Published in final edited form as:

Nat Struct Mol Biol. 2013 January ; 20(1): 10–12. doi:10.1038/nsmb.2483.

A Popular Engagement at the Ends

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Abstract

Three recent studies converged on a specific protein-protein interface between TPP1 and telomerase as being crucial for the regulation of both telomerase recruitment and processivity in mammalian cells. An equivalent interaction appears to exist in budding yeast, making this a nearly universal means of telomerase regulation.

Telomerase, the special reverse transcriptase dedicated to the synthesis of telomere repeat units, has attracted considerable attention owing to its critical function in maintaining chromosome ends and its potential applications in aging and cancer therapies ^{1, 2}. Although the nucleotidyl transferase activity of telomerase requires only two core components, a template-bearing RNA named TER and the catalytic protein named TERT, many additional regulatory factors are required to accomplish telomere extension *in vivo*. In particular, telomerase has to be recruited to chromosome ends and, once there, add a sufficient number of telomere repeats to compensate for DNA loss that is incurred as a consequence of incomplete replication or nucleolytic degradation. The ability of telomerase to add multiple repeats is referred to as its processivity, and the mechanisms that regulate both telomerase recruitment and processivity have long been major topics of research in the field.

Remarkably, three recent studies have converged on a single protein-protein interface as being central to both types of regulation in mammalian cells ^{3–5}. Moreover, an equivalent interaction appears to exist in budding yeast, though it is used for slightly different ends ^{6, 7}. This is all the more remarkable given the drastic divergence of telomere nucleoprotein structures of fungi and mammals ⁸, and thus points to one of the few unifying themes in the chemistry and biology of telomere maintenance.

A review of the telomere nucleoprotein structures in fungi and mammals is instructive to appreciate the remarkable convergence of findings. Telomeres terminate in single-stranded, G-rich 3' overhangs known as G-tails. The major G-tail binding protein in budding yeast is Cdc13, which recruits telomerase through an interaction with the Est1 subunit of the telomerase complex ^{9, 10} (Fig. 1). In mammals, however, the G-tails are bound by POT1, which is part of a six-subunit complex named shelterin that interacts both with G-tails and duplex telomere repeats and that shields telomeres from a variety of insults ¹¹. In contrast to Cdc13, POT1 does not appear to mediate telomerase recruitment. Rather, emerging evidence points to another subunit of the shelterin complex named TPP1, which tethers POT1 to the remaining subunits, as the main “recruiter” ¹². Purified POT1-TPP1 complex also has the

ability to stimulate telomerase activity and processivity *in vitro*, suggesting that it may influence multiple aspects of telomerase function¹³. However, analysis of the TPP1-telomerase nexus, particularly *in vivo*, is complicated by the role of TPP1 in telomere protection. In other words, telomere aberrations in TPP1 mutants are not necessarily caused by telomerase-dependent mechanisms¹⁴. This difficulty has now been overcome by three laboratories which each used targeted point mutations in the OB fold domain of TPP1 to demonstrate critical functions of this protein in controlling telomerase action³⁻⁵.

To minimize the complications introduced by other telomere proteins, Zhong et al. tested the ability of TPP1 to re-direct telomerase to a non-telomeric location using the combination of a LacO array and LacI-TPP1 fusion proteins. Strikingly, robust relocation of telomerase to the LacO locus was achieved with a fusion protein containing only the OB fold domain of TPP1. In addition, over-expression of the OB fold domain alone inhibited binding of telomerase to telomeres, presumably by competing with native TPP1 for the recruitment target site on telomerase. Zhong et al. then identified specific residues on TPP1 and TERT required for their mutual interaction in colocalization assays. In a complementary study, Sexton et al. used a variety of pull down assays to analyze the interaction between TPP1 and telomerase that was reconstituted *in vivo* by co-expression of TERT and TER. Consistent with the LacI fusion protein analysis, the OB fold domain of TPP1 recapitulated the binding affinity and specificity of full-length TPP1 for telomerase. Moreover, analysis of a large panel of point mutants strongly implicates a surface loop connecting beta strand 3 and 4 of the OB fold as playing a prominent role in TPP1-telomerase association. In a third study, the role of this interaction in regulating telomerase processivity was carefully examined by Nandakumar et al., who engineered their own set of TPP1 OB fold mutants and characterized their ability to bind telomerase and enhance processivity. This group also analyzed selected TPP1 mutants for their ability to support telomere elongation and telomerase-telomere co-localization *in vivo* by simultaneously knocking down endogenous TPP1 using shRNA and expressing shRNA-resistant TPP1 mutants. So what can be gleaned from this collection of studies? Considering the different methodologies employed by the three groups, their overall findings are remarkably congruent. Most importantly, all three studies implicated the loop connecting beta strand 3 and 4 (L34) of the TPP1 OB fold as a key determinant of telomerase interaction, which is in turn necessary for processivity enhancement (Fig. 2). Two of the studies additionally implicated the end of beta strand 5 (β 5) and the ensuing loop in the interaction. Notably, these two structural elements are in close physical proximity, suggesting a single contiguous surface for binding telomerase (named TEL patch by Nandakumar et al.). Also noteworthy is the fact this particular facet of the OB fold is distinct from the canonical DNA-binding surface of OB folds, and appears to be infrequently used for binding proteins¹⁵. With regard to the telomerase-side of the protein-protein interface, previous studies suggest the involvement of the TEN domain, an N-terminal DNA-binding domain of TERT that is believed to “anchor” telomerase to telomere DNA during processive synthesis^{16, 17}. Although these three papers did not address the target site in detail, two of them reported mutations in the TEN domain and residues near the C-terminus of TERT (CTE) that impaired the interaction in selected assays. A better definition of the structural determinants in telomerase that mediate binding to TPP1 is clearly a priority for future studies.

