



## Review

## POT1-TPP1 telomere length regulation and disease

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## ABSTRACT

Telomeres are DNA repeats at the ends of linear chromosomes and are replicated by telomerase, a ribonucleoprotein reverse transcriptase. Telomere length regulation and chromosome end capping are essential for genome stability and are mediated primarily by the shelterin and CST complexes. POT1-TPP1, a subunit of shelterin, binds the telomeric overhang, suppresses ATR-dependent DNA damage response, and recruits telomerase to telomeres for DNA replication. POT1 localization to telomeres and chromosome end protection requires its interaction with TPP1. Therefore, the POT1-TPP1 complex is critical to telomere maintenance and full telomerase processivity. The aim of this mini-review is to summarize recent POT1-TPP1 structural studies and discuss how the complex contributes to telomere length regulation. In addition, we review how disruption of POT1-TPP1 function leads to human disease.

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**Abbreviations:** POT1, Protection of telomere 1; TPP1 also known as ACD, Adrenocortical Dysplasia Protein Homolog; TRF1, Telomere Repeat binding Factor 1; TRF2, Telomere Repeat binding Factor 2; RAP1, Repressor/Activator Protein 1; TIN2, TRF1- and TRF2-Interacting Nuclear Protein 2; TERT, Telomerase Reverse Transcriptase; TERC, Telomerase RNA; CST, CTC1, Stn1 and Ten1; SMCHD1, Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1; CTC1, Conserved Telomere Capping Protein 1; Stn1, Suppressor of Cdc Thirteen; Ten1, Telomere Length Regulation Protein; ATR, Ataxia Telangiectasia and Rad3-related Protein; ATM, Ataxia Telangiectasia Mutated protein; RPA, Replication Protein A; TSPYL5, Testis-specific Y-encoded-like protein 5; USP7, ubiquitin-specific-processing protease 7.

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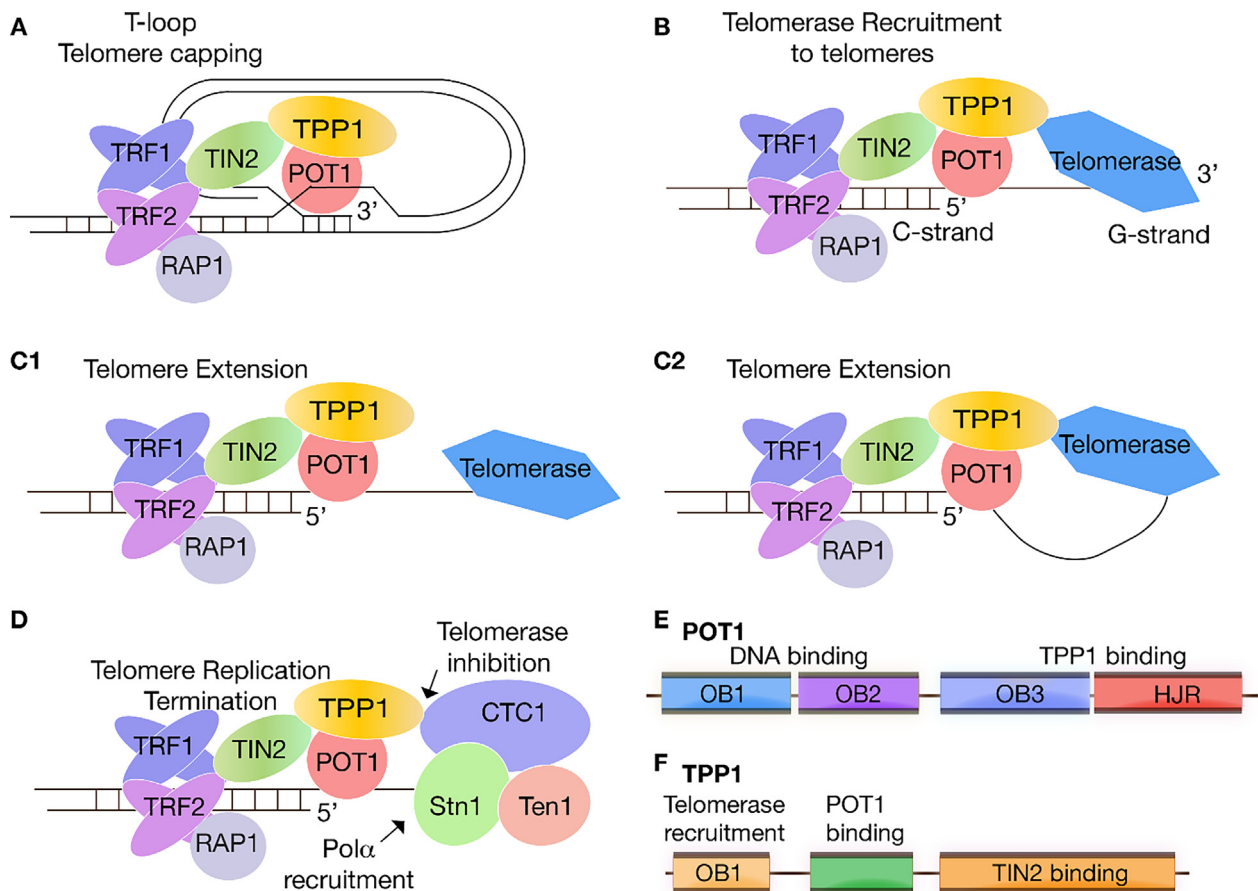
## 1. Introduction

