GETTING TO THE END: TELOMERASE ACCESS IN YEAST AND HUMANS

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In organisms with linear chromosomes, telomeres are essential to maintain genome integrity. However, inappropriate telomere addition, for example to double-stranded DNA breaks, might stabilize deleterious genetic changes. Therefore, telomere addition by telomerase is highly regulated, for example by mechanisms that determine the accessibility of telomeres to elongation by telomerase. These mechanisms, which have been studied mainly in budding yeast and human cell culture, can be subdivided into two classes: mechanisms that modulate the telomeric chromatin structure and those that sequester active telomerase from chromosome ends.

TELOMERASE
Specialized ribonucleoprotein, the catalytic core of which is composed of an RNA subunit and a reverse transcriptase subunit that facilitates the replication of linear chromosome ends or telomeres. The RNA subunit contains the template for sequence addition (3′ CACACACCCACACCAC 5′ in *S. cerevisiae* and 3′ CAAUCCCAAUC 5′ in humans).

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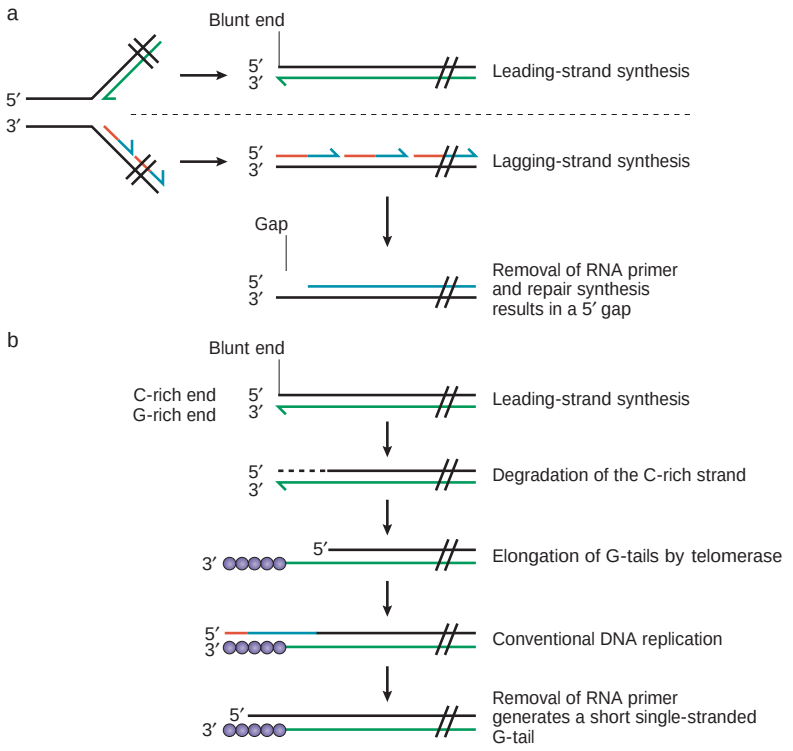
The extreme ends of linear chromosomes pose a unique problem to the eukaryotic DNA-replication machinery (BOX 1). To solve this 'end-replication problem', eukaryotes have evolved specialized structures at chromosome ends, known as telomeres, that are replicated by a unique mechanism using the TELOMERASE enzyme (see BOX 1 and FIG. 1). In addition to facilitating the complete replication of linear DNA molecules, telomeres also protect chromosome ends from degradation and fusions with other chromosome ends or DNA breaks.

Telomeres are comprised of DNA repeats, the sequence of which varies from organism to organism. Human telomeres bear precise C_3TA_2/T_2AG_3 repeats that can extend from 2 to up to 50 kilobase pairs, whereas *Saccharomyces cerevisiae* chromosomes end in 250–400 base pairs of a more heterogeneous sequence, abbreviated $C_{1-3}A/TG_{1-3}$. In both organisms, the G-rich strand extends in the 3′ direction to form a single-stranded overhang, known as the G-tail (FIG. 2a). In *S. cerevisiae*, G-tails of 50–100 bases are transiently detected in late S phase[1]; shorter G-tails are probably present during the rest of the cell cycle, as the TG_{1-3}-specific, single-stranded DNA-binding protein, Cdc13, is telomere associated at all times in the cell cycle[2,3]. In human cells, G-tails of 75–300 bases are detected throughout the cell cycle[4–6].

G-tails are the presumed substrate for telomerase (FIG. 1). However, in most human somatic cells, telomerase is undetectable and yeast cells can survive for 50–100 cell divisions in its absence[7]. So, it seems that genome integrity does not require telomerase-mediated lengthening of G-tails. However, there is increasing evidence, in both humans and yeast, that G-tails themselves are essential, because they serve as substrates for DNA-binding proteins that protect chromosome ends from degradation and end-to-end fusions[8–10,11].

The requirement for G-tails raises a second end-replication problem (BOX 1). When a DNA end is replicated by a conventional DNA polymerase, a short 8–12 base G-tail is created on the lagging strand after removal of the terminal RNA primer, and this tail could potentially be recognized and bound by G-tail-binding proteins. However, the chromosome end that is generated by the leading-strand polymerase is expected to be blunt ended, hence lacking a G-tail (see BOX 1 figure, part a). In yeast and mammals, the regeneration of G-tails on chromosome ends that are replicated by the leading strand occurs by a telomerase-independent mechanism[4,12–14]. In yeast, G-tails are probably generated by cell-cycle-regulated C-strand degradation, followed by C-strand re-synthesis and RNA primer removal to generate a short G-tail (see BOX 1 figure,

Box 1 | The 'end-replication problem'



Semi-conservative DNA synthesis presents an end-replication problem
Conventional DNA polymerases synthesize DNA in the 5′→3′ direction and cannot begin synthesis *de novo*. DNA polymerases use an 8–12-base segment of RNA as a primer (red). The leading strand can, in principle, be continuously synthesized (green). The lagging strand is synthesized in short, RNA-primed OKAZAKI FRAGMENTS (blue). After extension, the RNAs are removed and the gaps filled in by DNA polymerase priming from upstream DNA 3′ ends. Removal of the 5′-most RNA primer generates an 8–12-base gap. Failure to fill in this gap leads to a small loss of DNA in each round of DNA replication. See figure, part **a**.

Telomerase-dependent aspect of the end-replication problem
The chromosome end that is replicated by the leading-strand polymerase is not expected to result in DNA loss, but the blunt end creates a second problem for DNA replication. The ends of eukaryotic chromosomes bear single-stranded 3′ tails that are recognized by sequence-specific DNA-binding proteins that protect the ends from degradation and fusion. The 3′ overhang that is left on the end that is replicated by the lagging-strand polymerase is a telomerase substrate, however, telomerase cannot act on blunt ends. Both ends of yeast and human chromosomes have 3′ single-stranded G-tails, even in cells that lack telomerase[6,12,14] (FIG. 2). In yeast, the 3′ single-stranded tail (purple circles) on the end that is replicated by leading-strand synthesis is probably generated after DNA replication by regulated C-strand degradation[1,12,159]. In yeast, long G-tails of 50–100 bases are present only in late S phase[1], whereas in mammals, long G-tails are constitutively present[4–6]. In telomerase-deficient human cells in culture, it is unclear how G-tails are regenerated on the leading-strand telomere. Whatever the mechanism by which G-tails are generated, telomerase can extend them and the C-strand can be filled in by conventional DNA replication. Removal of the RNA primer results in a short G-tail. See figure, part **b**.

part b). In human and yeast cells, after conventional DNA replication, telomeres that are replicated by leading-strand polymerases are processed differently than DNA ends that are replicated by lagging-strand polymerases[15,16]. 5′ tails of C-rich telomeric DNA have recently been detected in replicating human cells[17]. The detection of these tails has led to the suggestion that leading-strand synthesis of the telomere might, in fact, be incomplete, so that blunt ends are never generated. How these 5′ C-rich tails would be processed to form G-tails is unclear.

