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The role of shelterin in maintaining telomere integrity

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1. ABSTRACT

The ends of eukaryotic chromosomes need to be protected from detection as DNA double strand breaks by the DNA damage response pathways. Failure to do so would have devastating consequences for genome integrity. Packaging of chromosome ends into protective structures called telomeres prevents checkpoint activation and DNA repair/recombination activities. Several studies on a variety of organisms have revealed that protein complexes with specificity for telomeric DNA protect chromosome ends from being recognized as DNA double-strand breaks and regulate telomere maintenance by the telomerase. In this review, we will discuss the consequences of telomere dysfunction and our understanding of how telomere integrity is maintained.

2. INTRODUCTION

Before the discovery of the double-helical structure of DNA, Hermann Muller and Barbara McClintock, working with fruit flies and maize, respectively, documented that native ends of linear eukaryotic chromosomes, unlike those arising from breakage at internal chromosome regions, were protected from joining reactions (1,2). In 1938, Muller called these protected DNA ends "telomeres" (from the Greek "telos" meaning end, and "meros" meaning part). Thus, by the first half of the century it was clear that the natural chromosome ends had special properties and structures that protected them from the frequent rearrangements that occur at broken DNA ends. This protective function, referred to as telomere "capping", depends on the presence of species-specific telomeric repeats, telomere-associated proteins and a proper terminal DNA end-structure.

The nature of the DNA sequences that confer telomere function on chromosome ends was discovered when Blackburn and Szostak showed that *Tetrahymena* telomeric repeat sequences were sufficient to stabilize a linear plasmid in the budding yeast *Saccharomyces cerevisiae* (3). This finding pointed to a novel mechanism for telomere replication and set up the conditions for the discovery of the telomerase enzyme. This specialized reverse transcriptase adds telomeric repeat sequences to the 3' ends of chromosomes by reverse-transcribing the template region of its tightly associated RNA moiety (4). In doing so, the telomerase ensures the presence of the same sequence at all telomeres and avoids sequence loss at the ends of chromosomes due to the inability of DNA polymerase to complete the lagging-strand synthesis of DNA ends. In most eukaryotes examined to date, telomeric DNA comprises tandemly repeated G-rich sequences (TG_{1-3} repeats in yeast and T_2AG_3 in vertebrates) (5). The G-rich strand forms a 3'-ended single-stranded overhang (G-tail or G-overhang), which allows the recruitment of telomerase (6-8).

Indeed, natural chromosome ends need to be distinguished from damage-induced intrachromosomal DNA double-strand breaks (DSBs) (reviewed in 9-12), which activate a DNA damage response (DDR), including checkpoint-mediated cell cycle arrest and DNA repair/recombination pathways. If cells were to react to the constitutive presence of chromosome ends as if they were DSBs, activation of the checkpoint could lead to perpetual cell cycle arrest, whereas repair/recombination attempts could result in chromosome end-to-end fusions, rearrangements and general genome instability. Hence, it is critical that telomeres escape the DDR and are protected from degradation, recombination, fusion and recognition by the checkpoint machinery (reviewed in 9-12). Nevertheless, some DDR proteins play important roles at telomeres as well. Thus, chromosome ends have evolved strategies to circumvent the harmful effects of the DDR, while at the same time exploiting those DDR activities that are necessary for their maintenance.

In mammalian cells, a complex formed by the TRF1, TRF2, POT1, TIN2, TPP1 and RAP1 proteins with specificity for the telomeric DNA repeats protects the ends of chromosomes from being recognized as DSBs and regulates telomerase-dependent telomere elongation (13). To denote these key roles in both telomere protection and telomere maintenance, this complex is referred to as "shelterin" (reviewed in 14,15). Analogous proteins bound to telomeric DNA exist also in other organisms such as *S. pombe* and *S. cerevisiae*.

3. THE CELLULAR RESPONSE TO DNA DOUBLE-STRAND BREAKS

DSBs induce a DDR that comprises both DNA repair to reverse DNA lesions and checkpoints to inhibit cell cycle progression until DNA lesions have been repaired (reviewed in 16). Two major pathways are devoted to repair a DSB: non-homologous end joining (NHEJ) directly rejoins broken DNA ends with no (or minimal) base pairing at the junction, whereas homologous recombination (HR) utilizes a homologous DNA template to restore the genetic information lost at the break site.

