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COMMUNICATION

Multiple POT1–TPP1 Proteins Coat and Compact Long Telomeric Single-Stranded DNA

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Telomeres are nucleoprotein complexes that cap and protect the ends of linear chromosomes. In humans, telomeres end in 50–300 nt of G-rich single-stranded DNA (ssDNA) overhangs. Protection of telomeres 1 (POT1) binds with nanomolar affinity to the ssDNA overhangs and forms a dimer with another telomere-end binding protein called TPP1. Whereas most previous studies utilized telomeric oligonucleotides comprising single POT1–TPP1 binding sites, here, we examined 72- to 144-nt tracts of telomeric DNA containing 6–12 POT1–TPP1 binding sites. Using electrophoretic mobility gel shift assays, size-exclusion chromatography, and electron microscopy, we analyzed telomeric nucleoprotein complexes containing POT1 alone, POT1–TPP1, and a truncated version of POT1 (POT1-N) that maintains its DNA-binding domain. The results revealed that POT1-N and POT1–TPP1 can completely coat long telomeric ssDNA substrates. Furthermore, we show that ssDNA coated with human POT1–TPP1 heterodimers forms compact, potentially ordered structures.

Telomeres are nucleoprotein complexes that comprise the ends of eukaryotic chromosomes. Telomeres contribute to genomic stability, in part, by preventing deleterious events such as chromosome end-to-end fusions and degradation. In addition, telomeres are intimately involved in recruiting and regulating enzymatic complexes necessary for telomeric DNA modification, replication, and repair.[1] Both the protective capabilities of telomeres and their regulation of cellular processes including senescence, DNA damage response, subnuclear localization, and gene expression involve specific proteins that bind to the telomeric DNA.[2–5]

In humans, telomeric DNA has a repeating, hexameric sequence of TTAGGG that extends for several thousand base pairs.[6] Telomeric DNA ends in 3′ single-stranded overhangs, which are about 50–300 bases in mammals.[7–9] Both the double- and single-stranded regions of the telomeric DNA are bound by various telomeric proteins. Telomere-repeat binding factors (TRFs) 1 and 2 are sequence-specific proteins that bind the double-stranded telomeric DNA.[10] The single-stranded portion of the telomere DNA is recognized and bound with nanomolar affinity by protection of telomeres 1 (POT1), also in a sequence-specific manner.[11–13] Other proteins interact with TRF1, TRF2, and POT1 to form a six-membered core complex called shelterin that contributes to chromosome-end

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Abbreviations used: ssDNA, single-stranded DNA; TRF, telomere-repeat binding factor; EMSA, electrophoretic mobility shift assay; EM, electron microscopy; POT1, protection of telomeres 1.