The catalytic core of telomerase is composed of a protein and an RNA subunit (FIG. 1). The protein subunit is a highly conserved REVERSE TRANSCRIPTASE, known as Est2 in *S. cerevisiae*[18] and TERT (telomerase reverse transcriptase) in humans[19,20]. The size and sequence of the RNA subunit — TLC1 in *S. cerevisiae*[21] and hTR in humans[22] — is divergent among species. Telomerase RNA contains the sequence 3′ CACACACCCACACCAC 5′ (in *S. cerevisiae*) or 3′ CAAUCCCAAUC 5′ (in humans) that serves as the template for the extension of the 3′ G-rich strand of the telomere by the reverse transcriptase[21,22] (FIG. 1). Following extension of the G-rich strand (FIG. 1b and see BOX 1 figure, part b), the conventional DNA replication machinery can fill in the C-rich strand, so that there is no net loss of DNA (FIG. 1c and see BOX 1 figure, part b; for a review of the relationship between telomere replication and the conventional, semi-conservative DNA-replication machinery, see REF. 23). Finally, the telomere end must be processed to remove this final RNA primer and regenerate the G-tail (FIG. 1d and see BOX 1 figure, part b).

Telomeres have a specialized chromatin structure that is important for telomere homeostasis. Sequence-specific, double-stranded (ds)DNA-binding proteins have been identified in several organisms, of which the best studied are Rap1 (repressor/activator-site binding protein) in *S. cerevisiae*[24] and TRF1 (telomeric-repeat binding factor 1) and TRF2 in humans[25,26]. In addition, telomeres bear single-stranded, sequence-specific G-tail-binding proteins, such as Cdc13 in yeast and POT1 (protection of telomeres protein 1) in humans[2,27–29]. Both types of telomere-binding proteins recruit additional proteins to the chromosome ends, thereby making the telomere a unique non-nucleosomal chromatin domain. These proteins are also important for the overall structure of the chromosome end. Studies in mammalian cells support a model in which G-tails loop back and invade the duplex telomere DNA forming a T-LOOP (FIG. 2b); *in vitro*, formation of this loop is dependent on TRF2 (REF. 30). Although t-loops have not been detected in yeast, yeast telomeres have a different higher-order chromatin organization in which the telomere folds back on the sub-telomeric DNA, a process that is thought to be Rap1 mediated (FIG. 2b)[31,32].

Although telomeres are essential for chromosome stability, the addition of telomeric DNA to a dsDNA break (DSB) can promote genome instability by stabilizing abnormal chromosomes. Indeed, deletion of *PIF1* in yeast increases the rate of telomere addition by telomerase[33] and results in a large increase in the types of gross chromosomal rearrangements that are associated with tumorigenesis in humans[34]. So, not surprisingly, telomerase action is highly regulated.

This review will focus on the regulatory mechanisms that determine the accessibility of the telomere to elongation by telomerase. These mechanisms can be

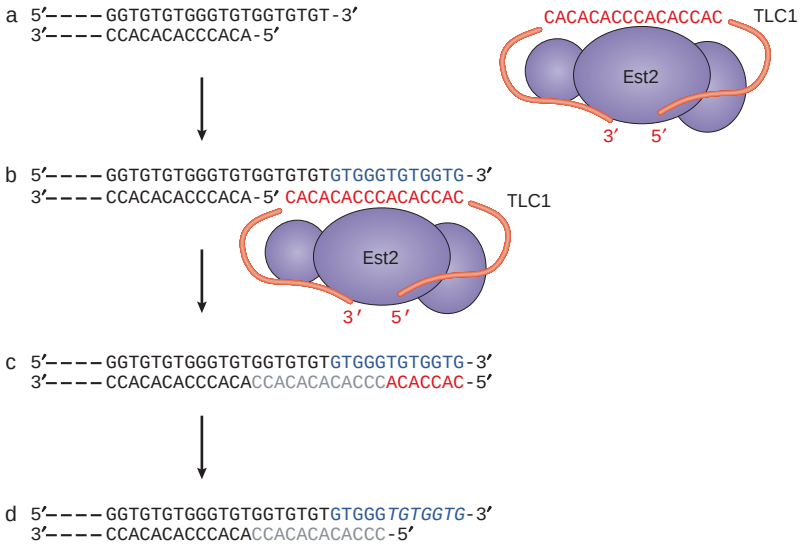


Figure 1 | **Telomerase-mediated telomere lengthening in yeast. a** | Telomeres that bear single-stranded 3′ G-tails are the presumed substrate for telomerase. The protein subunit is a highly conserved reverse transcriptase, known as Est2 in *Saccharomyces cerevisiae*[18] and TERT in humans[19,20]. The RNA subunit is TLC1 in *S. cerevisiae*[21] and TR in humans[22]. **b** | Telomerase RNA contains the sequence 3′ CACACACCCACACCAC 5′ (in *S. cerevisiae*) or 3′ CAAUCCCAAUC 5′ (in humans) that serves as a template for the extension of the 3′ G-rich strand of the telomere (shown in blue) by the reverse transcriptase[21,22]. **c** | Following extension of the G-rich strand, the conventional DNA replication machinery fills in the C-rich strand (shown in grey). **d** | Removal of the 5′ RNA primer (red) results in the regeneration of 3′ G-tails (italics) with no net loss of DNA. Please note that only a single elongation of the G-tail by telomerase is depicted.

subdivided into two major classes. The first class involves those that modulate the protein–DNA complexes (that is, the chromatin structure) at the telomere. These changes can affect telomerase in at least two, non-mutually exclusive ways — either in the direct recruitment of telomerase to the chromosome end, or by remodelling the chromatin to make G-tails more accessible to elongation by telomerase. The second class includes mechanisms that sequester active telomerase away from chromosome ends, thereby limiting telomere replication. These two methods of regulation will be discussed by comparing and contrasting experimental data primarily from *S. cerevisiae* and human cell-culture systems. Other mechanisms of telomerase regulation that are studied mainly in human cells include the transcriptional regulation of the TERT catalytic subunit (which seems to be the major form of regulation in human cells); maturation of the telomerase RNA component; the regulation of telomerase assembly; and post-translational modification of the telomerase ribonucleoprotein (RNP). These topics have been covered in recent reviews and will not be discussed here[35,36].