Once a DSB occurs, the highly conserved MRX/MRN complex, composed by the Mre11, Rad50 and Xrs2 (MRX) subunits in budding yeast and of Mre11, Rad50 and Nbs1 (MRN) in both fission yeast and mammals, binds the free ends very rapidly (17) (Figure 1). The Ku70/Ku80 (Ku) heterodimer is also loaded onto DNA ends and, together with MRX, mediates recruitment of downstream NHEJ factors in order to religate the DSB ends (Figure 1). This process requires the DNA ligase activity of the Dnl4-Lif1/XRCC4 heterodimer and the Nej1/XLF protein (reviewed in 18). In budding yeast, Ku binding also promotes NHEJ by protecting the DSB ends from degradation in the G1 phase of the cell cycle (19).

In both yeast and mammals, the presence of MRX/MRN onto DSB ends leads to recruitment/activation of the Tel1/ATM checkpoint kinase (20,21), which signals cell cycle arrest through its kinase activity. While human ATM is a strong activator of the checkpoint triggered by DSBs, yeast Tel1 has a very minor role in checkpoint activation and its signaling activity becomes apparent only after generation of multiple DSBs (22). The MRX/MRN complex is also important, in combination with the Sae2/CtIP protein, for initiating 5'-3' nucleolytic degradation (resection) of the DSB ends to yield 3'-ended single-stranded DNA (ssDNA) tails that initiate HR (Figure 1). In particular, end resection occurs through a biphasic mechanism: first MRX and Sae2 clip 50-100 nucleotides from the 5' DNA ends, and then Exo1 or Sgs1-Top3-Rmi1 and Dna2 process the early intermediate to form extensive ssDNA regions (reviewed in 23,24).

Initiation of DSB processing and subsequent ssDNA generation lead to recruitment and activation of Mec1/ATR (25,26), which binds with its partner Ddc2/ATRIP to ssDNA coated by Replication Protein A (RPA) (Figure 1) (27,28). Full checkpoint activation in response to DSBs also depends on the loading of the 9-1-1 protein complex, which is formed by the Rad17, Mec3 and Ddc1 proteins in *S. cerevisiae* and is loaded onto DNA by the Rad24-RFC complex (29).

The choice of the pathway for DSB repair is regulated during the cell cycle (Figure 1). While NHEJ is used in the G1 cell cycle phase, HR in haploid cells occurs during S and G2, when DNA replication provides a sister chromatid as a repair template. This cell-cycle specificity depends on cyclin-dependent kinases (Cdks; Cdk1 in *S. cerevisiae*), which promote resection of the 5' DSB ends to yield 3'-ended ssDNA tails that are necessary to initiate HR and concomitantly inhibit NHEJ (30-32). The Sae2 protein has been shown to be a Cdk1 target in promoting ssDNA generation at DNA ends (33,34), a mechanism that is conserved in the Sae2 vertebrate homolog CtIP. However, as Sae2 only resects a relatively small amount of DNA and other nucleases and helicases are required for efficient DSB resection, Cdk1 likely has additional targets in promoting this event.

