# Finding the end: recruitment of telomerase to telomeres

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Abstract | Telomeres, the ends of linear eukaryotic chromosomes, are characterized by the presence of multiple repeats of a short DNA sequence. This telomeric DNA is protected from illicit repair by telomere-associated proteins, which in mammals form the shelterin complex. Replicative polymerases are unable to synthesize DNA at the extreme ends of chromosomes, but in unicellular eukaryotes such as yeast and in mammalian germ cells and stem cells, telomere length is maintained by a ribonucleoprotein enzyme known as telomerase. Recent work has provided insights into the mechanisms of telomerase recruitment to telomeres, highlighting the contribution of telomere-associated proteins, including TPP1 in humans, Ccq1 in *Schizosaccharomyces pombe* and Cdc13 and Ku70–Ku80 in *Saccharomyces cerevisiae*.

Dyskeratosis congenita
A rare genetic disease, characterized by abnormal skin pigmentation, nail dystrophy and mucosal leukoplakia, caused by telomere shortening owing to mutations in telomerase core components (telomerase reverse transcriptase (TERT) and a template-containing RNA component (TR)), telomerase accessory factors (such as dyskerin, telomerase Cajal body protein 1 (TCAB1), NHP2 and nucleolar protein 10 (Nop10)), or the shelterin protein TRF1-interacting nuclear protein 2 (TIN2).

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The proper maintenance of linear chromosomes requires countering two biological problems: the chromosome end-protection problem and the chromosome end-replication problem. The ends of linear chromosomes, such as those in eukaryotes, must be distinguished from broken DNA ends that require repair. In the absence of such a distinction, linear chromosomes are prone to illicit DNA end-joining and recombinational events that result in deleterious consequences such as end-to-end chromosomal fusions. Such reactions define the chromosome end-protection problem[1]. The gradual loss of sequence information at the extreme end of chromosomes owing to incomplete replication by DNA polymerases (which only synthesize DNA in the 5′ to 3′ direction and are unable to fill in the gap left behind by the 5′ most RNA primer) defines the chromosome end-replication problem[2].

Telomerase, a unique enzyme that contains telomerase reverse transcriptase (TERT) and a template-containing RNA component (TR), facilitates the solution of both chromosome end-related problems. By synthesizing multiple tandem repeats of DNA (called telomeric DNA) encoded by its RNA template, telomerase compensates for the erosion of DNA ends during replication and provides the docking sites for telomeric proteins that bind specifically to the ends of chromosomes to distinguish them from broken DNA ends (FIG. 1). Although most protists, fungi, plants and animals use a telomerase, *Drosophila* species use a retrotransposon mechanism to overcome chromosome end protection and end replication[3].

The action of telomerase is required for the survival of continuously dividing cells such as those of unicellular eukaryotes. In mammals, telomerase is active in the germ line and in stem cells, but its expression in somatic cells may lead to or predispose to cancer. In the absence of a telomere maintenance mechanism, the telomeres of such proliferating cells shrink to the point when the cells stop dividing (replicative senescence). Telomerase has also been linked to ageing, as telomere loss may result in tissue atrophy, stem cell depletion and deficient tissue regeneration[4]. In humans, loss-of-function mutations in either TERT or TR have been associated with dyskeratosis congenita and cases of aplastic anaemia and pulmonary fibrosis[5].

Since its discovery, telomerase has been studied at the level of structure, function, biology and medicine[6–9]. In this Review, we focus on telomerase recruitment to telomeres, addressing the conceptual dilemma of how a chromosome end bound tightly by telomeric end-capping proteins also allows telomerase action. We describe, compare and contrast the mechanisms of telomerase recruitment in the human, budding yeast and fission yeast systems.

## Telomeres

***Telomeric DNA.*** Telomeric DNA is typically composed of multiple repeats of a short sequence that is often G/T-rich in the strand that extends 5′ to 3′ towards the chromosome end. The length and sequence of each DNA repeat are encoded by the telomerase RNA template (see below). The number of repeats per telomere varies widely among species, from a fixed 4.5 repeats of $G_4T_4$ in the ciliate *Oxytricha nova* to ~350–500 bp in *Saccharomyces cerevisiae*, and variable numbers of GGTTAG repeats encompassing 10–15 kb in humans and 20–50 kb in certain mouse and rat species[1].

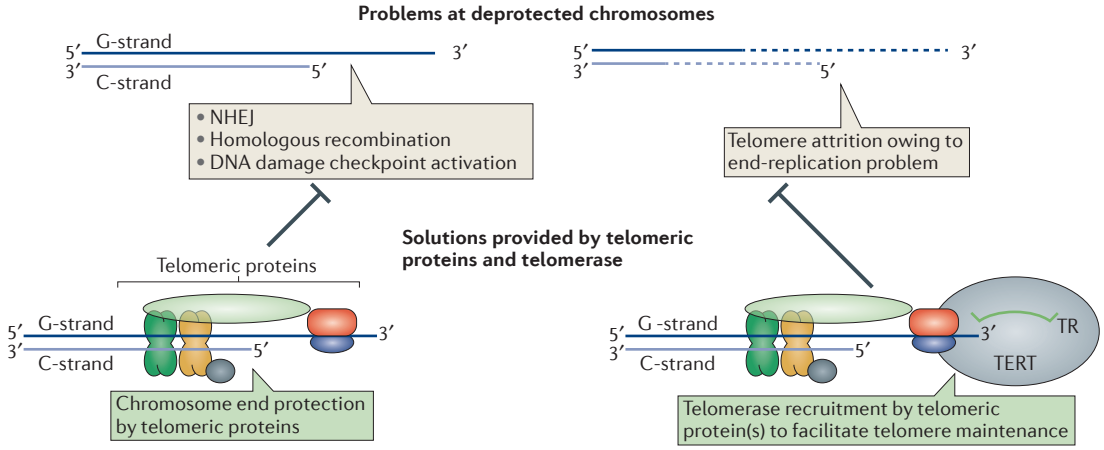



Figure 1 | **Chromosome end protection versus telomerase recruitment and action.** Deprotected, protein-free telomeric DNA (the G-strand and C-strand duplex is shown at the top) could be a target of illicit DNA end-joining and cell cycle checkpoint activation events at the telomere. By specifically binding telomeric DNA, telomeric proteins protect chromosome ends from such deleterious processes. DNA sequences are lost at the ends of chromosomes owing to incomplete replication by DNA polymerases. Telomerase is recruited to telomeres via interaction with specific telomeric proteins to extend chromosome ends and thereby counters DNA loss from incomplete replication. In the absence of telomerase recruitment or action, telomeric DNA shortens, ultimately leading to replicative senescence. Furthermore, shorter telomeric DNA results in a loss of bound telomeric proteins, which could result in deprotected chromosome ends. NHEJ, non-homologous end-joining; TERT, telomerase reverse transcriptase; TR, template-containing RNA component.

Telomeric DNA is mostly double-stranded (dsDNA) but ends with a single-stranded telomeric G-rich 3′ tail that serves two key functions: providing primers for extension by telomerase; and binding specific telomeric proteins to cap chromosome ends and ensure genome stability[1] (FIG. 2) (see below). Alternative structures formed by telomeric single-stranded DNA (ssDNA) include G-quadruplexes[10,11] and T-loops[12] (BOX 1).

***Proteins that cap telomeric dsDNA and ssDNA.*** The mammalian chromosome end is capped by a protein complex known as shelterin that is composed of six proteins: telomeric repeat-binding factor 1 (TRF1; also known as TERF1), TRF2 (also known as TERF2), repressor and activator protein 1 (RAP1; also known as TERF2IP), TRF1-interacting nuclear protein 2 (TIN2; also known as TINF2), protection of telomeres 1 (POT1) and TPP1 (also known as ACD). *Schizosaccharomyces pombe* telomeres contain a similar complex that contains Taz1 (a TRF homologue), Rap1, Pot1, Tpz1, Poz1 and Ccq1 (coiled-coil quantitatively-enriched 1). By contrast, *S. cerevisiae* does not have a shelterin-like complex, but instead contains chromosome end-binding proteins such as Rap1, Cdc13, Stn1 and Ten1 (REF. 9) (see below).