Telomeres are tandem nucleic acid repeats, (TTAGGG in vertebrates), located at the ends of eukaryotic chromosomes [1,2]. Telomeres are replicated by telomerase, a ribonucleoprotein reverse transcriptase. Its catalytic core consists of TERT, a reverse transcriptase, and TERC, an RNA sequence containing the template for telomere replication [3]. While TERC is expressed ubiquitously, TERT expression is tightly regulated and becomes suppressed in the somatic cells of adults. Telomeres allow cells to overcome two key problems associated with linear chromosomes: chromosome end protection and end replication. Telomeres protect chromosome ends from erroneous exonuclease degradation and DNA damage response (DDR), which result in chromosome end fusions and genomic instability [4]. Telomeres also prevent gene erosion by providing a solution to the end replication problem [5–8].

Telomere length regulation is extremely critical to organismal health because telomeres that are either too short or too long may result in genomic instability (and possibly oncogenesis). Apart from telomerase, telomere homeostasis is maintained via two separate multi-subunit nucleoprotein complexes, shelterin and CST [9–12] (Fig. 1). Shelterin consists of six protein subunits: TRF1, TRF2, TIN2, TPP1 (ACD), POT1 and RAP1, which are held together in a single nucleoprotein complex by TIN2 [10,13] (Fig. 1A-D). TIN2 makes direct contacts with TRF1, TRF2 and TPP1 [14,15]. Current evidence suggests that TIN2 does not bind POT1 or RAP1 [13].

The shelterin complex has a plethora of functions associated with both telomere capping/end protection, and telomere length regulation. It has been demonstrated that shelterin “caps” telomeres to prevent the single-stranded telomeric overhangs from being recognized as DNA breaks. This process effectively suppresses ATM- and ATR-dependent DNA damage signaling pathways, thus preventing unwarranted activation of DNA repair mechanisms, at telomeres including classical non-homologous end joining (c-NHEJ) and homology-directed repair (HDR). Both of these processes are associated with deleterious chromosomal end-to-end fusions in shelterin knockdown studies [16,17]. Shelterin achieves chromosome end protection from DDR in part by forming a lasso-like structure, known as the T-loop (Fig. 1A) [18,19]. T-loops are formed through the invasion of the single-stranded G-overhang into upstream double-stranded DNA. If the G-overhang is missing or too short to form the T-loop, shelterin recruits Apollo/SNM1B to telomeres [20]. Apollo/SNM1B facilitates the formation of the G-overhang through its 5'-to-3' DNA exonuclease activity, a process that, in turn, enables telomerase-dependent telomere replication and chromosome end capping [21,22].

