-overhang with 6 nucleotides or less, the complex is still inhibitory for telomerase extension. This could because of steric hindrance between hPOT1 and the telomerase (Fig. 7A).

On the other hand, when the unbound 3'-overhang is 8 nucleotides or longer, the hPOT1-ssDNA complex can be extended by telomerase, suggesting a distance of 8 nucleotides is required to avoid the steric hindrance (Fig. 7B). This result further emphasizes that telomeric DNA-binding proteins are not always telomerase inhibitors (35, 36). A possible explanation for the slightly increased activity and processivity is that hPOT1 could directly interact with telomerase in an ssDNA-dependent manner, facilitating telomere extension. In a recent paper, Kelleher et al. (37) also reported that hPOT1 inhibits telomerase activity in vitro by blocking substrate access to telomerase, consistent with our findings. These authors did not investigate the effects of hPOT1 on telomerase activity when it binds to different positions on ssDNAs relative to the 3'-ends.

The Proposed Interconversion of the Two States Would Rely on Other Factors in Vivo—We have used several point mutations of the telomeric DNA to control the location of hPOT1 relative to the 3'-end, which allowed us to observe that hPOT1 can either inhibit or facilitate telomerase action. However, whether these are in fact the relevant states in vivo, how the interconversion of these two states is regulated, and the mechanism by which the chromosome tail is handed off from hPOT1 to telomerase remain unknown.

Recently, a six-component complex (TTTRPP) was identified...
Regulation of Human Telomerase by hPOT1

A

Stably inactive

\[5' \text{OB1 OB2} 3'\]

\[5' \leq 6 \text{ nt} \]

Inactive

\[5' \text{OB1 OB2} 3'\]

\[5' \geq 8 \text{ nt} \]

Active, with higher processivity and activity

\[5' \text{OB1 OB2} 3'\]

\[5' \text{TTAGGGTTAGGG} \]

hPOT1 monomer

C-terminal domain

\[5' \text{telomerase} \]

\[5' \text{OB1 OB2} 3'\]

\[5' \text{TTAGGGTTAGGG} \]

FIG. 7. A model of telomere length regulation by hPOT1. A, schematic illustration showing how hPOT1 inhibits telomerase activity. The blue and yellow ovals represent the two oligonucleotide/oligosaccharide-binding folds of a single hPOT1 monomer. The kinked red bar represents the telomeric ssDNA. Each hPOT1 molecule binds two telomeric repeats. The 3' end of the overhang is prevented from interacting with telomerase. The C-terminal domain of hPOT1, which is not shown in the crystal structure, is represented by a green oval. B, schematic illustration showing that an hPOT1-bound single-stranded telomeric DNA with a 3'-overhang 8 nucleotides or longer is a preferred substrate for telomerase with improved activity and processivity. The purple arrow between A and B represents other factors, like hEST1A, which may catalyze the interconversion of the two regulatory states of hPOT1.

using mass spectrometry by several groups (12, 38–40). The components of this complex are TRF1, TRF2, TIN2, RAP1, PIP1/PTOP/TINT1, and hPOT1. It is proposed that these six proteins contribute to a regulatory network for telomere length regulation. Perhaps the position of hPOT1 relative to the very proteins contribute to a regulatory network for telomere length regulation. Perhaps the position of hPOT1 relative to the very

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