A dedicated set of proteins binds the double-stranded segment of telomeric DNA in mammals and in yeast. The mammalian proteins are TRF1 and TRF2, each of which contains a TRF homology (TRFH) domain that allows homodimerization[13] and a DNA-binding Myb domain that provides high-affinity binding to telomeric dsDNA[14–16] (FIG. 2a,b). Taz1 (REF. 17) binds telomeric dsDNA in *S. pombe*. Although no obvious structural homologues of TRF or Taz1 exist in *S. cerevisiae*, Rap1 binds telomeric dsDNA and certain internal sequences in this organism[18]. The carboxy-terminal portion of Rap1 binds Rap1-interacting factor 1 (Rif1)[19] and Rif2 (REF. 20) to form the telomeric dsDNA end-protection complex. Rap1 homologues in humans and *S. pombe* associate with telomeres, but these proteins lack the ability to bind DNA directly and are retained at telomeres via their interaction with TRF2 and Taz1, respectively[21,22].

The single-stranded telomeric G-rich 3′ tail of telomeres is protected from illicit DNA end-joining events by telomere-specific DNA-binding proteins in ciliated protozoa, yeast and mammals. The first discovered protein was a heterodimer comprising telomere end-binding protein-α (TEBPα) and TEBPβ in the ciliate *Oxytricha nova*, and the crystal structure of this complex bound to telomeric ssDNA revealed that the chromosome tail is essentially buried by the protein[23]. The OB (oligonucleotide- and oligosaccharide-binding)-fold domains in TEBPα–TEBPβ mediate DNA recognition and turned out to be a general feature of telomere end-capping proteins.

POT1 and TPP1 are evolutionarily related to TEBPα and TEBPβ, respectively. Indeed, mammalian POT1 binds with high affinity and specificity to the single-stranded telomeric G-rich 3′ tail using two OB-fold elements that collectively form the DNA-binding domain (DBD) of POT1 (FIG. 2c), and this interaction is enhanced by ternary complex formation with TPP1 (REFS 24–30). A centrally positioned domain of TPP1 binds POT1 (FIG. 2d), and this interaction is essential for the recruitment of POT1 to telomeres[31–34]. A recent electron microscopy study has shown that POT1–TPP1 can completely coat long stretches of telomeric ssDNA to form compact, ordered structures, which suggests that such structures may exist *in vivo*[35].

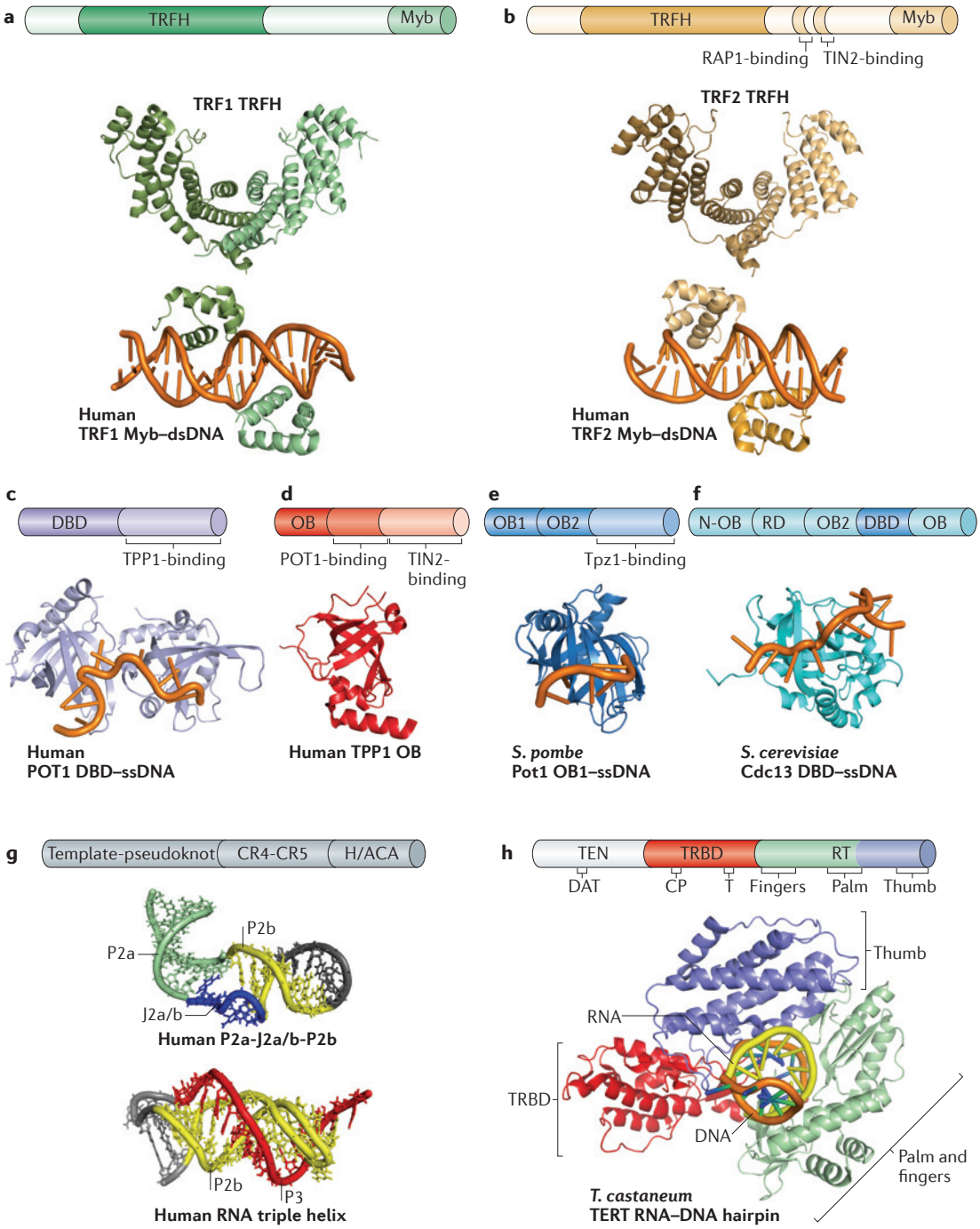
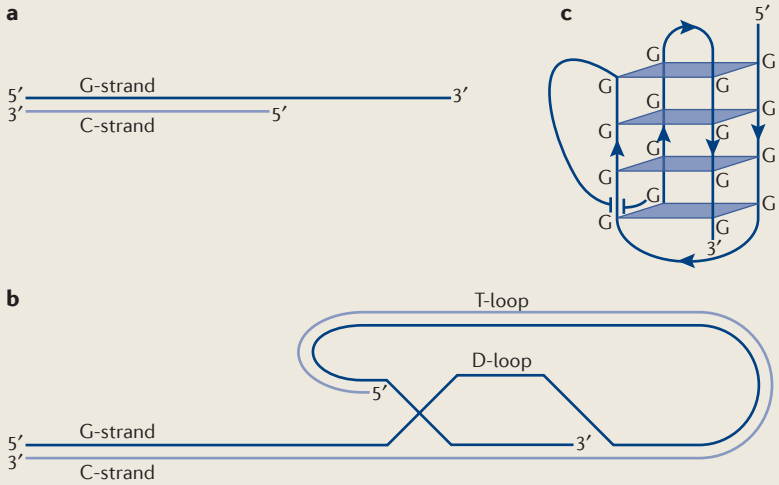



Figure 2 | **Structures of telomeric proteins and telomerase components.** **a** | Crystal structure of the TRF homology (TRFH) domain (Protein Data Bank identifier (PDB ID): 1H6O) and double-stranded DNA (dsDNA)-bound Myb domain of human telomeric repeat-binding factor 1 (TRF1; PDB ID: 1W0T). **b** | Crystal structure of the TRFH domain (PDB ID: 1H6P) and dsDNA-bound Myb domain of human TRF2 (PDB ID: 1W0U). **c** | Crystal structure of human protection of telomeres 1 (POT1) bound to single-stranded DNA (ssDNA) via its DNA-binding domain (DBD). The DBD is composed of two OB (oligonucleotide- and oligosaccharide-binding)-fold domains (PDB ID: 1XJV). **d** | Crystal structure of the amino-terminal OB-fold domain of human TPP1 (DB ID: 2I46). **e** | Nuclear magnetic resonance (NMR) structure of the ssDNA-bound DBD of *Saccharomyces cerevisiae* Cdc13 (PDB ID: 1S40). **f** | Crystal structure of ssDNA-bound structure of the first OB-fold domain (OB1) of *Schizosaccharomyces pombe* Pot1 (PDB ID: 1QZG). **g** | NMR structures of template-pseudoknot fragments of human template-containing RNA component (TR) with the secondary structural elements indicated (PDB IDs: 2L3E (top) and 2K96 (bottom)). The 'P' and 'J' elements are RNA double-helical paired regions and joining segments. **h** | Crystal structure of *Tribolium castaneum* telomerase reverse transcriptase (TERT) with a hybrid RNA–DNA hairpin representing a putative telomerase–primer–template ternary complex (PDB ID: 3KYL). CP, ciliated protozoan motif; DAT, dissociates activities of telomerase; N-OB, N-terminal OB-fold domain; RD, recruitment domain; RT, reverse transcriptase; T, telomerase-specific motif; TIN2, TRF1-interacting nuclear protein 2; TRBD, TERT RNA-binding domain.