The subcomplex POT1-TPP1 is critical to telomere capping and telomere length regulation. Depletion of POT1 leads to catastrophic ATR-dependent DDR, significantly elongated telomeres, cell cycle arrest and is embryonically lethal [24–26]. TPP1 loss leads to reduced levels of POT1 at telomeres, diminished telomerase pro-



**Fig. 1.** Schematic of shelterin and CST activities at telomeres. A. Shelterin facilitates T-loop formation at the ends of our chromosomes. B. Telomerase is recruited by the POT1-TPP1 processivity factor. C1 and C2. Two possible mechanisms of telomerase-dependent telomere extension: telomerase may have a transient interaction with TPP1 (C1) or remain tethered to TPP1 during telomere replication (C2). D. Inhibition of telomerase dependent telomere replication by the CST complex. E. POT1 contains three OB folds (OB1, OB2, OB3) and one Holliday Junction Resolvase (HJR) domain. While the N-terminal OB1 and OB2 bind ssDNA, OB3 and the HJR domain bind TPP1. F. TPP1 contains an N-terminal OB-fold involved in telomerase recruitment to telomeres, and a POT1- and TIN2-binding domains.

cessivity, ATR-dependent DDR, and p53-dependent cell growth arrest [27–29] (Fig. 1C1, C2). Although much is known on how shelterin protects telomeres directly, recent studies have uncovered new players involved in shelterin-mediated chromosome end protection. It was recently found that SMCHD1, a protein implicated in a variety of cellular functions including DNA damage repair, promotes ATM-dependent DDR at uncapped telomeres. Current data indicates that SMCHD1 acts upstream of the ATM-dependent DDR by promoting ATM activation [23]. The recent discovery of novel shelterin partners suggests that our current understanding of telomere maintenance remains incomplete.

Telomere length is further regulated by the CST complex. CST is a heterotrimeric nucleoprotein complex composed of Stn1, Ten1 and CTC1 in higher eukaryotes [12,30] (Fig. 1D). The main functions of CST include termination of the telomerase extension reaction [31] and recruitment of pol $\alpha$ /primase to telomeres for C-strand fill-in [32–36] (Fig. 1D). CST inhibits telomerase activity potentially through sequestration of the single-stranded telomeric overhang and possibly by interfering with POT1-TPP1-dependent recruitment of telomerase to telomeres [12,37].

Although both of these complexes function primarily at telomeres, shelterin and the CST proteins may have “moonlighting” functions. The shelterin component RAP1 has been found in extratelomeric sites and it's been shown to protect against obesity in mice [38,39]. This suggests it may act as a transcriptional regulator in metabolic signaling pathways. Another example, the mammalian CST complex rescues stalled DNA replication forks at non-telomeric sites. It also promotes origin firing during replication restart and some suggest it may be responsible for loading Pol $\alpha$ /primase to these sites [40]. Nevertheless, the non-telomeric functions of the shelterin and CST complexes remain poorly understood.

Missense mutations that render shelterin and CST dysfunctional, lead to telomere length dysregulation and a host of diseases referred to as telomere syndromes or telomeropathies. These include bone marrow failure (BMF), dyskeratosis congenital (DKC), idiopathic pulmonary fibrosis (IPF), and Coats Plus (CP) [41–46]. For example, specific mutations of POT1 or TPP1 have been identified in BMF and CP, and in a number of cancers including familial melanoma, glioma, and chronic lymphocytic leukemia as well as breast, stomach and parathyroid cancers [47–52]. Structural data is now beginning to shed light on precisely how such mutations lead to disease [53–55].

This review aims to evaluate recent structural and functional studies of POT1-TPP1 and outline our current understanding of its role in telomere maintenance and disease.