Telomere proteins and substrate access

Not surprisingly, the requirements for telomerase action are more complicated *in vivo* than *in vitro*. For example, in *S. cerevisiae*, the telomerase-associated proteins Est1 and Est3 are both essential for telomerase action *in vivo*, but when using the conventional primer-extension assay for telomerase activity, extracts prepared from cells lacking either protein are as active as extracts from wild-type cells[37,38]. Similar differences in the *in vivo* versus *in vitro* requirements for Est1 have been observed in *Schizosaccharomyces pombe*[39]. In addition, mutations or modifications of both the human and yeast reverse-transcriptase subunits can compromise telomerase activity *in vivo* without affecting the *in vitro* activity[40–44]. Proteins or mutations that only affect the *in vivo* action might define interactions that are required for telomerase to interact productively with telomeric chromatin. Consistent with this possibility, in both yeast and humans, mutations that impair telomerase activity *in vivo*, but not *in vitro*, can be rescued by specifically targeting telomerase to the telomere by fusion with telomere-specific DNA-binding proteins[45–47]. Examples of yeast and human telomere-binding proteins that regulate telomere accessibility are described below and summarized in TABLE 1.

Duplex-telomeric-DNA-binding proteins. Rap1 is the main double-stranded telomere-binding protein in *S. cerevisiae* (TABLE 1). Approximately 10–20 molecules of Rap1 bind to each telomere through two MYB-LIKE DOMAINS[24,48–50]. Numerous experiments support a negative role for Rap1 in telomere-length regulation; for example, deletion of the Rap1 carboxyl terminus results in telomere lengthening[51,52]. The inclusion of Rap1-binding sites that are internal to a telomere 'seed' results in the addition of fewer $TG_{1–3}$ repeats to the end bearing these sites, which indicates that Rap1 acts in *cis* to negatively regulate telomere length[53,54]. These observations led to a 'counting' model in which the number of Rap1 binding sites at the chromosome end determines its overall length. Although the precise mechanism by which Rap1 negatively regulates telomere length is unclear, it probably involves recruitment of the Rap1-interacting factors, Rif1 and Rif2 (see below). Paradoxically, Rap1 which is bound more internally at the telomere seems to promote telomerase-mediated telomere addition by a Rif-independent mechanism[55]. So, telomerase access might be regulated both positively and negatively by Rap1.

In human cells, TRF1 seems to be the functional homologue of *S. cerevisiae* Rap1 (TABLE 1). TRF1 binds double-stranded telomere repeats *in vitro* as a dimer through its Myb domains and localizes to chromosome ends, as shown by immunofluorescence[25,56,57]. Like Rap1, TRF1 negatively regulates telomere length: when TRF1 is targeted to an artificial human telomere, it inhibits telomere length in *cis*[58]. In addition, overexpression of TRF1 leads to gradual telomere shortening in a telomerase-positive tumour cell line, whereas overexpression of a DNA-binding-deficient TRF1 results in telomere lengthening[59,60]. These changes in telomere homeostasis are independent of any detectable changes in telomerase activity *in vitro*, which indicates that TRF1 has a role in regulating access of telomerase to the telomere. Overexpression of TRF1 results in the reduced association of TERT with telomeres, as shown in chromatin-immunoprecipitation experiments[61]. So,

OKAZAKI FRAGMENT
Short DNA fragment that is formed during DNA replication due to the discontinuous synthesis of the lagging strand. Okazaki fragments are initiated with an 8–12-base stretch of RNA.

REVERSE TRANSCRIPTASE
An enzyme that copies single-stranded RNA into a single-stranded DNA.

T-LOOP
Duplex telomeric loop that results from invasion of 3′ G-rich overhangs into duplex telomeric regions. T-loops have been found on eukaryotic telomeres and range in size from 0.3 kb to >30 kb.

MYB-LIKE DOMAIN
Highly conserved DNA-binding domain that is composed of tandem repeats of a helix-turn-helix motif.

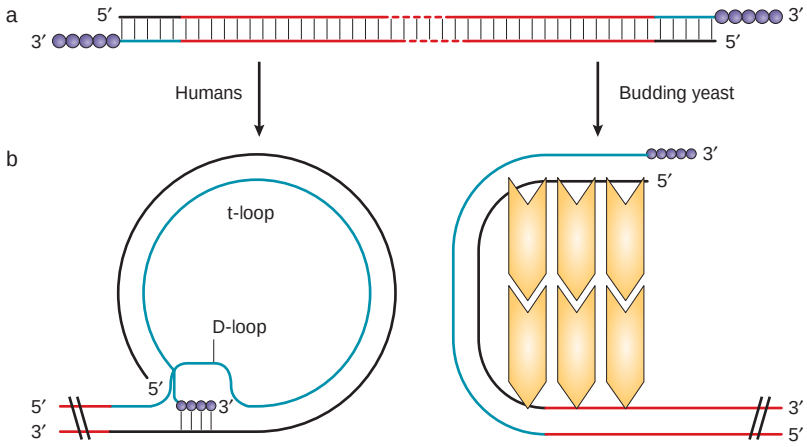


Figure 2 | **Telomere ends contain G-rich overhangs. a** | Chromosome ends are comprised of stretches of repeated C/G-rich DNA (C-rich strand shown in black and G-rich strand shown in blue; non-telomeric DNA is in red). In both humans and yeast, the G-rich strand is longer, so that it generates a 3′ single-stranded overhang or G-tail (purple circles). **b** | Higher-order chromatin structures at the telomere in human cells and in yeast. Human telomeres end in t-loops that are formed when G-tails loop back and invade the duplex telomere DNA, displacing the G-rich strand to form a single-stranded displacement (D)-loop[30]. Yeast telomeres have a different higher-order chromatin structure whereby the telomere folds back on the sub-telomeric DNA to form a ~3-kb region of core heterochromatin[31,32]. This higher-order chromatin structure is mediated by protein–protein interactions (double-stranded DNA-binding proteins that mediate looping are shown in yellow).

TRF1 probably acts by inhibiting the association of TERT with telomeric DNA.

TRF2 — a second, human, double-stranded telomere-DNA-binding protein — was identified on the basis of its amino-acid similarity to TRF1. Like TRF1, TRF2 binds duplex, vertebrate, telomeric DNA *in vitro* and localizes to the ends of metaphase chromosomes *in vivo*[26]. Overexpression of full-length TRF2 leads to telomere shortening in telomerase-positive cells, which is similar to the effects of TRF1 overexpression[60]. Again, this inhibition occurs without any measurable effect on telomerase activity *in vitro*. However, the overexpression of a form of TRF2 that is unable to bind DNA, and containing a deletion in an amino-terminal basic domain, reveals that TRF2 has telomere functions that are unique from those of TRF1. High-level expression of this version of TRF2 leads to the loss of single-stranded G-tails, and a dramatic increase in end-to-end fusions without a substantial loss of duplex telomeric DNA at the fused ends[11]. Since TRF2 mediates t-loop formation *in vitro*[30], the G-tail loss that is seen in cells expressing the dominant-negative version of TRF2 could be a consequence of t-loop loss. Alternatively, the primary effect of mutant TRF2 expression may be G-tail loss, which in turn prevents t-loop formation. Taken together, these data demonstrate a crucial role for TRF2 in telomere-end protection and further indicate that TRF2-mediated t-loops might be essential for the genome stability function of human telomeres. By contrast, loss of Rap1-mediated telomere folding in yeast does not affect chromosome-loss rates, suggesting that telomere folding in yeast is not essential for genome stability[62].