Box 1 | **Alternative telomeric DNA structures**




Telomeric DNA ends have a single-stranded G-rich 3′ tail that is bound by telomeric proteins and that is a substrate for telomerase (see the figure, part **a**). However, alternative structures can be adopted. Telomere loops (T-loops) are one such example. They are lariat-like configurations that arise by strand invasion of the telomeric single-stranded DNA (ssDNA) tail into the upstream telomeric double-stranded DNA (dsDNA) (see the figure, part **b**). Electron microscopy of photocrosslinked telomeric DNA first showed the existence of T-loops[12], and electron microscopy studies on native (uncrosslinked) telomeric chromatin from chicken erythrocytes and mouse lymphocytes revealed similar structures. T-loop formation is not thermodynamically favourable, so it is not surprising that protein factors have been implicated in T-loop assembly[12,165]. Because the T-loop would be a highly protected, inert structure, future work is needed to address the question of how T-loops are opened up to allow telomerase to access the chromosome end.

Another common structure is G-quadruplex DNA (see the figure, part **c**). This configuration is built from G-quartets, which are square, planar arrays of four Gs that are hydrogen-bonded by Hoogsteen base-pairing. The atomic structures of both parallel and antiparallel G-quadruplexes have been solved. Intramolecular G-quadruplexes form spontaneously *in vitro*, as long as the DNA sequence contains at least four blocks of Gs and the ionic conditions are appropriate. Given the thermodynamic imperative for G-quadruplex formation, it is perhaps not surprising that telomeric proteins can catalyse G-quadruplex folding and unfolding[166–168]. All of the known chromosome end-capping proteins bind unfolded telomeric DNA and ignore the G-quadruplex[169,170], which provides a strong argument that most telomeric DNA in cells is not folded into quadruplexes. However, there is evidence that these structures occur *in vivo*[171]. They serve to limit telomerase extension *in vitro*[166,172,173], and this could certainly be useful *in vivo*. Another possibility is that they provide an emergency solution to the end-protection problem when capping proteins have been displaced.

A big challenge to interrogate the function of G-quadruplex and T-loop DNAs is that it is difficult to 'knock out' a DNA structure using genetics and to determine the phenotype. Although G-quadruplexes can be knocked out by changing the telomerase template sequence, the resultant novel repeats do not bind telomeric proteins, so the phenotypes are indirect.

Hoogsteen base-pairing
Non-Watson–Crick pairing that involves the N7 atom of a purine and is important in stabilizing triplex and quadruplex nucleic acid structures.

*S. pombe* Pot1–Tpz1 (FIG. 2e) is functionally homologous to the mammalian POT1–TPP1 complex in that it binds telomeric ssDNA with high affinity and specificity[24,36–38]. However, whereas human POT1 binds telomeric ssDNA as a monomer using two amino-terminal tandem OB-fold domains[27], *S. pombe* Pot1 can bind 2.5 repeats of the *S. pombe* telomeric DNA sequence $[(GGTTAC)_2]GGT$ as a monomer[39] or two repeats $[(GGTTAC)_2]$ as a dimer[38].

Budding yeast also cap their telomeres with OB-fold domain-containing proteins, but they are evolutionarily distinct from POT1–TPP1. The single-stranded telomeric G-rich 3′ tail in *S. cerevisiae* is bound by the CST (Cdc13–Stn1–Ten1) complex[40–42]. Cdc13 binds telomeric ssDNA with high affinity and specificity using a centrally positioned OB-fold domain[40,43–45] (FIG. 2f) and homodimerizes using an N-terminal OB-fold domain[46]. Thus, the CST complex may exist as a dimer. Because all CST members are composed mainly of OB-fold domains analogous to the replication protein A (RPA) heterotrimer, the CST complex has been proposed to be a telomere-specific RPA[47].

***The shelterin complex.*** The mammalian shelterin complex (TABLE 1; yeast homologues are also indicated) brings together telomeric ssDNA- and dsDNA-binding proteins by bridging protein partners to protect the natural ends from being recognized as sites of DNA damage. TIN2 is a crucial bridging component that not only links TRF1 and TRF2 but also connects TRF1 and TRF2 to POT1–TPP1 (REFS 48–52) (FIG. 3a). TIN2 uses an N-terminal region to bind TRF2, and a FxLxP amino acid motif at the C-terminus of TIN2 facilitates TRF1 binding[50,53]. Bridging of TRF1 and TRF2 by TIN2 is crucial for the assembly of a complete shelterin complex because TRF1 does not heterodimerize or otherwise interact with TRF2 (REF. 13). TIN2 uses a region in its N-terminal domain to bind to the C-terminal portion of TPP1 to recruit it to telomeres[33,51,52] (FIG. 2d). Because the TRF2- and TPP1-binding sites reside in the N-terminal domain of TIN2, TIN2 binding to TRF2 and TPP1 could potentially occur in a mutually exclusive manner, but data showing stabilization of TRF1–TIN2–TRF2 in the presence of TPP1 support a model whereby the four shelterin components coexist in a single complex[54].

Although the various binary interactions that join the six shelterin complex components to one another are known, the number and identity of shelterin subcomplexes are not established. Removal of mammalian TRF1 led to a depletion of TRF2 from telomeres, an observation that is unanticipated if TRF1 and TRF2 were present in mutually exclusive complexes[50,55]. Moreover, co-immunoprecipitation and gel-filtration analysis have shown that TRF1 and TRF2 are part of the same complex that also contains RAP1, TIN2, TPP1 and POT1 (REF. 55). However, similar studies have also shown the presence of both stable TRF2–RAP1 and TRF2–RAP1–TIN2–POT1 complexes in cell extracts, which suggests that subcomplexes of shelterin might coexist in cells along with the complete complex[50]. Furthermore, a study that used quantitative immunoblotting to determine the stoichiometry of shelterin complex components in human cells showed that TRF1, TRF2, RAP1 and TIN2 are present at comparable molar equivalents, but POT1 and TPP1 are present at lower levels[56]. Thus, some TRF1–TRF2–RAP1–TIN2 may not be associated with POT1–TPP1.

Regardless of whether the shelterin complex functions as one complex or as multiple subcomplexes, its importance in protecting chromosome ends cannot be understated. Knockdown of individual components of this complex leads to deprotection of chromosome ends and the activation of DNA damage response pathways at telomeres[34]. A recent study using conditional TRF1–TRF2