## 2. POT1-TPP1 structure

POT1 consists of four domains: three OB folds and a Holliday junction resolvase domain (HJRD) (Fig. 1E). It is the only shelterin

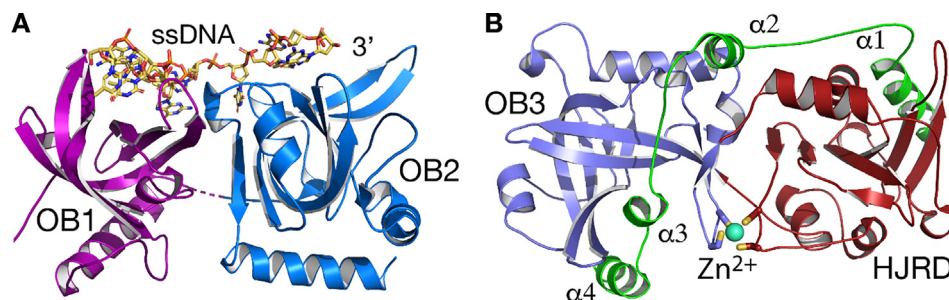
component that binds single-stranded telomeric DNA (Fig. 1A). The two N-terminal OB folds bind the single-stranded telomeric overhang [56,57] while the C-terminal portion binds TPP1 [54] (Figs. 1E, F and 2A, B). Interestingly, a 4-cysteine zinc binding cluster (C382, C385, C503, C506), located between the two C-terminal domains, appears to stabilize the extended conformation adopted by this portion of POT1 (Fig. 2B). It is worth noting that the POT1 HJRD, unlike other known holliday junction resolvase domains, does not bind double stranded DNA (Fig. 2B) [54,58].

POT1-TPP1 binding is mediated by the C-terminal OB-fold (OB3) and HJR domains of POT1 (PDB ID: 5UN7) (Fig. 2B). Both domains make extensive, primarily hydrophobic, interactions with the TPP1 central peptide, residues 266–332, which form an extended coil with four equally distributed  $\alpha$ -helices ( $\alpha$ 1–4). The TPP1 peptide straddles the two POT1 domains, stretching across the length of the whole molecule. Contacts between TPP1 and the POT1 HJRD are mediated primarily by helix  $\alpha$ 1 of TPP1. Helix  $\alpha$ 2 binds at the interface of the POT1 OB3 and HJRD, while helices  $\alpha$ 3 and  $\alpha$ 4 bind the POT1 OB3 fold (Fig. 2B). Although most of the POT1-TPP1 contacts involve the four helices of TPP1, limited contacts do occur throughout the loop regions connecting these four helices.

TPP1 contains, in addition to POT1-binding domains, an N-terminal OB fold and a TIN2 binding domain at its C-terminus (Fig. 1F). The TPP1 N-terminal OB fold (PDB ID: 2I46), comprising residues 86–250, contains the ‘TEL-patch’ - a group of surface residues involved in the recruitment of telomerase to telomeres and stimulation of telomerase processivity [59]. The N-terminal tail of 86 residues is not conserved across organisms but is important for stimulating telomerase processivity in primates [29,60,61]. The C-terminal region of TPP1 contains a short sequence of residues (510–540) shown to be necessary and sufficient for TIN2 binding [62]. In fact, recent evidence shows that TIN2-TPP1 binding leads to increased telomerase activity, suggesting TIN2 cooperates with the POT1-TPP1 complex to stimulate telomerase processivity [15].

## 3. The POT1-TPP1 complex is highly specific for single-stranded telomeric DNA

POT1 binds the telomeric overhang with high affinity in a specific manner, a process mediated by its two N-terminal OB folds. The OB fold nearest the N-terminus binds six-nucleotides while OB2 binds 3 nucleotides. POT1 ssDNA binding, positions the 3'-end of the DNA within the canonical binding pocket of OB2, thus protecting it from exonuclease degradation as well as from access by telomerase for telomere replication. The affinity of human POT1 for telomeric DNA is in the range of 10–20 nM [54,56,57] and is enhanced by TPP1 binding. This could be attributed to the fact that



**Fig. 2.** POT1 structures in complex with TPP1 or DNA. A. POT1 N-terminal 2 OB folds (OB1 and OB2) in complex with single-stranded telomeric DNA (PDB: 1XJV) B. POT1 C-terminal OB fold (OB3) and holliday junction resolvase domain (HJRD) in complex with TPP1 (green). The cysteine cluster is coordinated to a  $Zn^{2+}$  ion shown as blue sphere. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

TPP1 tethers POT1 to the shelterin complex and therefore places POT1 in proximity to the telomeric overhang.