Telomerase regulators recruited through protein–protein interactions. The yeast Rif1 and Rif2 proteins were identified by their ability to interact with the carboxyl terminus of Rap1 in two-hybrid assays[63,64] (TABLE 1). Both proteins are telomere bound *in vivo*[2,65,66]. Deletion of either gene results in telomere lengthening, whereas deletion of both results in a dramatic, synergistic lengthening, similar to that seen in cells expressing a version of Rap1 that lacks its carboxyl terminus[52,63,64]. The telomere lengthening that is observed in response to Rap1 overexpression is presumably due to the titration of Rif proteins from the telomere[24,64,67]. Since the loss of Rif proteins causes lengthening only in telomerase-proficient cells, these proteins are thought to limit access of telomerase to chromosome ends[68]. In support of this model, Rap1–Rif1 and Rif2 show inverse patterns of telomere association, with Rap1 and Rif1 association peaking in late-S/G2 phase when telomerase acts, whereas the Rif2 association decreases as S phase progresses. These data led to the proposal that Rap1, Rif1 and Rif2 might remodel telomeric chromatin during the cell cycle, thereby restricting the access of telomerase to the telomere[66].

In human cells, TRF1 interacts with several proteins including PINX1 (Pin2-interacting protein X1; REF. 69), TIN2 (TRF1-interacting nuclear protein 2; REF. 70), tankyrase 1 (REF. 71) and the KU heterodimer[72], all of which localize to telomeres *in vivo* (TABLE 1). Since PINX1 binds TERT (as well as TRF1) and inhibits telomerase activity *in vitro*, it probably does not function by affecting the access of telomeres to telomerase[69]. Although *S. cerevisiae* encodes a PINX1-related protein, this protein affects ribosomal-RNA processing and, so far, has not been shown to inhibit telomerase[73].

Human TIN2, tankyrase 1 and the Ku heterodimer are each thought to regulate telomerase access to the telomere. Overexpression of an amino-terminal-truncation mutant of TIN2, which retains the TRF1-binding domain, leads to telomerase-dependent telomere lengthening by an unknown mechanism[70]. Tankyrase 1 is an ADENOSINE-DIPHOSPHATE-RIBOSE POLYMERASE (PARP), which ADP-ribosylates TRF1 *in vitro*. This modification reduces the ability of TRF1 to bind telomeric DNA[71]. Overexpression of a nuclear-targeted tankyrase 1 results in PROTEASOME-mediated degradation of TRF1 and a gradual lengthening of telomeres without effects on *in vitro* telomerase activity[74,75]. By contrast, expression of a tankyrase 1 carboxy-terminal-truncation allele, which eliminates both PARP activity and a domain that is involved in protein–protein interactions, has no effect on TRF1 levels or telomere length. UBIQUITYLATION of TRF1 is inhibited *in vitro* by the addition of telomeric DNA, which suggests that only unbound TRF1 is a target for the proteasome[75]. So, it is thought that ADP-ribosylation of TRF1 results in its dissociation from telomeres and that degradation is necessary to prevent premature re-association of TRF1 with telomeric DNA[75]. Since TERT binding to telomeres is inversely correlated with the telomeric presence of TRF1 (REF. 61), these observations suggest a model in

KU PROTEIN
A highly conserved heterodimer consisting of ~70- and ~80-kDa subunits that binds at double-stranded DNA breaks and at telomeres and is important for DNA repair and telomere functions.

ADENOSINE-DIPHOSPHATE-RIBOSE POLYMERASE
An enzyme that uses NAD^+ as a substrate to produce peptidyl-glutamic acid poly-ADP-ribose-modified proteins. This modification regulates various processes such as differentiation, proliferation and the repair of single-stranded DNA breaks.

PROTEASOME
A multi-protein complex that degrades proteins marked for destruction by ubiquitylation.

UBIQUITYLATION
The addition of the small evolutionarily conserved polypeptide, ubiquitin, to proteins that are targeted for destruction.

Table 1 | Telomerase and telomere-associated proteins in budding yeast and humans*

Factors	Budding yeast	Human	Functions and interactions
Telomerase catalytic core	TLC1 Est2	hTR TERT	RNA subunit Reverse transcriptase subunit
Telomerase accessory factors	Est1, Est3	EST1A, EST1B	Associates with telomerase (Sc, Hs) Binds TLC1 RNA (Sc)
G-tail-binding factors	Cdc13	POT1	Thought to bind DNA using OB-fold (Sc, Hs) Interacts with TRF1, TIN2, tankyrase 1 (Hs)
Duplex-telomere-binding proteins	Rap1	TRF1 TRF2	Binds telomeres (Sc, Hs) Telomere length regulator (Sc, Hs) Binds telomeres; role in t-loops (Hs)
Telomere proteins brought to telomeres by protein –protein interactions	Rif1, Rif2 Stn1, Ten1	 RAP1 TANK1, TANK2 TIN2	Recruited by Rap1 (Sc) Recruited by TRF2 (Hs) Binds TRF1; PARP activity (Hs) Binds TRF1 (Hs) Recruited by Cdc13; end protection (Sc)
Others	Ku heterodimer Mre11/Rad50/Xrs2 Pif1	Ku heterodimer MRE11/RAD50/NBS1 PIF1	Telomere localization (Sc, Hs) Ku binds TLC1 RNA (Sc) Associates with telomerase (Hs) Telomere localization (Hs) ATP binding (Sc, Hs) Nuclease activity (Sc,Hs) Helicase activity (Hs) 5′→3′ helicase activity Associates with telomeres *in vivo* (Sc)

*See text for references. Cdc, cell division cycle; Est, ever shorter telomeres; Hs, *Homo sapiens*; NBS1, Nijmegen breakage syndrome protein 1; OB-fold, oligonucleotide- and oligosaccharide-binding fold; PARP, adenosine-diphosphate-ribose polymerase; Rap1, repressor/activator-site binding protein 1; Rif, Rap1-interacting factor; Sc, *Saccharomyces cerevisae*; TANK, tankyrase; TERT, telomerase reverse transcriptase; TIN2, TRF1-interacting nuclear protein 2; TLC, telomerase component; hTR, human telomerase RNA component; TRF, telomeric-repeat binding factor; Xrs, X-ray sensitive.

HELICASE
An enzyme that uses the energy of ATP hydrolysis to unwind duplex nucleic acids

EXONUCLEASE
An enzyme that hydrolyzes ester linkages within nucleic acids. They can remove nucleotides from either the 3′ or 5′ end of the molecule.

MRE11 COMPLEX
A highly conserved protein complex that is composed of MRE11, RAD50 and NBS1 (in humans) and Mre11, Rad50 and Xrs2 (also known as the MRX complex, in yeast) and that is involved in detection, signalling and repair of DNA damage. In humans, mutations in *ATM*, *MRE11* and *NBS1* are associated with increased predisposition to cancer and cause ataxia–telangiectasia (AT), AT-like disorder (AT-LD) and Nijmegen breakage syndrome (NBS), respectively.