Table 1 | **Telomere and telomerase components in humans, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe***

| Humans | *Schizosaccharomyces pombe* | *Saccharomyces cerevisiae* | Function |
|---|---|---|---|
| ***Telomeric proteins*** | | | |
| TRF1 | Taz1 | NA | Bind dsDNA |
| TRF2 | Taz1 | NA | Bind dsDNA |
| RAP1 | Rap1 | Rap1 | *S. pombe* Rap1 binds dsDNA |
| TIN2 | Poz1 | NA | Bridge telomeric proteins via protein–protein interactions |
| POT1 | Pot1 | NA | Bind ssDNA |
| TPP1 | Tpz1 | NA | Enhance ssDNA binding of POT1; human TPP1 recruits telomerase |
| NA | Ccq1 | NA | Recruits telomerase |
| CTC1 | NA | NA | Part of a telomeric RPA-like complex |
| STN1 | Stn1 | Stn1 | Part of a telomeric RPA-like complex |
| TEN1 | Ten1 | Ten1 | Part of a telomeric RPA-like complex |
| NA | NA | Cdc13 | Binds ssDNA; part of a telomeric RPA-like complex |
| ***Telomere-associated proteins or complexes*** | | | |
| KU70–KU80 | Ku70–Ku80 | Ku70–Ku80 | Contribute to telomeric heterochromatin formation |
| XPF–ERCC1 | NA | NA | Endonuclease; mediates 3′ overhang processing |
| EXO1 | NA | Exo1 | Mediate 5′ end resection |
| Apollo | NA | NA | Mediates 5′ end resection |
| MRN complex | MRN complex | MRX complex | Involved in telomere maintenance |
| RAD51D | NA | NA | Protects telomeres against attrition and fusion |
| Tankyrase | NA | NA | Mediates poly(ADP-ribosyl)ation of TRF1 and resolution of sister telomere cohesion prior to mitosis |
| ORC | NA | NA | Prevents telomere circles |
| 9-1-1 complex | NA | NA | Involved in telomere maintenance |
| RecQ helicases | NA | NA | Bind TRF2 |
| HP1 | NA | NA | Involved in sister telomere cohesion |
| ***Telomerase core components*** | | | |
| TERT | Trt1 | Est2 | Telomerase reverse transcriptase |
| TR | Ter1 | Tlc1 | Telomerase RNA |
| ***Telomerase accessory factors*** | | | |
| Dyskerin | NA | NA | Binds and stabilizes TR |
| EST1 | Est1 | Est1 | Promote telomerase recruitment |
| NA | NA | Est3 | Associates with Est2; essential for telomere maintenance *in vivo* |
| NA | NA | Ku70–Ku80 | Mediates nuclear localization of telomerase |
| NA | Sm, Lsm | Sm | Involved in telomerase biogenesis and telomerase nuclear localization |
| TCAB1 | NA | NA | Localizes telomerase to Cajal bodies |
| Reptin | NA | NA | Associates with TERT in a cell cycle-dependent manner |
| Pontin | NA | NA | Associates with TERT in a cell cycle-dependent manner |
| PINX1 | NA | NA | Inhibitor of telomerase |
| HSP90 | NA | Hsp82 | Associates with telomerase |
| p23 | NA | Sba1 | Associates with telomerase ; HSP90 co-chaperone |

Ccq1, coiled-coil quantitatively-enriched 1; CTC1, conserved telomere maintenance component 1; dsDNA, double-stranded DNA; ERCC1, excision repair cross-complementing protein 1; EST, ever shorter telomeres; EXO1, exonuclease 1; HP1, heterochromatin protein1; HSP, heat shock protein; Lsm, SM-like; MRN complex, MRE11–RAD50–NBS1; MRX complex, Mre11–Rad50–Xrs2; NA, not applicable; ORC, origin recognition complex; PINX1, PIN2-interacting protein X1; POT1, protection of telomeres 1; RAP1, repressor and activator protein 1; RPA, replication protein A; Sba1, sensitive to benzoquinoid ansamycins 1; ssDNA, single-stranded DNA; Stn1, suppressor of *cdc* thirteen; TCAB1, telomerase Cajal body protein 1; TERT, telomerase reverse transcriptase; TIN2, TRF1-interacting nuclear protein 2; TR, template-containing RNA component; TRF, telomeric repeat-binding factor 1; Trt1, telomerase reverse transcriptase 1; XPF, xeroderma pigmentosum group F-complementing protein.

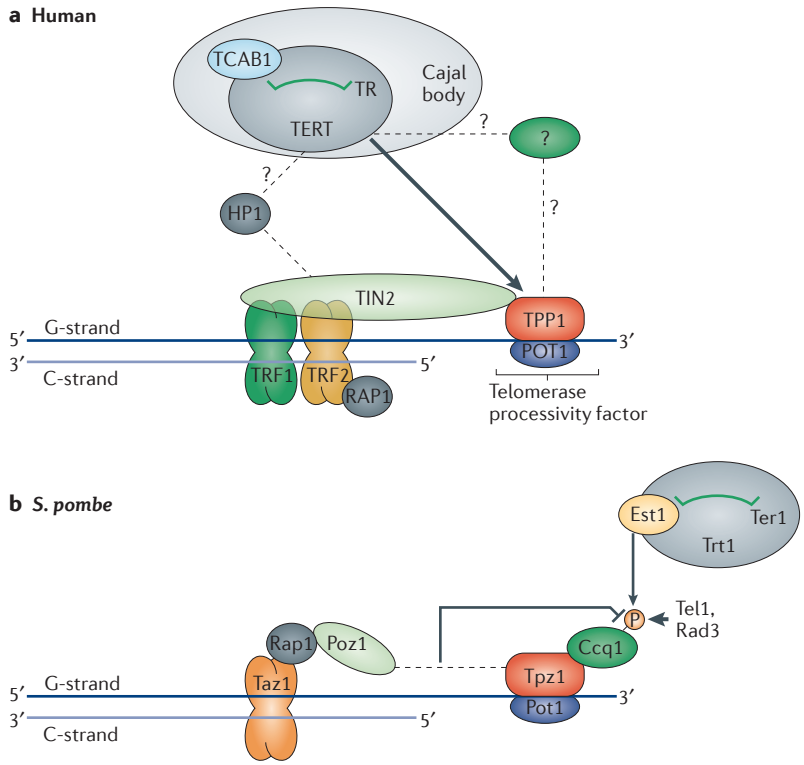



Figure 3 | **Models for telomerase recruitment in humans and *S. pombe*.** Mammalian telomeres are capped by the shelterin complex, which is composed telomeric repeat-binding factor 1 (TRF1), TRF2, repressor and activator protein 1 (RAP1), TRF1-interacting nuclear protein 2 (TIN2), protection of telomeres 1 (POT1) and TPP1. *Schizosaccharomyces pombe* contains a similar complex comprising Taz1, Rap1, Pot1, Tpz1, Poz1 and Ccq1 (coiled-coil quantitatively-enriched 1). These proteins have a key regulatory role in telomerase recruitment. **a** | Model for telomerase recruitment in humans. Accumulation of the recruitment-competent telomerase ribonucleoprotein in Cajal bodies requires TCAB1 (telomerase Cajal body protein 1). Telomerase recruitment to telomeres involves the redistribution of telomerase from Cajal bodies to telomeres, which is driven by the telomerase–TPP1 interaction[105,107,108]. This recruitment depends on TIN2, because TIN2 is required for recruiting TPP1 to telomeres[107]. It is possible that the TPP1–telomerase interaction is mediated by a yet-to-be identified homologue of *S. pombe* Ccq1 in humans. Once at telomeres, telomerase action during S phase is coordinated with sister telomere cohesion, which is facilitated by the recruitment of heterochromatin protein 1 (HP1). The recruitment of HP1 is mediated by the HP1-binding motif (PTVML) of TIN2 (REFS 122,123) (not shown). Telomerase extension then proceeds in a processive manner facilitated by the bound POT1–TPP1 complex, which reduces the primer dissociation rate and increases the translocation efficiency of telomerase[30,106,115]. **b** | Model for telomerase recruitment in *S. pombe*. The Pot1–Tpz1–Ccq1 complex (which is telomerase stimulatory) associates with the Taz1–Rap1–Poz1 complex (which is telomerase inhibitory) at long telomeres[37]. Such an association is facilitated by the presumably high local concentration of Taz1–Rap1 at such long telomeres and results in the inhibition of Ccq1 phosphorylation at Thr93 by telomere length regulation protein 1 (Tel1) and Rad3 (REFS 37,127,128). At short telomeres, however, the Pot1–Tpz1–Ccq1 connection to Taz1–Rap1 is lost owing to lower protein concentration of Taz1–Rap1, which results in Ccq1 phosphorylation. This phosphorylation event leads to ever shorter telomeres 1 (Est1) binding to Ccq1 via the 14-3-3-like domain of Est1 and the phosphorylated Thr93 residue of Ccq1 (REFS 37,127,128). Although telomerase is recruited to telomeres via interaction of telomerase reverse transcriptase 1 (Trt1) to Ccq1-bound Est1, this state would be in a 'closed' configuration with regard to telomerase action, possibly because telomerase tethered to the shelterin complex is unable to extend telomeres. A conformational switch that replaces phosphorylated Ccq1 with Ter1 at the surface of the phosphorylated Ccq1- and Ter1-binding 13-3-3-like domain of Est1 would relieve the telomerase–shelterin complex tether and lead to an 'open' configuration of telomerase that allows it to carry out telomere elongation[134] (not shown). Putative interactions are indicated by dashed arrows.

double knockout mice in which the shelterin complex was completely removed from telomeres revealed six independent DNA damage response pathways that the shelterin complex normally prevents from occurring at chromosome ends[57].