The ability of POT1 to bind single-stranded telomeric DNA with high affinity and specificity also allows it to discriminate against TERRA, which would deplete POT1 binding to the telomeric overhang and risk telomere exposure. TERRA is the single-stranded RNA transcribed from telomeric DNA and therefore could contain multiple POT1 binding sites [63–65]. The specificity of POT1 for ssDNA over ssRNA is attributed to the substitution of the second deoxythymidine nucleotide in the 5'-TTAGGG-3' repeat for ribouridine in TERRA [65]. Interestingly, TPP1 binding to POT1 enhances POT1's specificity for DNA over RNA [65].

#### 4. POT1 suppresses DNA damage response

The ends of eukaryotic chromosomes can be recognized as DNA strand breaks. DNA breaks induce cell cycle arrest and activate DNA repair via ATM- and ATR-dependent signaling pathways, which involve NHEJ and HDR mechanisms [1,4]. NHEJ results in chromosome end-to-end fusions [1,16,66–69] while HDR results in telomere sister chromatid exchanges, loss of telomeric DNA, and the formation of unstable telomere-free chromosome ends [70,71]. Eukaryotic chromosome ends avoid recombination mechanisms through the shelterin mediated formation of the T-loop. T-loop formation is facilitated by the shelterin complex, and most recently it was shown that TRF2 alone is sufficient for this process [1,16,72].

T-loop formation involves the invasion of the single-stranded overhang into upstream double stranded telomeric DNA. Apollo, recruited to telomeres by TRF2 [21], uses its exonuclease activity to process telomeres immediately after replication, generating the 3' single-stranded overhang. In doing so, it promotes T-loop formation as well as telomerase-dependent telomere replication. It also eliminates blunt-end telomeres (that activate DDR) and promotes POT1 binding [73] thus preventing ATR-dependent DDR [74]. ATR signaling is activated by the binding of the replication protein A (RPA) heterotrimer to the single-stranded DNA [75]. POT1 suppresses ATR signaling by directly outcompeting RPA for binding to the telomeric overhang [76]. TPP1 facilitates POT1 recruitment to telomeres and increases its affinity for ssDNA tenfold [29], which could explain why TPP1 knockdown results in ATR-dependent DDR.

#### 5. POT1-TPP1 is a telomerase processivity factor

The POT1-TPP1 complex plays a critical role in telomere length regulation. POT1 inhibits telomere extension by sequestering the telomeric overhang, thus preventing access of telomerase to telomeres. POT1-TPP1 together facilitate telomere extension by recruiting of telomerase to telomeres for DNA synthesis [29,59,77]. POT1-TPP1 therefore acts like a switch either promoting or inhibiting telomerase activity. It is worth noting that short telomeres are preferentially extended by telomerase [78]. Long telomeres are coated with a large number of POT1-TPP1 complexes which render the telomeric overhang inaccessible to telomerase thus inhibiting telomerase-dependent telomere extension [79]. These results may suggest a model whereby POT1-TPP1 recruits and stimulates telomerase recruitment during late S-phase triggering telomere extension. After telomere elongation, a switch may occur leading to telomerase inhibition.

POT1-TPP1 dependent telomerase recruitment to telomeres involves contacts between the TEL-patch and the N-terminal domain of TERT (TEN) [77]. Interestingly, recent evidence shows that the ubiquitin-specific-processing protease 7 (USP7) also interacts with the same TPP1 N-terminal OB fold. The proximity of the USP7 and telomerase binding sites on TPP1 suggests a possible reg-

ulatory mechanism of telomerase access to telomeres [80]. The facts that POT1-TPP1 binding to ssDNA decreases the dissociation of the ssDNA from telomerase [81] and that the presence of POT1-TPP1 during telomere replication increases the rate of telomerase translocation provide additional evidence supporting this mechanism [81]. Another recent study shows that TIN2 cooperates with POT1-TPP1 to stimulate telomerase processivity [15]. This finding is not surprising since TIN2 interacts with TPP1 and TPP1 binds POT1, thus TIN2 assists with the localization of the POT1-TPP1 complex to telomeres [15]. It remains to be seen whether TIN2 also increases telomerase processivity by stabilizing the POT1-TPP1 interaction with TERT.