WRN HELICASE/EXONUCLEASE
WRN is a member of the RecQ helicase subfamily and has 3′→5′ helicase and 3′→5′ exonuclease activities. Mutations in human WRN result in Werner syndrome, an autosomal-recessive disease that is characterized by premature ageing, chromosome instability and telomere–telomere fusions.

which tankyrase 1 acts catalytically to promote telomerase access by decreasing the binding of TRF1 (REF. 74). A second, less well-studied PARP activity — tankyrase 2 — also ADP-ribosylates TRF1 *in vitro* and releases TRF1 from telomeres when overexpressed in the nucleus[76,77]. The role of the Ku heterodimer in telomerase access is described below.

The major TRF2-interacting protein identified to date is the human orthologue of yeast Rap1. Although human RAP1 has a Myb-type DNA-binding domain, unlike yeast Rap1, it does not bind telomeric DNA directly[78]. Instead, human RAP1 localizes to telomeres *in vivo* only in the presence of functional TRF2. Overexpression of human RAP1 in a telomerase-positive cell line results in telomere elongation in the absence of any effect on *in vitro* telomerase activity. By analogy with yeast Rap1, human RAP1 is thought to be a negative regulator of telomere elongation that acts through the interaction with additional, as yet unidentified, proteins[78].

TRF2 also co-immunoprecipitates with several enzymes that act on DNA, such as the WRN DNA HELICASE/EXONUCLEASE[79] and the MRE11 COMPLEX[80], which is composed of the MRE11, RAD50 and NBS1 proteins and has both DNA helicase and endonuclease activity[81]. Although the WRN HELICASE can unwind G-quadruplex DNA *in vitro*, it is not known whether this activity affects telomere structure or function *in vivo*[82]. However, mutations in WRN that cause Werner syndrome, a premature ageing disorder, show telomere-maintenance defects in cells derived from affected persons[83,84]. The yeast homologue of WRN, Sgs1, also affects telomeres, although it may function solely during telomerase-independent, recombinational lengthening of telomeres[85–87]. The Mre11 complex is discussed below.

G-tail-binding proteins. The *S. cerevisiae* Cdc13 protein is a sequence-specific, single-stranded TG_{1-3} DNA-binding protein[88,89] that localizes to telomeres *in vivo*[2,27] (TABLE 1). Although Cdc13 is not required for telomerase activity *in vitro*, specific *CDC13* alleles, such as *cdc13-2*, confer a standard telomerase-deficient phenotype that is characterized by progressive telomere shortening and eventual cell death[89]. In addition to a positive role in promoting telomerase, Cdc13, in concert with its interacting proteins Stn1 and Ten1, is essential for protecting chromosome ends from degradation[8–10,90]. Cdc13 probably also promotes C-strand resynthesis by helping recruit DNA polymerase α to the telomere[91].

Est1 is a telomerase-RNA-binding protein[92–94] that is essential for telomerase activity *in vivo* but not *in vitro*[7,37,38]. Like Cdc13, Est1 binds single-stranded TG_{1-3} DNA *in vitro*, although with considerably lower affinity[95]. Unlike Cdc13 (REFS 88,89), Est1 requires a free 3′ end for binding, which indicates that it might associate with the very end of the G-tail[95]. Est1 and Cdc13 interact in both yeast two-hybrid and biochemical assays[91]. A search for *EST1* mutations that suppress the telomerase defect of a *cdc13-2* strain identified the *est1-60* allele[90]. Like *cdc13-2* cells, *est1-60* cells are telomerase defective. However, a *cdc13-2, est1-60* double-mutant strain is telomerase proficient. The *est1-60* allele converts a lysine residue to glutamine[90], whereas the *cdc13-2* allele substitutes lysine for a glutamine residue[89]. So, the reciprocal suppression of mutant phenotypes is

due to compensatory charge changes, which makes a compelling argument for a direct interaction between the two proteins. Together, these results imply that Cdc13 recruits a telomerase holoenzyme — which consists of Est1, Est2, Est3 and telomerase RNA — to the telomere by its ability to interact with Est1. According to this 'recruitment' model, the telomerase defects of a *cdc13-2* strain result from the inability of the mutant Cdc13-2 to bind to Est1. In support of this model, a Cdc13–Est2 fusion protein bypasses the need for Est1 *in vivo*[45].

Additional experiments indicate that the recruitment of telomerase to telomeres is, in fact, more complex. In contrast to the expectations of the recruitment model, Est1 and Est2 are still telomere bound in a *cdc13-2* strain[3]. In addition, the mutant Cdc13-2 interacts with Est1, as shown by both two-hybrid analysis and glutathione *S*-transferase (GST) pull-down assays[91]. These data indicate that the *in vivo* telomerase activity requires a functional interaction, not just a physical interaction, between Cdc13 and Est1. According to this alternative model, the functional interaction between Cdc13 and Est1 is restored by the charge-swap mutations. The Cdc13–Est1 interaction that is defective in *cdc13-2* cells might be important during late S phase, as the pattern of Est1 and Est2 association with telomeres is almost identical in wild-type and *cdc13-2* cells, except that there is a loss of the Est2 signal at the telomere during late S phase, which is precisely the time when telomerase acts[3]. So, the functional interaction between Cdc13 and Est1 that is lost in *cdc13-2* cells might be required to retain Est2 at the telomere.

Surprisingly, Est2 is telomere associated throughout most of the cell cycle[3,66], even in G1 and early S phase when telomerase is not active[96]. However, it is not clear whether Est2 is associated with the very end of the G-tail or is bound to duplex telomeric DNA — perhaps as proposed[97] — by an interaction between Ku and telomerase RNA[98]. Unlike Est2, Est1 binds telomeres only in late S phase, when telomerase acts[3]. The cell-cycle-limited nature of Est1 binding is explained, in part, by the fact that the abundance of Est1 is also cell-cycle regulated, peaking in late S phase. However, even though levels of Est1 are low in G1 phase cells, in *cdc13-2* cells there is a modest binding of Est1 to telomeres at this time[3]. Cdc13 also interacts with Stn1, an association that is required for its end-protection function[9,10]. The Cdc13–Stn1 interaction is eliminated in *cdc13-2* cells[99]. So, perhaps the presence of Stn1 prevents Est1 from binding telomeres in G1 phase. In summary, the association of Est1 with telomeres at the time of telomerase action suggests a new working model in which Est1 is a cell-cycle-regulated activator of telomere-bound Est2 (REF. 3).