In addition to the telomeric proteins discussed above, other proteins that are not telomere-specific have also been shown to be present at telomeres and to carry out important functions there (TABLE 1).

## Telomerase structure and function

***The RNA subunits: a conserved core with elaborations.*** TR is a core subunit of telomerase that includes, among other important elements, the template for DNA synthesis[58]. TRs from different organisms vary substantially in length. Ciliate TRs are short (*Tetrahymena thermophila* TR is 159 nucleotides), mammalian TRs are intermediate in length (human TR is 451 nucleotides and mouse TR is 397 nucleotides)[59] and TR subunits in yeast such as *S. cerevisiae* (Tlc1 is 1,157 nucleotides)[60] and *S. pombe* (Ter1 is 1,213 nucleotides)[61,62] are much longer.

TR is involved at multiple stages of telomerase biogenesis and function. For example, TR provides a template boundary element that limits the extent of reverse transcription[63–65]. In the absence of such an element, telomerase would 'read-through' past the template to incorporate extra non-telomeric nucleotides. The pseudoknot and triple helix of TR[66,67] (FIG. 2g) contribute to catalysis, perhaps by orienting the primer–template duplex in the enzyme active site[68]. TR has also been shown to contribute to the processivity of the enzyme[69,70], which allows multiple rounds of telomeric DNA additions after a single primer-binding step. Using biochemical experiments and single-molecule Förster resonance energy transfer (FRET) measurements, *T. thermophila* TR has been proposed to position the template within the active site and to aid template translocation during repeat addition processivity[71]. An alternative, more passive model proposes that the strand separation and template realignment processes of *T. thermophila* TR translocation occur outside the telomerase active site, followed by binding of the realigned hybrid to the active site[72].

Another essential property of TR is to bind TERT. Vertebrate TRs bind their cognate TERT subunit using the template-pseudoknot domain and an additional domain known as the CR4-CR5 domain[73–76] (FIG. 2g). In addition to binding TERT, TRs have distinct RNA structural and sequence elements that bind various telomerase accessory proteins. The 3′ ends of mammalian TRs contain an H/ACA box that is associated with the RNA-binding proteins dyskerin, NHP2, NOP10 (nucleolar protein 10) and GAR1, and this interaction ensures TR stability and telomerase ribonucleoprotein (RNP) biogenesis[8,77]. Also included in the 3′ domain of mammalian TRs is a Cajal box (CAB) motif, which allows binding to a WD repeat-containing protein known as TCAB1 (telomerase Cajal body protein 1; also known as WDR79) and consequent localization of TR in Cajal bodies, where the telomerase RNP is packaged for delivery to telomeres[78,79].

Similarly, yeast TRs bind various protein cofactors. *S. cerevisiae* Tlc1 binds the Est1 (ever shorter telomeres 1)

regulatory subunit[80] and the dsDNA-binding Ku70–Ku80 heterodimer[81,82] (see below), as well as Sm proteins[83]. Sm proteins are required for *S. cerevisiae* telomerase function, probably because they are is necessary for nuclear import of Tlc1 (REF. 83). In *S. pombe*, the Sm complex first binds to the Sm-binding site on Ter1 to facilitate spliceosomal cleavage and trimethylguanosine synthase 1 (Tgs1)-mediated 5′ capping of the RNA[84,85]. Next, the Lsm (Sm-like) complex Lsm2–8 replaces the Sm complex on Ter1. This allows binding of the catalytic Trt1 (the *S. pombe* homologue of TERT) subunit to Ter1 and protects the mature Ter1 RNA from exonucleolytic degradation[85]. Complexes containing human telomerase and Sm proteins have also been observed but seem dispensable for telomerase function[86].

Yeast TRs contain long protein-binding 'arms' that protrude from the catalytic core of the RNA. These arms are variable with respect to their sequence, length and positional requirements[87,88]. It has therefore been hypothesized that Tlc1 binds to its protein partners in a 'beads' (protein partners) on a 'string' (RNA linkers) manner such that the main function of the RNA arms is to recruit the protein partners and retain them in the same complex to facilitate telomerase function. To evaluate the importance of conformational flexibility of the RNA arms, a recent study replaced the natural bulges and internal loops in Tlc1 with perfect double-helical RNA. The resulting mutant, TSA-T, fully reconstituted telomerase activity *in vitro* and, although deficient in RNA accumulation, it could carry out telomere length maintenance *in vivo*[89]. When the RNA expression levels were equalized, the telomeres of cells expressing the TSA-T mutant were longer than those of wild-type cells, suggesting that the flexibility of wild-type Tlc1 comes at some cost of telomerase activity[89].

***The TERT subunit provides the active site for catalysis.*** In addition to the RNA subunit, the telomerase core enzyme contains the reverse transcriptase subunit TERT[90,91]. TERT contains roughly 1,000 amino acids and is conserved among organisms as disparate as humans[92], *S. cerevisiae* (in which it is known as Est2)[90], *S. pombe* (in which it is known as Trt1)[92] and *T. thermophila*. On the basis of its structure and function, the TERT polypeptide can be subdivided into three major domains: the telomerase essential N-terminal (TEN) domain; the TERT RNA-binding domain (TRBD); and the reverse transcriptase domain, which contains the fingers, palm and thumb (also known as the C-terminal extension) subdomains and the active site for reverse transcription[90] (FIG. 2h).

The crystal structure of a putative TERT from the red flour beetle *Tribolium castaneum*[93,94] has provided more detailed structural information. Although definitive identification of this protein as an authentic telomerase subunit awaits the discovery of its RNA subunit, the presence of a T-motif provides confidence that it is either TERT or a closely related protein. The structure revealed close contacts between the TRBD and the thumb subdomain of reverse transcriptase. This interaction results in a closed ring-like tertiary structure with a large cavity at its centre, which is sufficiently large to bind the primer–template duplex[93]. A co-crystal structure of the *T. castaneum* protein bound to a RNA–DNA hairpin mimicking a template–primer pair confirmed this idea and provided further insights into the various molecular contacts at and near the active site[94] (FIG. 2h). The structure showed that the fingers and palm of the reverse transcriptase interact with the backbone of the RNA arm of the hairpin to place the template in the active site, whereas two motifs of the reverse transcriptase — the T-pocket (which contains the T-motif residues) and the ciliated protozoan (CP)-pocket — bind a region upstream of the template on the hairpin that presumably mimics the template boundary element of TR. Although the fingers and palm hold the RNA template, the thumb of the reverse transcriptase binds and secures the DNA primer part of the hairpin.

The TEN domain of TERT has been implicated in providing the 'anchor site' that binds telomeric DNA upstream from the primer–template interaction[95,96], although other domains also contribute[97]. The crystal structure of the isolated TEN domain of *T. thermophila* TERT revealed a novel protein fold with a groove on the surface that is important for DNA–primer binding and telomerase activity. A photocrosslink between a DNA primer maps to Trp187 within the groove, which suggests that the surface encompassing this residue is involved in DNA binding[98]. A similar anchor site resides within the TEN domain of human TERT[99,100]. In addition, the human TEN domain contains the DAT motif[101], which has been implicated in telomerase recruitment, and the yeast TEN domain contributes to binding of the Est3 accessory subunit[102].

In addition to the catalytic core of telomerase composed of TERT and TR, several telomerase accessory factors assist in mammalian and yeast telomerase assembly, maturation, recruitment and activation (TABLE 1). Additional components of *T. thermophila* telomerase have been recently reviewed[8].