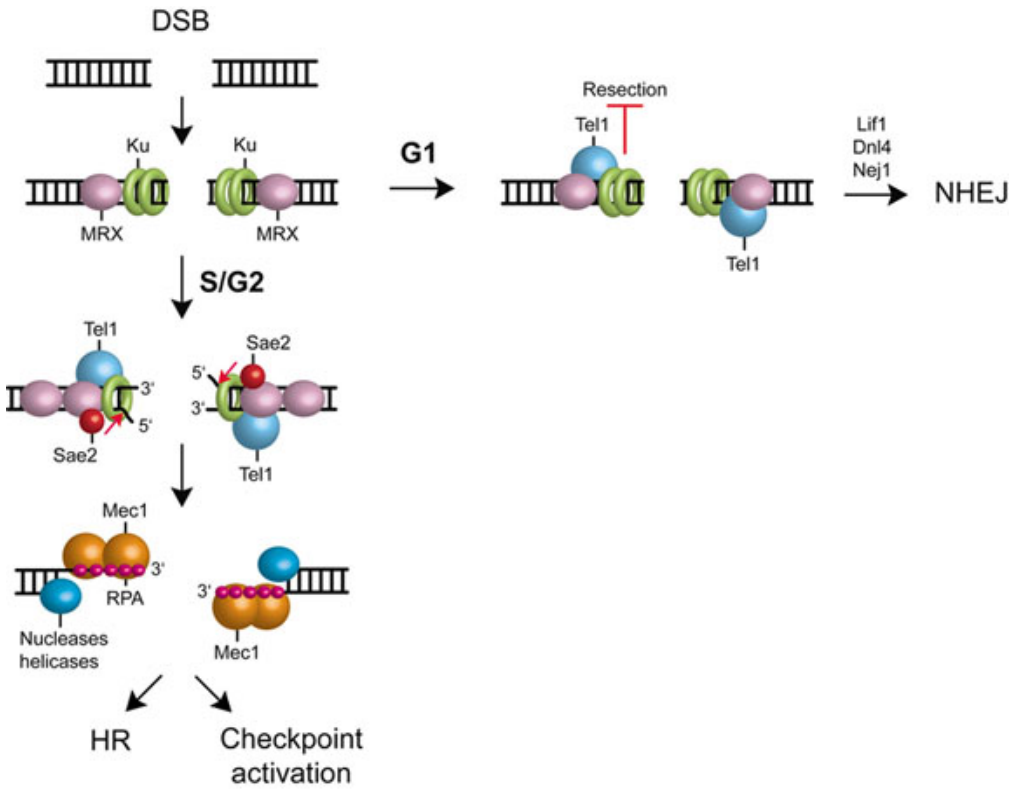

Figure 1. DNA damage response to a DSB in S. cerevisiae. Both MRX and Ku bind the DSB ends. In G1, Ku and MRX mediate recruitment of the NHEJ proteins (Lif1, Dnl4 and Nej1), which allow religation of the DSB ends. Recognition of the DSB by MRX also leads to Tel1 recruitment. Both Ku and the NHEJ proteins prevent initiation of resection. In S/G2, Sae2 is activated by Cdk1-dependent phosphorylation events. MRX and Sae2 then catalyze the initial processing of the 5' strand possibly by endonucleolytic cleavage (red arrows), which reduces the ability of Ku to bind the ends and promotes extensive 5'strand resection by Sgs1, Exo1 and Dna2. The 3'-ended ssDNA tails coated by RPA channel DSB repair into the HR pathway and eventually allow recruitment of Mec1 that leads to DNA damage checkpoint activation.

4. CHECKPOINT AND DNA REPAIR PROTEINS AT TELOMERES

Although natural chromosome ends are hidden from detection by the DNA damage recognition machinery, their features (DNA ends with ssDNA overhangs) could be subjected to DNA repair/recombination activities and could elicit a checkpoint response. While resection at DSBs is a prelude to HR, generation of 3'-ended ssDNA is a key step at telomeres because it provides the substrate for telomerase activity. In *S. cerevisiae*, the G overhang length ranges from 12-14 nucleotides in the G1 cell cycle phase to ~50-100 nucleotides during late S/G2 (35). Lagging-strand replication at the telomere will inevitably lead to a 3' single-stranded overhang due to removal of the terminal RNA primer, whereas the product of leading-strand replication requires further processing to convert blunt ends into 3' overhang structures (Figure 2). At such, it is still unclear whether a 5' resection activity also processes the lagging-strand telomere. The existence of distinct types of end processing at telomeres has been supported by studies that observed (i) chromosomal fusions only among the telomeric products of leading-strand replication in mammalian cells carrying mutant TRF2 (36,37), (ii) much longer G-tails in lagging-strand telomeres than in leading-strand telomeres in human cells lacking active telomerase (38), and (iii) shortening of G-tails by C-strand fill-in synthesis at mammalian lagging telomeres (39).

Notably, the nuclease requirements to resect DSBs and telomeres are similar. In fact, MRX, Sae2, Sgs1, Exo1 and Dna2, which are all required for generation of ssDNA at intrachromosomal DSBs, are also involved in the generation of the G-strand overhangs at telomeres (34,40-42). It has been recently shown that the MRX complex is present only at the leading-strand telomere (43), suggesting that MRX resects the products of leading strand DNA replication, while 3' ssDNA at lagging-strand telomeres could be generated by RNA primer removal and/or MRX-independent processing. In any case, as ssDNA accumulation at DSBs invokes an ATR/Mec1-dependent DDR when it exceeds a certain threshold, telomeres should display an inherent resistance to exonuclease attack to limit the amount of ssDNA at their ends.