Est1-like proteins were recently discovered in humans[100,101], as well as in several other yeasts[39,102], despite their limited sequence similarity with *S. cerevisiae* Est1 (TABLE 1). Three Est1-like proteins are present in humans — EST1A, -1B and -1C — although the effects of EST1C on telomeres have not yet been examined[100,101]. Like yeast Est1, human EST1A and EST1B are associated with telomerase activity[100,101]. Also similar to *S. cerevisiae* Est1, EST1A, but not EST1B, exhibits weak single-stranded telomeric-DNA-binding activity[101]. However, human EST1A binds *S. cerevisiae* telomeric-DNA sequences with a higher affinity than it binds human telomeric-DNA sequences, so it is not clear if this DNA binding has physiological significance[101]. Overexpression of EST1A alone results in telomere shortening, whereas co-overexpression of EST1A and TERT results in telomere lengthening[101]. These results indicate that the telomere shortening that is observed after overexpression of EST1A alone is due to titration of TERT from telomere ends. In another study, overproduction of EST1A alone led to an increase in chromosome end-to-end fusions, which indicates that human EST1 interacts with a protein that is required for telomere capping[100]. Likewise, the *Candida* and *S. pombe* Est1 proteins seem to affect both telomerase activity and end protection[39,102]

The first G-strand-specific telomere-binding activity, which is a heterodimeric protein complex, was identified in the ciliated protozoan, *Oxytricha nova*[103]. Although *S. cerevisiae* has no evident homologue for the ciliate proteins, *S. pombe* and humans both express POT1 proteins, which were identified by their sequence similarity to the α subunit of the ciliate complex[28]. Structural analysis and secondary-structure predictions indicate that the *Oxytricha nova* protein Cdc13 and *S. pombe* and human POT1 bind single-stranded DNA through a common motif, known as the OB-fold (oligonucleotide- and oligosaccharide-binding fold)[28,104,105]. Chromatin-immunoprecipitation studies showed that human POT1 is telomere bound *in vivo* and that this binding is correlated with the presence of G-tails[106]. The telomere association of POT1 is further supported by its co-localization with TRF2 and RAP1 (REF. 29). POT1 interacts biochemically with TRF1, TIN2 and tankyrase 1, and expression of nuclear-targeted tankyrase 1, which decreases telomere-bound TRF1, also reduces the telomeric association of POT1 (REF. 106). Overexpression of a POT1 mutant with a deletion in the OB domain results in rapid elongation of telomeres[106]. Although the OB domain of POT1 is thought to be essential for DNA binding *in vitro*, chromatin-immunoprecipitation and immunofluorescence experiments showed that the OB-mutant POT1 is telomere associated. So, the localization of POT1 to telomeres could be mediated through protein–protein interactions[106]. These observations imply that POT1, in concert with TRF1, inhibits telomere length by limiting the accessibility of telomeres to telomerase. In contrast to these results, another study showed that overexpression of POT1 resulted in telomerase-dependent telomere lengthening, which suggests that POT1 is a positive regulator of telomerase[107].

Double-stranded-DNA breaks and telomeres

Telomeres and DSBs share the common feature of being physical ends of chromosomes. However, unlike DNA breaks, normal-length telomeres do not activate DNA-damage checkpoints[108,109]. Telomeres are normally protected from non-homologous recombination and therefore do not fuse with other telomeres or with

random DNA breaks. In addition, whereas the 5′ ends of both telomeres and DSBs are degraded to generate 3′ single-stranded overhangs at telomeres, this processing is limited, probably due to the presence of G-tail-binding proteins[8,10,90]. So, genome integrity requires that DNA breaks be recognized as DNA damage to provide time for their repair by recombination, whereas telomeres must be shielded from both checkpoint recognition and repair. Given the different fates of DSBs and telomeres, it is remarkable that several proteins with roles in NON-HOMOLOGOUS END JOINING (NHEJ) — a process that is prohibited at telomeres — also function at telomeres. A dramatic example of this paradox was recently described in *S. cerevisiae* where Nej1 was found to be required for efficient NHEJ at DNA breaks, but to inhibit NHEJ at telomeres[110].

The highly conserved Ku heterodimer binds with high affinity to dsDNA ends, regardless of their sequence or structure, and has a crucial role in NHEJ in yeast and mammals[111]. In *S. cerevisiae*, Ku is also telomere bound *in vivo*[112]. Cells that lack Ku show multiple telomere defects including reduced telomere length[113], long constitutive single-stranded G-tails[112,114], altered expression of telomere proximal genes[112,115–117] and increased telomere–telomere recombination at elevated temperatures[114]. Given that the absence of Ku exacerbates the telomere shortening of *est* mutants[112,114,117], Ku was initially thought to affect telomere length by a mechanism that is distinct from telomerase. However, more recently, Ku has been shown to interact specifically with a stem-loop portion of the telomerase RNA[98,118]. Deletion of the part of TLC1 that binds Ku, resulting in the *tlcΔ48* mutant, leads to shortened telomeres in the absence of any effects on the *in vitro* telomerase activity[118]. Furthermore, a separation-of-function allele of Ku80, which disrupts the ability of Ku to interact with telomerase RNA but is competent for DNA binding, was identified[98]. Although these strains have normal chromosome-end protection and DNA repair, telomere addition is compromised[98]. These results indicate that Ku promotes the access of telomerase to telomeres by its ability to bind telomerase RNA[98,118]. Consistent with a role for Ku in telomerase recruitment, the expression of a Cdc13–Ku70 fusion protein resulted in a hyperlengthening of telomeres[119].

The Ku heterodimer also associates with human telomeres[72,120,121]. However, this association might not be direct, but rather, is mediated through interaction with TRF1 (REF. 72). Although Ku has not been shown to interact with mammalian telomerase RNA, the Ku heterodimer co-immunoprecipitates with TERT[122]. The functions of Ku at human telomeres have not yet been described. However, in the mouse, cells that lack the Ku86 subunit show an increase in chromosome fusions, which is consistent with a role for mammalian Ku in telomere capping[72,123–125]. Ku also seems to have a role in telomere-length maintenance in the mouse, although its exact function is unclear[121,124,125]. So, although mammalian Ku might also have multiple telomeric functions, additional experiments are required to determine its precise roles.

The evolutionarily conserved Mre11, Rad50 and Xrs2 proteins form a complex — the Mre11 complex — that functions in DSB repair and, at least in yeast, in NHEJ (reviewed in REF. 126). In vertebrates, the Mre11 complex has a crucial role in preserving the integrity of replication forks during DNA replication[127], and in both yeast and mammals, the complex functions in the INTRA-S-PHASE CHECKPOINT[126]. In addition, the yeast and human complexes affect telomeres, although their mechanism of action is obscure. In yeast, loss of the complex results in short but stable telomeres[128,129], whereas cells that lack both the Mre11 complex and the Mec1 DNA-checkpoint protein kinase have the same telomere phenotype as telomerase-deficient cells[130]. As Mre11 is a nuclease, one appealing possibility is that the Mre11 complex generates the 3′ single-stranded G-tails on the chromosome ends that are replicated by the leading-strand polymerase (see BOX 1 figure, part b). Results that were obtained using an assay that monitors telomerase-mediated telomere addition, after induction of a DSB near an internal telomeric tract, support this view[131]. In cells arrested in mitosis, the absence of the Mre11 complex results in reduced Cdc13 binding and the inability to form new telomeres at the DSB. So, on the basis of these results, the Mre11 complex seems to facilitate the loading of Cdc13 onto the single-stranded G-tail and subsequent telomerase recruitment[131].

However, cycling cells do not require the Mre11 complex for telomere addition, even though telomere addition is considerably delayed in its absence. Paradoxically, Mre11 is a 3′→5′ nuclease, whereas processing of the DSB requires a 5′→3′ exonucleolytic activity. Furthermore, *in vivo*, yeast strains that lack the nuclease activity of Mre11 have normal-length telomeres, do not senesce in the absence of Mec1 and have wild-type (or higher) levels of telomere-bound Cdc13 (REFS 27,132). The conflicting results with regard to telomere-bound Cdc13 levels in Mre11-complex mutants could reflect the different assay systems that were used in these studies. So, taken together, these data are consistent with a role for the Mre11 complex in promoting telomerase, although its precise mechanism is still unclear.