## Human telomere–telomerase interactions

***Telomerase recruitment.*** One predictable outcome of loss of chromosome end protection would be telomere elongation, resulting from unregulated access of telomerase to telomeres. Indeed, expression of mutated components of the shelterin complex that are defective in end protection or siRNA-mediated knockdown of shelterin complex components lead to over-elongation of telomeres[26,48,51,103–105]. By contrast, cells carrying a mutant TPP1 that lacks the OB-fold domain (TPP1ΔOB) do not show enhanced telomere elongation; instead, TPP1ΔOB seems to protect telomeres as efficiently as the wild-type protein[105]. Furthermore, TERT–TPP1 binding has been observed in pull-down experiments, and this interaction is lost in cells expressing TPP1ΔOB[105]. Consistent with a direct telomerase–TPP1 interaction, telomerase has been shown to act preferentially on telomeric primers coated with POT1–TPP1 when such substrates are present together with protein-free primers *in vitro*[106]. Thus, the OB-fold domain of TPP1 (FIG. 2f) is proposed to be the telomerase-binding component of the shelterin complex which facilitates telomerase association with

Cajal bodies
Conserved nuclear organelles that are involved in the biogenesis of small nuclear ribonucleoprotein (snRNP) particles and the maturation of the telomerase RNP.

Sm proteins
Proteins that form a ring which binds to a specific U-rich sequence near the 3′ ends of small nuclear RNAs involved in mRNA splicing, allowing nuclear import of the ribonucleoprotein.

T-motif
An amino acid sequence preceding the reverse transcriptase motif that is conserved among telomerase reverse transcriptase (TERT) proteins but not apparent in other reverse transcriptases.

Photocrosslink
The formation of covalent adducts between nucleotides and/or amino acids that are adjacent in a complex using light energy, generally in the ultraviolet range.

chromosome ends that are otherwise protected by this complex (FIG. 3a).

Furthermore, cell biological studies have confirmed that TPP1 recruits telomerase to chromosome ends. Immunofluorescence and fluorescence *in situ* hybridization (IF–FISH) to telomeres as well as telomere chromatin immunoprecipitation (ChIP) using antibodies against TERT were carried out to assess and quantify telomerase recruitment[107]. RNAi-mediated knockdown of individual components of the human shelterin complex revealed that TPP1 and TIN2, but not POT1, were essential for recruiting telomerase to telomeres in HeLa cells. The loss of telomerase recruitment owing to TPP1 silencing resulted in telomerase accumulation in Cajal bodies, and recruitment was rescued by the expression of a wild-type construct that was resistant to the silencing RNA. This role of TPP1 in telomerase recruitment is conserved in other mammals, as conditional deletion of TPP1 prevented telomerase localization to mouse telomeres[108]. In agreement with the telomerase-binding data presented above, expression of the TPP1ΔOB construct in a TPP1 knockdown background failed to rescue telomerase recruitment to telomeres[107]. More recently, the TPP1 OB-fold domain was shown to be sufficient to recruit telomerase to an artificially constructed non-telomeric locus in the human genome[109], and particular amino acids on the surface of the TPP1 OB-fold domain were required for both telomerase binding[110,111] and recruitment to telomeres[109,111]. Thus, the TPP1 OB-fold domain facilitates binding of telomerase to TPP1 and recruitment of telomerase to telomeres, highlighting this domain as a key component in the shelterin complex that facilitates telomerase action at telomeres (FIG. 3a).

*In vitro*, TPP1 may be bound to telomeric ssDNA through its interaction with POT1. However, recruitment via POT1 is not rate-limiting *in vivo*, suggesting that TPP1 is recruited to telomeres primarily through its interaction with TIN2. Indeed, knockdown of TIN2 results in reduced telomerase recruitment to telomeres to a similar level as what is observed upon TPP1 knockdown[107].

Although an interaction with TPP1 is central to the recruitment of telomerase to mammalian telomeres, this event must be regulated by the cell cycle because telomerase accumulates at telomeres at detectable levels only during S phase[112,113]. Hence, akin to fission yeast and budding yeast (see below), it seems highly probable that cell cycle-dependent phosphorylation of telomeric components provides a switch that triggers the recruitment of telomerase in mammals. However, the telomeric protein substrates and the involved cell cycle-related kinases remain unidentified in mammals.

***Telomerase activation and processivity.*** The assignment of POT1–TPP1 as the mammalian counterpart of the ciliate telomeric ssDNA-binding complex TEBPα–TEBPβ led to the simplistic prediction that POT1–TPP1 would be an inhibitor of telomerase; after all, POT1–TPP1 and telomerase bind to the same single-stranded telomeric G-rich 3′ tail of the chromosome. This prediction turned out to be partly true, as telomerase failed to efficiently extend a primer that was bound by POT1 at its 3′ end (the site of action of telomerase) *in vitro*[30,114]. However, the addition of both POT1 and TPP1 to telomerase preparations resulted in a 2–3-fold increase in telomerase processivity, and the addition of TPP1 alone resulted in a twofold increase in total telomerase activity[30,106,115]. Although it has not yet been possible to determine the importance of this POT1–TPP1-mediated enhanced processivity *in vivo*, we note that only with such enhanced processivity would one or two rounds of telomerase binding and extension be sufficient to generate the 60 nucleotides of telomeric DNA per telomere end that are seen in a single cell cycle[116]. Are the processivity-stimulation and telomerase-recruitment activities of TPP1 a manifestation of the same TPP1–telomerase interaction? Recent studies have revealed that the same patch of amino acids on the surface of the TPP1 OB-fold domain that is crucial for telomerase binding and recruitment also mediates telomerase processivity stimulation[111] and telomere elongation *in vivo*[109,111].

What is the mechanism by which POT1–TPP1 increases the processivity of telomerase? A step-by-step analysis of the telomerase catalytic cycle using a combination of kinetic and equilibrium approaches[106] revealed that POT1–TPP1 increases telomerase processivity by decreasing primer dissociation and improving template translocation. Furthermore, a single POT1–TPP1–ssDNA interaction is necessary and sufficient for stimulating telomerase processivity. This suggests that once the POT1–TPP1–telomerase tether is formed on telomeric ssDNA, it is maintained throughout telomerase action on that DNA, independently of additional POT1–DNA binding events. Both the decrease of primer dissociation and the increase of template translocation by POT1–TPP1 are most easily explained by a physical TPP1–telomerase interaction on the primer that is being extended. An alternative model is that POT1–TPP1-mediated stimulation of telomerase processivity is solely due to its DNA-binding activity (which, for example, prevents G-quadruplex formation). If that were the case, stimulation would be achieved by POT1–TPP1 from another organism. However, each organism's POT1–TPP1 is most efficient at stimulating its cognate telomerase[115], arguing that processivity stimulation does not arise mainly from primer DNA–protein interactions.

TIN2 also has a role in promoting telomerase action that is independent of its interaction with TPP1. Indeed, mutations in TIN2 (REFS 117–119) outside its TPP1-binding site are associated with dyskeratosis congenita, a disease in which telomerase function is compromised[120]. The TIN2 mutations that lead to dyskeratosis congenita were shown to cause telomere shortening without any signs of chromosome end deprotection, suggesting that the mutations interfere with telomerase function[121]. Furthermore, TR and TPP1 immunoprecipitated with TIN2, but the levels of TR, but not TPP1, associated with TIN2 were decreased in the presence of a TIN2 mutant carrying dyskeratosis congenita-associated mutations[121]. This further confirmed that the telomerase defect in the TIN2 mutants carrying dyskeratosis congenita-associated mutations is not due to disruption of the TIN2–TPP1 interaction.

**Immunofluorescence**
Localization of a protein (or other macromolecule) within cells or tissues using a specific antibody that is derivatized with a fluorescent probe (direct immunofluorescence) or using a fluorescently labelled secondary antibody (indirect immunofluorescence).

**Fluorescence *in situ* hybridization**
(FISH). Localization of specific nucleic acid sequences in the cell by specific annealing of fluorescently labelled antisense oligonucleotide probes.

**Chromatin immunoprecipitation**
(ChIP). A technique that allows the isolation of DNA sequences bound to a protein of interest using specific antibodies.