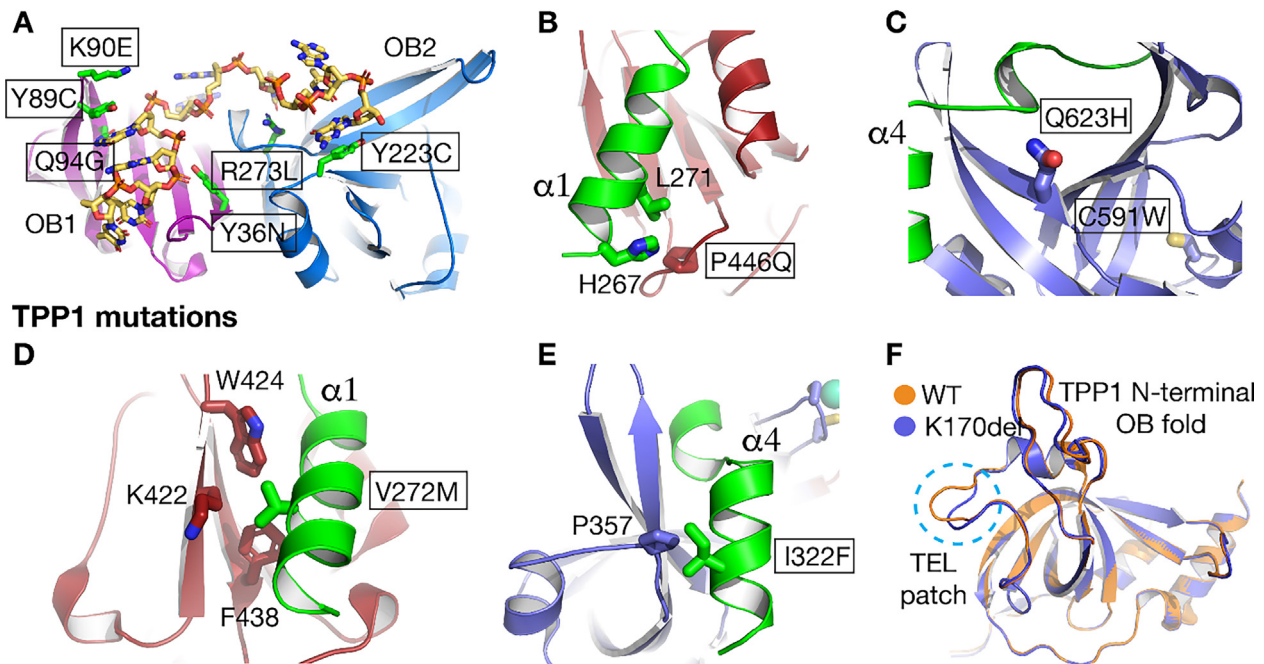
#### 6. POT1-TPP1 and cancer

Telomere elongation is crucial to the continued division and survival of cancer cells [82–84]. In somatic cells, telomerase is inactive and telomeres get shorter with each round of cell division, eventually triggering replicative senescence [85–87]. Cells that have lost tumor suppressor pathways, such as p53, may bypass senescence and continue proliferating, further shortening telomeres to a critical length [88,89]. At this 'crisis' stage, cells exhibit chromosome recombination and genomic instability, leading to extensive cell death. Rarely, a cell may escape crisis by reactivating telomerase or, in some cases, via homologous recombination based telomere replication, known as the ALT mechanism [90,91].