In addition to the MRE11-encoded nuclease, the human Mre11 complex has DNA helicase activity, which is conferred by NBS1, the human counterpart of *S. cerevisiae* Xrs2 (REF. 81). As in yeast, the human Mre11 complex has a positive role in telomere length: individuals with Nijmegen breakage syndrome (NBS) — a rare recessive genetic disorder that is caused by mutation of NBS1 — show accelerated telomere shortening[133,134]. Moreover, in humans, both RAD50 and MRE11 are constitutively telomere associated, and are perhaps recruited through their ability to bind TRF2, whereas NBS1 is telomere associated only during S phase[80]. It is thought that the human Mre11 complex works together with TRF2 to modulate t-loop formation by the same mechanism by which the complex affects DSB repair[80].

NON-HOMOLOGOUS END JOINING (NHEJ). A double-stranded DNA break (DSB) repair pathway that involves the largely homology-independent ligation of two DNA ends.

INTRA-S-PHASE CHECKPOINT Pathway that responds to stalled replication forks and other DNA damage during S phase by activating the ATM-like kinases Mec1 and Rad53. Checkpoint activation results in delayed S-phase progression and inhibits spindle elongation.

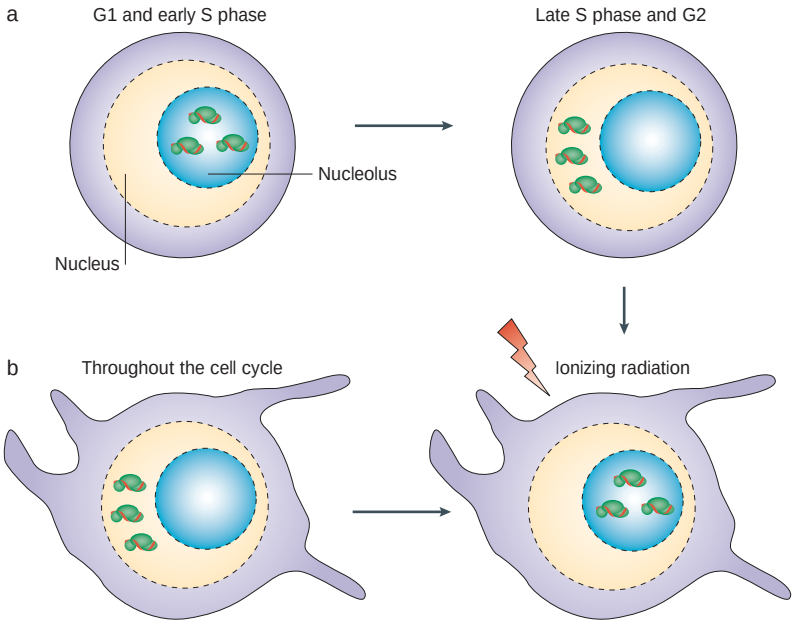


Figure 3 | **Sequestration of telomerase in the nucleolus.** The nucleolar localization of TR (red strands) and TERT (green ovals) might sequester active telomerase away from chromosome ends when telomerase action is not needed. **a** | In primary cells, TERT is localized in the nucleolus in G1 and early-S-phase cells but is mainly excluded from the nucleolus in late-S/G2 phase[148]. **b** | In telomerase-positive tumour cell lines, TERT is excluded from the nucleolus at all stages of the cell cycle[148]. The association of TERT with the nucleolus increases after treatment with ionizing radiation to induce double-stranded DNA breaks (DSBs) in both primary and tumour cells. Sequestration of telomerase into the nucleolus might serve to inhibit the action of telomerase on DSBs[148].

Removal of telomerase from chromosome ends

Although telomeres are essential for maintaining genome integrity, the addition of telomeric DNA to a DSB contributes to genome instability. For example, if a telomere is added to a DSB, the DNA distal to the break is lost, generating an aneuploid cell for that region of the genome. By contrast, if the DSB is repaired by homologous recombination, normal ploidy is maintained.

In *S. cerevisiae*, telomere addition after chromosome breakage is rare and essentially undetectable in cells that are proficient in homologous recombination[135,136]. Even in cells that are recombination deficient, fewer than 0.1% of broken chromosomes are healed by telomere addition, and virtually all of these additions occur near long tracts of telomere-like DNA — tracts that are quite rare in internal regions of yeast chromosomes[136].

Telomere addition in yeast is actively inhibited by Pif1, a 5′→3′ DNA helicase, the absence of which results in an enormous increase in telomere addition to spontaneous and induced DSBs[136,137]. The absence of Pif1 also reduces the stringency that is required for telomere addition, such that long stretches of telomeric DNA are no longer needed to promote telomere addition[136,137]. Pif1 also has strong effects in an assay that detects gross chromosomal rearrangements (GCRs), such as translocations and deletions[34]. Consistent with the idea that inhibition of telomere addition promotes genome integrity, *pif1Δ* cells exhibit a 1,000-fold increase in the rate of GCR generation. Analysis of these GCR events shows that they all fall into a particular category — the deletion of a chromosome end followed by telomere addition.

Pif1 affects not only *de novo* telomere addition but also the lengths of existing telomeres: reduced Pif1 causes telomere lengthening and overexpression results in telomere shortening[33,137]. The effects of Pif1 on telomere addition and lengthening of existing telomeres require both telomerase and the helicase activity of Pif1[33,34], which indicates that Pif1 is a catalytic inhibitor of telomerase. As Pif1 is telomere associated *in vivo*, its effects on telomerase are likely to be direct[33]. Together, the data suggest a model in which Pif1 limits telomerase activity; either by preventing initiation of telomerase-mediated telomere lengthening, or by limiting telomerase processivity by dissociating the telomerase-RNA–telomeric-DNA hybrid formed during telomere replication. Although a human homologue of *S. cerevisiae* Pif1 has been identified, it is not known whether it has a role in telomere homeostasis[33].

Sequestration of telomerase from telomeres

Telomerase access to chromosome ends might also be regulated by sequestering the telomerase RNP away from telomeres at times when its action is not appropriate. Recent data suggest that the biogenesis of human telomerase might occur in the nucleolus, and that the release of active telomerase from this compartment might be an important step in the regulation of its activity (FIG. 3). Both the human telomerase RNA (hTR) and its catalytic subunit (TERT) are partially enriched in the nucleolus[138–141]. hTR contains a box H/ACA domain near its 3′ end that is characteristic of box H/ACA SMALL NUCLEOLAR (SNO)RNAS and which is important for stability and 3′ end processing of the transcript, as well as for *in vitro* activity of the telomerase RNP[138]. In addition, hTR immunoprecipitates with several snoRNA-associated proteins, including dyskerin[142–145]. Mutant dyskerin results in a reduction in hTR levels, telomerase activity and telomere length, and is a cause of the human genetic disease dyskeratosis congenita, a condition that is characterized by bone marrow failure, genetic instability, elevated cancer risk and other abnormalities[142].

The presence of the box H/ACA domain led to the proposal that hTR may be localized to, or processed in, the nucleolus. Subcellular fractionation experiments in HeLa cells and hTR localization experiments in *Xenopus* oocytes indicate that at least a portion of hTR is specifically localized to the nucleolus, and that this localization is mediated by the box H/ACA domain of hTR[138,139].