As outlined above, the recruitment mechanism for telomerase in budding yeast, which is based on interactions between Cdc13 and Est1, is quite distinct from that of mammals, as would be expected given the drastically different protein complexes that assemble on telomeres in these two groups of organisms (Fig. 1). Yet surprisingly, a potential TPP1 homologue does exist in budding yeast, namely Est3^{6,7}. Est3 is not fully equivalent to TPP1; it is considerably smaller and corresponds to just the OB fold domain of TPP1. Moreover, Est3 has been shown to be a subunit of the yeast telomerase holoenzyme rather than a subunit of the telomere nucleoprotein complex¹⁸. Nevertheless, if one disregards the recruitment role of TPP1, then the effects exerted by Est3 on telomerase are remarkably similar to those of TPP1; like TPP1, Est3 can stimulate telomerase activity, and in selected assays, telomerase processivity^{19,20}. Moreover, Est3 has been shown to bind the TEN domain directly^{21,22}, and residues both in L34 and near the end of $\beta 5$ have been implicated in this physical interaction, although the mutagenic studies are not as extensive in the case of Est3^{6,7} (Fig. 2). The remarkable confluence of findings on the TPP1-TERT and Est3-Tert interactions reflect either a deeply rooted evolutionary kinship⁸ or represent a striking example of convergent evolution at the molecular level.

Just how universal is the TPP1/Est3-TERT interface? In the ciliate *Stylonychia lemnae*, the TPP1 ortholog TEBP β has also been shown to mediate telomerase recruitment, but the protein surface responsible has not been mapped²³. An important test of the extent of evolutionary conservation could be provided by analysis of *S. pombe* Tpz1-TERT interaction. Tpz1, a clear ortholog of TPP1, is known to associate with telomerase in cell extracts, but the association is dependent upon Ccq1, another shelterin component which binds *S. Pombe* Est1^{24,25}. Whether the Ccq1-Est1 interaction obviates or reinforces direct Tpz1-TERT binding is an interesting issue for future investigation. Also intriguing is the recent discovery of a putative TPP1 ortholog, Tpt1, in the ciliated protozoan *Tetrahymena*²⁶. Identifying an interact

The extent of evolutionary conservation is far from the only unresolved issue concerning TPP1-TERT association. It is almost certainly the case that this interaction would be subject to extensive regulation. For example, telomerase recruitment and telomere addition is confined to the S-phase of the cell cycle. Could this be due to cell cycle-dependent regulation of TPP1-TERT interaction? Equally interesting is the mechanism of processivity enhancement by TPP1, which also remains incompletely understood. In this regard, it may be instructive to consider what is known about Est3. In the case of the yeast protein, “separation-of-function” mutants suggest that a second function unrelated to TERT-binding is required for telomere maintenance⁷. A recent study in *Candida* further supports the existence of a cryptic and functionally important DNA-binding activity that is unmasked when Est3 forms a complex with the TEN domain²². Does TPP1 possess such an activity, and does it play a role in telomerase activity enhancement? Even though TPP1 has long been known to stimulate the DNA-binding activity of POT1, evidence for direct TPP1-DNA contacts has only very recently emerged from a single-molecule study²⁷. Indeed, the same study also suggests that TPP1 slides along DNA, a property that may enable TPP1-bound telomerase to “hang on” to DNA stably and dynamically during processive synthesis. In summary, now that the nature of the engagement at chromosomal ends is better understood, there is no shortage of topics to investigate concerning the regulation and consequences of this engagement.

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Budding Yeast *Mammals*

Figure 1. The telomere-telomerase interaction network in budding yeast and mammals

In budding yeast, the G-tails are bound by Cdc13, which recruits telomerase through an interaction with Est1. Est1 also binds Est3 to facilitate the incorporation of Est3 into the telomerase holoenzyme. Est3 stimulates telomerase activity through an interaction with the TEN domain (anchor site) of TERT. In mammals, the G-tails are bound by POT1, which is tethered to the rest of the shelterin complex by TPP1. The OB fold domain of TPP1 recruits telomerase by touching both the TEN domain and CTE of TERT, and also stimulates telomerase activity and processivity.

Figure 2. The telomerase-binding surface of TPP1 and ScEst3

Structural representations of OB fold domain of The TPP1 OB fold domain and Est3 are shown in the same orientation and in both surface and backbone representations. The TPP1 structure is from Wang et al.¹³, whereas the *ScEst3* structure is obtained by homology modeling using the (PS)² server²⁸. All residues required for telomerase interaction are shown in colors. Residues implicated by three studies are shown in red (E169 in TPP1), by two studies shown in orange (E171, L183, and E215 in TPP1; D166 in Est3), and by one study shown in yellow (D166, W167, K170, F172, R180, and L212 in TPP1; W21, D86, E114, T115, and N117 in Est3). In the backbone representations, the loop L34 and residues near the end of beta strand 5 ($\beta 5$) are shown in green and blue, respectively.