Mutations in the shelterin complex that lead to telomere dysfunction and dysregulation are prevalent in cancer [92]. POT1-TPP1 mutations are implicated in multiple cancer types including melanoma, glioma, and chronic lymphocytic leukemia (CLL) [51,52,93–96]. Recent studies have shown that POT1 mutations are also found in breast, stomach and parathyroid cancers [50,97,98]. Missense mutations at the N-terminal two OB folds of POT1, such as Y36N, Y89C, Q94G, Y223C, and R273L, render POT1 unable to bind telomeric ssDNA [49,51,52] (Fig. 3A). The crystal structure of POT1 with telomeric ssDNA shows Y89 and Y223 engaged in pi-stacking interactions with guanine nucleotides of the telomeric DNA that would be disrupted by mutation to a non-aromatic residue (PDB: 1XJV – Fig. 3A). Y36, Q94, and R273 make electrostatic interactions with the telomeric DNA. The much shorter, non-polar or hydrophobic side chains of the mutant Y36N, Q94G, and R273L lead to complete loss of interaction with the ssDNA (Fig. 3A). The biological consequences of CLL-associated Y36N and Y223C were observed in human fibrosarcoma cells expressing the POT1 variants. These cells exhibited chromosomal abnormalities associated with telomere uncapping, such as irregular telomere length, end-to-end fusions, and telomere fragility [49].

To this end, research from our lab and others (PDB ID: 5UN7, 5H65 – Fig. 3B and C) shows how several of the reported cancer mutations that localize to the C-terminal portion of POT1 (POT1C) disrupt the POT1-TPP1 complex [54]. Several of these mutations directly alter the ability of POT1 to bind TPP1, while others are likely to disrupt the fold of the protein. In our study, we specifically interrogated the POT1C cancer mutations L343F, P446Q, P475L, R477T, A532P, I535F, C591W and Q623H found in patients with either familial glioma, melanoma, or CLL [49,52]. Three of these mutations, P446Q, C591W, and Q623H (Fig. 3B and C), exhibited a reduction in POT1C-TPP1 binding, consistent with the structural data showing that these residues made direct contacts with TPP1. Biochemical studies show that these mutant proteins bind telomeric DNA with lower affinity than the wild type POT1 [79]. A decrease in DNA binding is expected to translate into defective telomere capping and an increase in DDR. In addition, defective DNA binding will most likely lead to an increase in telomere length due to persistent telomere replication by telomerase. Long

## POT1 mutations



**Fig. 3.** Structures of POT1-DNA and POT1-TPP1 showing selected mutations implicated in cancer and telomere syndromes. A. Structure of the POT1-N-terminal OB folds in complex with single-stranded telomeric DNA (PDB ID: 1XJV). The telomere syndrome mutation K90E is shown in stick. Mutations described in detail in the text are denoted by boxes. B. Holliday Junction resolvase domain (HJRD – red) of POT1 in complex with helix  $\alpha 1$  of the TPP1 peptide (green). The cancer mutation P446Q and the TPP1 interacting residues H267 and L271 are shown in stick. C. POT1 (OB3) (blue) in complex with TPP1 (green) showing the cancer mutations C591W and Q623H. C591 is buried and contributes to the fold of the protein. In the C591W mutant, the much larger tryptophan side chain would perturb the fold of the OB fold. Q623 makes direct contact with the amide backbone of the TPP1 peptide. D. POT1 (HJRD) (red) in complex with TPP1 (green) showing TPP1 V272 and its interactions with POT1 K422, W424 and F438. E. I322 interacts with the P357 of the POT1 (OB3). F. Overlay of wild type (WT – PDB ID: 2I46) TPP1 and K170 deletion (K170del PDB ID: 5I2Y and 5I2X) of the N-terminal TPP1 OB fold. The region of the K170del in the TPP1 structure is highlighted with a blue dashed circle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

telomeres are fragile and tend to collapse, generating telomere free ends. Cells with telomere free ends are prone to senescence, apoptosis and, in rare cases, genomic instability leading to cancer.

POT1 is also implicated in the ALT mechanism utilized by cancer cells (primarily osteosarcomas) to maintain their telomeres through homologous recombination [99–102]. Promyelocytic leukemia (PML) nuclear bodies cluster around ALT telomeres and are directly involved in telomere recombination [103,104]. In addition, shelterin and DNA repair proteins (TRF1, TRF2, TIN2, RAP1, MRE11, RAD50, NBS1) are required for the formation of ALT-associated PML bodies (APBs) at telomeres [105]. Testis-specific Y-encoded-like protein 5 (TSPYL5) was recently identified as another component of the PML body crucial to the viability of ALT<sup>+</sup> cells [106]. TSPYL5 prevents POT1 proteasomal degradation in ALT cells by inhibiting USP7-dependent POT1 deubiquitylation [106]. The fact that TSPYL5 is essential to POT1 protection and the survival of ALT<sup>+</sup> cells make it a promising target for ALT<sup>+</sup> cancer therapies [106].