The nucleolar localization of TERT was demonstrated by expressing yellow or green fluorescent protein (YFP/GFP)–TERT fusion proteins in telomerase-negative cells[140,141] and by localizing endogenous TERT to the nucleolus using polyclonal TERT antibodies[141]. TERT localization to the nucleolus is not dependent on the presence of hTR, which suggests that TERT possesses its own nucleolar targeting signal[140,141]. Indeed, nucleolar targeting of TERT seems to be dependent on multiple domains in its amino-terminus[140,141].

SMALL NUCLEOLAR RNA (snoRNA). Stable RNA species in the eukaryotic nucleolus, most of which function to target the major nucleotide modifications in ribosomal RNA or are involved in rRNA processing. There are three classes of snoRNAs: box H/ACA snoRNAs, box C/D snoRNAs and 7-2/MRP snoRNAs.

It is unclear whether the biogenesis of yeast telomerase involves the nucleolus. The box H/ACA domain of hTR is not conserved in the telomerase-RNA component of *S. cerevisiae*, TLC1. Instead, TLC1 possesses an Sm domain that is common among small nuclear ribonucleoprotein (snRNP) particles that are involved in mRNA splicing[146]. Mutations in the TLC1 Sm domain result in decreased levels of telomerase RNA, indicating its importance in RNA accumulation. *In situ* hybridization analysis of overexpressed TLC1 reveals a nuclear localization pattern with no preferential accumulation in the nucleolus[147]. However, when overexpressed, both Est1 and Est2 show a preferential nucleolar accumulation that is independent of expression of the other protein or TLC1 (REF. 147). Co-overexpression of Est2 and TLC1 leads to a redistribution of Est2 from the nucleolus to the nucleoplasm, which implies that active telomerase is in the nucleoplasm.

The nucleolar localization of hTR and TERT might serve to sequester active telomerase from chromosome ends when telomerase action is not needed (FIG. 3). In support of this possibility, primary human fibroblasts that express limiting amounts of functional GFP–TERT show cell-cycle-dependent changes in its localization[148]. GFP–TERT localization is predominately nucleolar in G1 and early S phase cells. However, in late-S/G2 phase, GFP–TERT localization is no longer nucleolar limited and it might even be excluded from the nucleolus (FIG. 3). This re-localization of TERT does not correlate with changes in telomerase activity, as determined by *in vitro* assays. These data suggest a model in which nucleolar compartmentalization restricts telomerase action on chromosome ends to late-S/G2 phase of the cell cycle.

In contrast to primary cells, in telomerase-positive tumour cell lines, the localization of GFP–TERT does not vary upon cell-cycle progression, but rather, is excluded from the nucleolus throughout the cell cycle[148] (FIG. 3). These observations indicate that increased telomerase access might be advantageous during tumorigenesis, either to stabilize frequent chromosome rearrangements or to ensure that telomeres are maintained at a minimal length despite rapid cell division. However, when either primary cells or tumour cells that express GFP–TERT are treated with ionizing radiation to induce DSBs, association of the fusion protein with the nucleolus increases. This result implies that telomerase localization is also affected by cellular DNA-damage pathways, and that these pathways are still functional in the types of tumour cells examined (FIG. 3). So, the sequestration of telomerase in the nucleolus in normal cells might serve to inhibit the action of telomerase on DSBs and hence promote genome stability[148].

Other situations in which telomerase might be regulated by subcellular localization have also been described. Following immune stimulation, T lymphocytes show increased telomerase activity that is independent of TERT transcription[149–151]. Rather, phosphorylation of TERT is correlated with its translocation from the cytoplasm to the nucleus[152]. Another example of signal-dependent subcellular movement of TERT is seen in TNF-α-treated multiple myeloma cells[153], where TNF-α treatment results in the shuttling of TERT from the cytoplasm to the nucleus. This shuttling increases telomerase activity in nuclear versus cytoplasmic extracts. Finally, in tissue-culture cells, telomerase is exported from the nucleus to the cytoplasm in response to both exogenous and endogenous oxidative stress[154].

In yeast, it is unknown what role, if any, subcellular compartmentalization has in the regulation of telomerase activity. In fact, although telomerase acts at yeast telomeres only during late S phase, Est2 is bound to telomeres even in G1 phase, which makes it unlikely that yeast telomerase activity is regulated by altered subcellular localization[3,66]. Moreover, even though Est1 is not telomere bound when telomerase is inactive, its absence at the telomere does not seem to be due to sequestration in a subnuclear compartment such as the nucleolus. Rather, its telomere association parallels its cell-cycle-regulated expression[3,155]. However, it is possible that yeast Est2 is telomere bound in a manner that sequesters it from the 3′ end of the G-rich strand — for example, by its association with Ku[98] — and requires other factors for its movement to the telomere end in late S phase[97].

Conclusions

Yeast and humans share several mechanisms for regulating telomerase, yet other aspects of this regulation are clearly different between the two organisms. Both yeast and human telomerase might mature in the nucleolus[138–141,147] (FIG. 3). However, although nucleolar sequestration of human telomerase might limit its access to chromosome ends except during S phase[148], yeast telomerase is telomere bound throughout most of the cell cycle[3,66]. In both organisms, telomere-binding proteins have important functions in regulating the accessibility of telomeres to telomerase (TABLE 1). Human and yeast encode sequence-specific, duplex-telomere-DNA-binding proteins — Rap1 in budding yeast and TRF1 and TRF2 in humans[24–26]. Although the human and yeast proteins lack significant sequence similarity, they contact DNA in a similar manner and share common functions in negatively regulating telomere length. In both cases, this regulation occurs in *cis* through the recruitment of additional proteins[58,156,157]. The association of TRF1 with telomeres is also regulated by ADP-ribosylation[74], whereas post-translational modulation of Rap1 binding has not been reported.

In addition to acting as a negative regulator of telomerase, TRF2 also has a crucial role in end protection, as inferred from the phenotypes of cells expressing a non-DNA-binding version of TRF2 that probably acts by disturbing t-loop formation[11]. By contrast, yeast Rap1 does not seem to have a major role in end protection[65,158]; instead, this function is mediated largely through the Cdc13–Stn1–Ten1 complex[90]. POT1 is the best candidate for a human functional homologue of Cdc13, yet its role in end protection is unknown and its action on telomere length is disputed[106,107]. In both organisms, telomeres assume a higher-order organization that is promoted by duplex-telomere-binding proteins (FIG. 2)[30–32]. However, there is little

similarity in the details of these higher-order structures and it is unclear at present if they share any common functions. Whereas the human t-loop is mediated through DNA base pairing and associated telomeric proteins, the folded yeast telomere is probably maintained solely by protein–protein interactions. Moreover, human t-loops seem to be important for telomere function[11], whereas telomere looping in yeast is dispensable for chromosome stability[62]. In both organisms, proteins that function in DSB repair and NHEJ are inexplicably telomere associated. Although the significance of these associations is not understood, perhaps these proteins help shield telomeres from DNA checkpoints by making them appear as DSBs that are undergoing repair. The recent discoveries of human counterparts of *S. cerevisiae* Est1 (REFS 100,101), Cdc13 (REF. 28) and Pif1 (REF. 33) indicate that insights from yeast will continue to inform our understanding of human telomerase.

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