The involvement in dyskeratosis congenita of TIN2 mutations that do not affect its interaction with other components of the shelterin complex suggested that either TIN2 directly binds telomerase (using the protein region that harbours mutations) or that TIN2 binds another factor that regulates telomerase at telomeres. Evidence for the recruitment of another regulatory protein came from studies showing binding of heterochromatin protein 1 (HP1) to a HP1-binding motif (PTVML) in the region of TIN2 that also harbours the dyskeratosis congenita-associated mutations[122] (FIG. 3a). Indeed, TIN2 from patient cells carrying TIN2 mutations that give rise to dyskeratosis congenita failed to bind HP1, which suggests that disruption of the HP1–TIN2 interaction contributes to the dyskeratosis congenita phenotype[122]. The HP1-binding site on TIN2 was shown to be required for sister chromatid cohesion at telomeres, as mutation of the PTVML motif resulted in an increased separation of sister telomeres[122]. HP1 was also shown to colocalize with TIN2 and to participate in cohesion at telomeres in the S phase of the cell cycle[122]. Importantly, disruption of the HP1–TIN2 interaction led to a moderate decrease in telomere length, suggesting that failure to undergo telomeric cohesion leads to some telomere shortening. Strikingly, expression of an N-terminal deletion mutant of TIN2 resulted in drastic telomere elongation in a telomerase-dependent manner but did not lead to telomere lengthening in the background of mutated PTVML[122]. It is possible that cohered sister telomeres are better substrates for telomerase[122,123], which has been proposed to function as a dimer[124–126], but more work is required to understand how these activities are related.

## Fission yeast telomere–telomerase interactions

Chromosome end protection in *S. pombe* is similar to what occurs in mammals (see above). Recent studies have identified three proteins in *S. pombe* — Tpz1, Ccq1 and Poz1 (REF. 37) — that, together with Pot1, Taz1 and Rap1, form a shelterin-like complex in this species (FIG. 3b). As mentioned above, Pot1–Tpz1 is functionally homologous to mammalian POT1–TPP1 and mediates chromosome end protection[24,37,38]. In addition, deletion of either Pot1 or Tpz1 leads to depletion of telomeric DNA and circularization of chromosomes (a common mode of survival in *S. pombe* when chromosome end protection is compromised). However, telomerase recruitment to telomeres in *S. pombe* seems to occur through a distinct mechanism that involves Ccq1, which is the *S. pombe*-specific component of the shelterin-like complex[37] (FIG. 3b). Interestingly, unlike TPP1, Ccq1 does not seem to affect *S. pombe* telomerase activity, as Trt1 or Tpz1 immunoprecipitates from *ccq1Δ* cells showed telomerase activity that was comparable to that of wild-type cells[37].

***Ccq1 is the telomerase recruitment factor in fission yeast.*** The first indication that Ccq1 may have a role in facilitating telomerase action at telomeres came from the observation that Ccq1 deletion led to a ~200 bp reduction in telomere length[37]. By contrast, Poz1 deletion resulted in a drastic increase (up to 2 kb) in telomere length, suggesting that Poz1 is a negative regulator of telomerase[37]. In addition, the telomerase activity in Tpz1 immunoprecipitates (which is similar to the telomerase activity in Trt1 immunoprecipitates) was lost when Ccq1 was deleted, but not when Poz1 was deleted. The effects of Ccq1 on telomere length occurred through the telomerase pathway because cells lacking both Ccq1 and Trt1 showed similar reduction in telomere length as cells lacking just Trt1 (REF. 37). Finally, epistasis experiments examining Poz1–Taz1 double mutants or Poz1–Rap1 double mutants confirmed that Taz1, Rap1 and Poz1 function along a single telomerase-inhibition pathway, as none of the double deletions exacerbated the telomere shortening phenotype of the respective single deletions[37].

Moreover, Ccq1 was important for telomerase recruitment to telomeres when assayed by Trt1–telomere ChIP[127,128]. Hence, Ccq1 seems to be a recruitment factor for *S. pombe* telomerase (FIG. 3b). Surprisingly, Ccq1–Poz1 double mutant cells lost telomeres more rapidly than Trt1–Poz1 double mutants, which suggests that Ccq1 also has a telomerase-independent contribution to telomere maintenance[37]. It is possible that Ccq1 homologues might exist in higher eukaryotes, mediating the recruitment of telomerase to telomeres in those organisms[37]. However, such a protein would possibly have to bridge TPP1 and telomerase, because TPP1 has been shown to pull down telomerase from cell lysates[105,110,111].

***Regulation of telomerase recruitment by phosphorylation.*** The roles of ataxia telangiectasia mutated (ATM) and ATM Rad3-related (ATR) kinases in mammalian and fission yeast telomere biology are complex. Although they are normally inhibited by the shelterin complex from triggering a DNA damage response at telomeres, they are also recruited to telomeres during S phase and G2 phase for telomere maintenance[1,34,129–131]. Deletion of both telomere length regulation protein 1 (Tel1; the fission yeast homologue of mammalian ATM) and Rad3 (the fission yeast homologue of mammalian ATR) in *S. pombe* led to a failure to recruit telomerase to telomeres and a complete loss of telomeres, leading to chromosome circularization[132,133]. The same double deletion also resulted in a decrease in Ccq1 recruitment to telomeres, suggesting that Ccq1 might be a substrate for Tel1 and Rad3 and that phosphorylation of Ccq1 could be crucial for telomerase recruitment to telomeres.

Further work revealed that Ccq1 interacts with the telomerase accessory subunit Est1 via the N-terminal half of Ccq1 and three basic amino acids at the N-terminal 14-3-3-like domain of Est1 (REF. 127). Phosphorylation of Thr93 in Ccq1 was crucial for the Ccq1–Est1 interaction, for telomerase recruitment to telomeres and for telomere length maintenance[127,128]. Thr93 was shown to be phosphorylated by Tel1 and Rad3, resolving the role of these kinases in *S. pombe* telomerase regulation[127,128]. This phosphorylation event is inhibited by the telomerase inhibitory complex Taz1–Rap1–Poz1 (REF. 127) (FIG. 3b). Consistent with this, Ccq1 hyperphosphorylation was observed at shorter telomeres, probably because they have a lower concentration of the Taz1–Rap1–Poz1 complex[127].

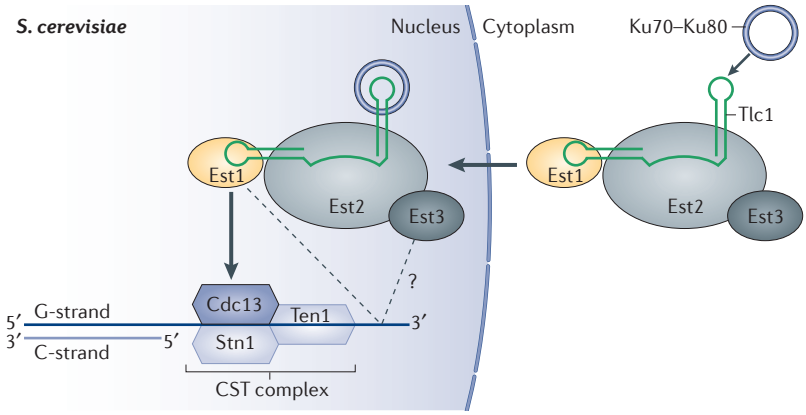



Figure 4 | **Model for telomerase recruitment in *Saccharomyces cerevisiae*.** The Ku70–Ku80 heterodimer binds Tlc1 (the telomerase RNA) and promotes nuclear import and retention of the ribonucleoprotein enzyme. The interaction between telomeric single-stranded DNA (ssDNA)- bound Cdc13 and ever shorter telomeres 1 (Est1) is responsible for recruiting telomerase to *Saccharomyces cerevisiae* telomeres. It is not known whether Cdc13 is part of the Cdc13–Stn1–Ten1 (CST) complex when it recruits telomerase to telomeres. The Est1–ssDNA interaction may further strengthen the telomerase–telomere tether. A putative Est2-dependent Est3–ssDNA interaction proposed for certain *Candida* species could serve as yet another mechanism for telomerase recruitment. Putative interactions are indicated by dashed arrows.

How does Ccq1 binding to Est1 lead to telomerase recruitment to telomeres? A fragment of Ter1 (nucleotides 415–507) has been shown to bind specifically to Est1 in a yeast three-hybrid screen. Through an unbiased random mutagenesis screen of Est1, the authors of this study found that three amino acids (Leu48, Arg194 and Lys252) that reside in the Thr93-binding 14-3-3-like domain of Est1 are crucial for the interaction of Est1 with Ter1 (REF. 134). This raised the intriguing possibility that Est1 binding to Ter1 and to Ccq1 may be mutually exclusive, with important ramifications for telomerase recruitment to telomeres in *S. pombe*; substitution of phosphorylated Ccq1 by Ter1 at the 14-3-3-like domain of Est1 may convert telomerase to its active or open state[134] (FIG. 3b).