Regarding TPP1 mutations, a study on familial melanoma found that mutations V272M and I322F located in the POT1-binding domain (Fig. 3D and E) were also associated with the disease [93].

## 7. POT1-TPP1 and telomere syndromes

POT1 mutants implicated in telomere syndromes are defective in efficient chromosome end capping and telomerase recruitment to telomeres. Both of these defects lead to significantly short telomeres and, in some cases, telomere free ends. In cells with high turnover, such as bone marrow, short telomeres lead to premature senescence and are associated with telomeropathies.

There are four known mutations within the POT1-TPP1 complex implicated in the telomere syndromes BMF, IPF, and CP. These include the TPP1 A72E, K170E and K170 $\Delta$  [107–109] and the POT1 K90E and S322L mutants [110]. A72E is located at the N-terminal 86 residues of TPP1. Structural data on this region of TPP1 is lacking, however we can speculate that A72E affects telomerase processivity in primates. K170 is located away from the canonical binding pocket of the N-terminal TPP1 OB fold and forms part of the TEL-patch (Fig. 3F). The K70 deletion (K70 $\Delta$ ) distorts the location and organization of adjacent amino acids (Fig. 3F) resulting in reduced telomerase processivity at telomeres [107].

The POT1 K90E mutation (Fig. 3A) localizes to the very N-terminal OB fold of POT1 and at the tip of the indentation comprising the canonical OB pocket. Although this pocket is involved in DNA binding, K90 is not (Fig. 3A). Although K90E does not appear to affect POT1-TPP1 or POT1-ssDNA binding, one study found that it did affect CST complex localization to telomeres [111]. This observation raises questions about a possible cooperation between POT1-TPP1 and the CST complex. In mice, the POT1b paralog recruits the CST complex to telomeres [24,73,112,113] and, in humans, interaction between POT1 and the CST complex has been reported [31,114]. However, direct binding between POT1 and CST has yet to be shown and the details of this mechanism remain unclear.

The POT1 mutant S322L is located at the intersection of the DNA and TPP1 binding domains of POT1 but its precise role remains unknown [115]. Identified in siblings with CP, the POT1 S322L mutant was expressed at normal levels, bound TPP1, and suppressed ATR-dependent DDR. However, the S322L POT1 mutant failed to regulate telomerase, resulting in extended telomeric overhangs, defective C-strand fill-in, and telomere truncations [115].

The current hypothesis is that mutations like S322L may interfere with possible cooperation between shelterin and CST [115], presumably needed to maintain a healthy length of telomeres. Structural and secondary structure prediction analysis suggest that S322L is located in the linker connecting the N- and C-terminal portions of POT1. It is common for unstructured loop regions of proteins to contain serine or threonine residues that are post-translationally modified. One, possibility is that S322 is a phosphorylation site and that the leucine mutant disrupts this process.

## 8. Summary and outlook

Apart from telomerase, telomere length regulation has been attributed to two major protein complexes, shelterin and CST. In depth understanding of the function of these complexes may hold the key to understanding and halting disease progression. A plethora of work from multiple labs has shed light into the functions of POT1-TPP1. Despite the wealth of data, there are still significant questions that remain to be addressed. For example, we still do not fully understand how the full length POT1-TPP1-DNA complex assembles at telomeres. Structural elucidation of the full length complex will be invaluable to our understanding of how these two proteins carry out their functions. Similarly, evidence now suggests that there is crosstalk between shelterin and CST. Undertesting how these two complexes cooperate to regulate telomerase and telomere homeostasis will be invaluable. Finally, understanding how the POT1-TPP1 mutations lead to cancer and telomere syndromes will be instrumental in our effort to identify and tailor therapies for these human diseases.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Author statement

The review was written by TA and ES with contributions from SP.

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