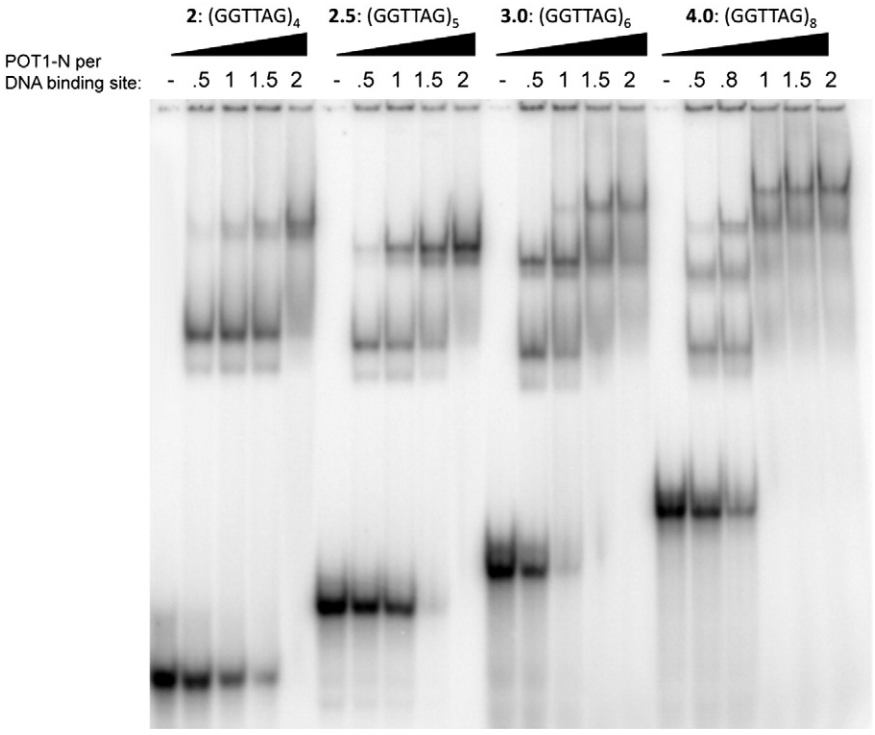

Fig. 1. EMSAs depicting the ability of POT1-N to coat long tracts of telomeric DNA. ssDNA oligos were synthesized by Integrated DNA Technologies. Oligos ranged from 24 nt to 48 nt and contain four to eight telomere hexameric repeats. Every two hexameric repeats (5′-GGTTAG GGTTAG-3′) is suitable for binding individual POT1 molecules. DNA was 5′-radiolabeled, and gel shifts were performed essentially as previously described.[33] For each reaction, a 10-μL solution was prepared containing 400 nM ssDNA spiked with approximately 4% 5′-^{32}P-labeled DNA. POT1-N was added to each reaction at molar ratios indicated on top of the gel. The molar ratio indicated is calculated for the ratio of protein added per dodecameric repeat (5′-GGTTAG GGTTAG-3′) in the oligonucleotide in each lane. Reactions were conducted in buffer containing 25 mM Tris (pH 8.0), 150 mM NaCl, 10 mM DTT, and 5% glycerol. After 30 min on ice, reactions were analyzed by gel electrophoresis on 6% acrylamide gels in 0.5× Tris–borate–ethylenediaminetetraacetic acid buffer. Gels were dried and imaged on a Typhoon phosphorimager (GE Life Sciences).

protection and telomere homeostasis.[2,14] Sub-complexes have been identified that consist of only three to five of the shelterin components.[15,16] The multimerization state may be important for conducting interactions with enzymes functioning at the telomere. For example, the binding of one shelterin protein (TIN2) has been shown to protect another member of the shelterin complex, TRF1, from selective ubiquitination.[17]

It is proposed that telomere length in the yeast *Saccharomyces cerevisiae* is regulated by the number of Rap1p proteins coating the double-stranded, telomeric DNA.[18] In such a protein-counting mechanism, a greater number of Rap1p molecules stabilize the formation of a compact structure that prevents access of telomerase, the ribonucleoprotein enzyme that synthesizes telomeric DNA. As telomere length increases, more Rap1p molecules coat the telomeric DNA, and telomerase activity is inhibited. A similar counting mechanism has been proposed in mammals, where the number of TRF1 and TRF2 proteins regulates the length of the double-stranded region of telomeres.[19,20] How the length of the single-stranded region of the telomere is regulated and whether it is coated by multiple telomeric proteins are less clear. TPP1 (formerly named PTOP/PIP1/TINT1[21–23]) heterodimerizes with POT1 and binds the single-stranded 3′ overhang of human telomeres.[24] The *in vivo* stoichiometry of POT1–TPP1 (50–100 copies per telomere) is potentially more than enough to coat the single-stranded DNA (ssDNA),[25] although such coating has not been previously demonstrated.

Information regarding the role of the POT1–TPP1 heterodimer in telomere extension and protection indicates a fascinating balance of function. One role of TPP1 is to recruit telomerase to its natural substrate, the telomere.[26,27] In addition, the POT1–TPP1 heterodimer stimulates the processivity of telomerase, at least *in vitro*.[24,28] In a second opposing role, the POT1–TPP1 heterodimer binds single-stranded, telomeric DNA and shields it from degradation, repair, and recombination.[29–31]

Multiple splicing variants of human POT1 have been identified *in vivo*.[11,32] The primary products of the splicing variants are the full-length protein and a