## Budding yeast telomere–telomerase interactions

The protein components of budding yeast telomerase were discovered through pioneering genetic screens that identified four *est* genes, *est1–est4* (REFS 135,136). Est2 (which corresponds to mammalian TERT) and the regulatory subunits Est1 and Est3 together with Tlc1 (which corresponds to mammalian TR)[60] make up the telomerase holoenzyme of *S. cerevisiae*. Est4 was found to be Cdc13, a telomeric DNA-binding protein that was previously identified in a cell cycle regulation screen[135,136]. Cdc13 is not considered a telomerase subunit because it does not co-immunoprecipitate with Tlc1 RNA or with active telomerase[137], but instead associates with Stn1 and Ten1 to form the trimeric CST complex[41] (see above).

***Cdc13 and Est1 mediate telomerase recruitment.*** How is budding yeast telomerase recruited to telomeric DNA? Cdc13 and Est1 were initially implicated in this event because of the following observations. First, Cdc13 and Est1 bound telomeric ssDNA[40,138] (FIG. 4). Next, Cdc13 and Est1 were shown to weakly interact with each other in yeast two-hybrid experiments[40,139]. Finally, Est1 was shown to bind Tlc1 (REF. 140), thereby establishing a possible Est2–Tlc1–Est1–Cdc13–DNA connectivity for telomerase recruitment in *S. cerevisiae* (FIG. 4). One prediction of this model is that Cdc13 mutations that disrupt its interaction with telomerase could be rescued if the Cdc13 mutant were fused covalently to Est1. Indeed, a covalent fusion of Est1 to a telomerase-defective *cdc13* mutant rescued the *est* phenotype (that is, short telomeres)[141]. Furthermore, covalently tethering Est2 to Cdc13 eliminated the requirement of Est1 for telomerase recruitment, suggesting that the primary function of Est1 in telomerase recruitment is to bridge Cdc13 to telomerase[141]. Specifically, genetic data indicated that Glu252 of Cdc13 forms a salt bridge with Lys444 of Est1 such that single charge reversals at either of these positions lead to the disruption of the Est1–Cdc13 connection (leading to telomere shortening), whereas double charge reversals at these positions restore the salt bridge and rescue Est1–Cdc13 binding[142]. This salt bridge may perhaps not be required for binding *per se* but for a subsequent step[143].

Additional results support Cdc13–Est1 functioning in telomerase recruitment in *S. cerevisiae*. Using an engineered system, tethering either Cdc13 or Est1 near a double-strand DNA break promoted telomerase recruitment and *de novo* telomere formation[144]. Furthermore, although Est2 is first recruited to telomeres in G1 to early S phase by the Ku-dependent pathway (see below), those levels are insufficient to prevent an *est* phenotype; wild-type Est2 levels at telomeres require Est1 to be both telomere-associated and bound to Tlc1 RNA[145]. The interaction between Est1 and Cdc13 is facilitated by cyclin-dependent kinase 1 (Cdk1)-mediated phosphorylation of Cdc13 (REFS 146,147).

In addition to its interaction with Cdc13, Est1 has been reported to promote telomerase activation[148], possibly by recruiting the Est3 subunit[149] (see below). Because Est1 can bind telomeric ssDNA directly, it has been proposed that Est1 may present the 3′ end of the chromosome in a conformation conducive for telomerase action[141] and/or stabilize telomerase on telomeres[150,151].

***The Est3 subunit.*** The telomerase regulatory Est3 subunit of budding yeast is predicted to fold into an OB-fold domain that resembles the OB-fold domain of the mammalian telomere protein TPP1 (REFS 152,153). This suggests that Est3 might have some function in the recruitment of telomerase to telomeres. A group of amino acids in Est3 has been found to bind to the TEN domain of Est2, which helps to explain how Est3 is incorporated into the telomerase holoenzyme without binding Tlc1. The inclusion of Est3 in the telomerase preparation stimulates telomerase activity *in vitro*[102]. These observations alone do not implicate Est3 in telomerase recruitment. However, the Est3 subunit of *Candida* spp., which is a larger protein than that of *S. cerevisiae* with additional structural elements, has been proposed to be functionally analogous to the OB-fold domain of TPP1 (REF. 154). These authors proposed that binding of Est3 to Est2 causes a

Non-homologous end-joining
(NHEJ). A repair pathway of DNA double-strand breaks by directly ligating the broken ends without the need for a homologous template.

conformational change that reveals a DNA-binding site that may be involved in telomerase recruitment (FIG. 4). Additional studies are needed to test this model.

***Ku70–Ku80 promotes telomerase nuclear localization.*** The Ku70–Ku80 heterodimer (Ku), best known for its DNA-binding function in the non-homologous end-joining (NHEJ) pathway, binds a 48 nucleotide stem–loop in Tlc1 to facilitate telomerase accumulation at telomeres during the G1 phase of the budding yeast cell cycle[82,145,155]. It was initially proposed that Ku could bind the end of a chromosome (dsDNA) and telomerase (Tlc1) simultaneously, suggesting an elegant model for telomerase recruitment[81,155,156]. However, purified *S. cerevisiae* Ku is unable to form a ternary complex with a DNA substrate (that mimics the end of a chromosome) and Tlc1 RNA; binding of these two nucleic acids is mutually exclusive[157]. Thus, it seems unlikely that Ku directly bridges telomerase with telomeric DNA.

It is now apparent that the role of Ku in facilitating telomerase recruitment is first and foremost at the level of nuclear localization (FIG. 4). Tlc1 biogenesis involves export of the nuclear RNA to the cytoplasm followed by import back into the nucleus[158]. Using FISH to visualize Tlc1 localization, it was shown that the nuclear retention of Tlc1 was lost in the absence of Ku70, suggesting that Ku70 is important for importing Tlc1 back into the nucleus[158]. Moreover, Ku80-deletion mutants that are deficient in Tlc1-binding (and DNA binding) failed to accumulate telomerase in the nucleus[157]. Although nuclear localization of telomerase by Ku is necessary for telomerase recruitment in G1, it is presumably not sufficient; other factors such as monopolar spindle protein 3 (Mps3; a membrane-spanning SUN domain-containing protein that is found at the nuclear periphery) are involved[159,160].

Although the telomerase-associated functions of Ku have been described only in certain budding yeast species, Ku has additional roles in forming telomeric heterochromatin and repressing gene expression, and these functions require its dsDNA-binding activity and may be broadly conserved[161–163]. In budding yeast these telomeric functions have been genetically separated from the NHEJ function of Ku[164]. Ku also contributes to the localization of telomeres at the nuclear envelope, and this in turn affects telomerase extension[160].

## Conclusion

Recent biochemical and functional studies have revealed salient features of telomerase recruitment in humans, budding yeast and fission yeast. The recruitment mechanisms in humans and *S. pombe* are similar but also exhibit organism-specific nuances. Homologous proteins bind the ssDNA tails of chromosomes in humans and *S. pombe* (POT1–TPP1 and Pot1–Tpz1, respectively) and are involved in telomerase recruitment. However, an *S. pombe*-specific protein, Ccq1, bridges telomerase to Pot1–Tpz1 in this organism. Although TPP1 seems to directly connect human telomerase with telomeres, it is also possible that humans have a Ccq1-like protein that has not been identified. If a functional Ccq1 homologue were discovered in humans, it would provide a unifying principle for telomerase recruitment in these two organisms.

Another important aspect of telomerase recruitment in *S. pombe* is the phosphorylation and activation of Ccq1 by the ATM- and ATR-like kinases Tel1 and Rad3. It remains to be seen how protein modifications may regulate the recruitment of human telomerase.

In *S. cerevisiae* the telomeric ssDNA-binding protein Cdc13 binds the Est1 subunit of telomerase to facilitate telomerase recruitment. In addition, the Ku70–Ku80 heterodimer binds the telomerase RNA subunit to mediate nuclear retention. The different architecture of the telomeric protein complex and the unique roles of Est1 and the Ku70-Ku80 heterodimer separate the budding yeast telomerase recruitment mechanism from that of fission yeast and humans. The recently discovered structural homology between budding yeast Est3 and the OB-fold domain of human TPP1 has provoked the re-examination of possible mechanistic conservation between some aspects human and budding yeast telomerase recruitment, a subject of current research.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**Competing interests statement**
The authors declare competing financial interests: see Web version for details.

**FURTHER INFORMATION**
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