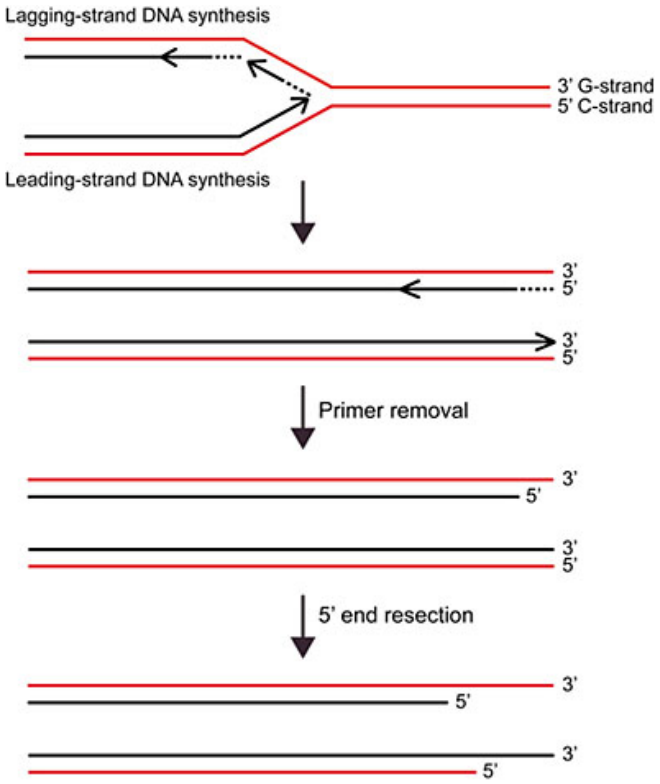

Figure 2. Telomere DNA replication. A telomere is replicated by a fork moving in a single direction, implying that the G-rich 3' strands are replicated discontinuously by the lagging-strand machinery, while the C-rich 5' strands are replicated continuously by the leading-strand machinery. While the strand replicated by lagging-strand synthesis will lead to a 3' single-stranded overhang due to removal of the last RNA primer and/or to incomplete synthesis of the last Okazaki fragment, a 5' resection activity is needed to create a G-tail on the leading-strand telomere, where replication generates a blunt end. Whether a 5' resection activity also processes the lagging-strand telomere is still unknown.

Telomeres and DSBs not only have common features but they also share a number of proteins (44), suggesting that, rather than excluding DNA repair/recombination/checkpoint activities, telomeres have evolved to harness the harmful consequences of the DDR. In both yeast and mammals, the checkpoint kinase Tel1/ATM is found at telomeres during S phase in an MRX/MRN-dependent manner and contributes to maintain telomere length (45-47). In S. cerevisiae, both Tel1 and MRX are required for recruitment of the telomerase subunits Est1 and Est2 specifically to short telomeres (48-52), which are preferentially elongated by telomerase (53). These findings suggest that MRX binding is the critical step that marks telomeres for elongation by telomerase.

The Ku heterodimer, which is necessary for NHEJ, is also involved in maintaining telomere length in S. cerevisiae, S. pombe and humans, and this function appears to be distinct from Ku function in NHEJ (reviewed in 54). Budding yeast Ku performs different functions at telomeres: i) it is important for recruiting telomerase to telomeres by binding to a stem-loop portion of TLC1 RNA (55-59), and ii) it protects telomeres from nuclease activities (60-63).

5. THE SHELTERIN COMPLEX

The shelterin complex plays an important role in maintaining telomere identity. In mammals, this complex is composed of the six core proteins TRF1, TRF2 (telomeric repeat binding factors 1 and 2), TIN2 (TRF1-interacting protein 2), POT1 (protection of telomeres 1), TPP1 (TIN2-POT1 organizing protein) and RAP1 (repressor/activator protein 1) (Figure 3). TRF1 and TRF2 are related to each other, as they both harbor a C-terminal Myb domain of the homeodomain subfamily, but differ at their N terminus that is acidic in TRF1 while it is basic in TRF2 (64-72). TRF1 and TRF2 bind the TTAGGG sequences in double-stranded DNA and recruit TIN2 and RAP1, respectively (73,74). The single-stranded G-overhang is bound by the POT1-TPP1 heterodimer. Both POT1 and TPP1 proteins contain OB (oligonucleotide/oligosaccharide binding)-fold domains (75-79). TPP1 also interacts with TIN2, and therefore these two proteins are postulated to bridge the shelterin components that bind double-stranded and single-stranded telomeric DNA (78,80,81). The TPP1 binding site in TIN2 appears distinct from its TRF2 binding site (82), raising the possibility that TIN2 might switch between TRF1/TRF2-bound and TPP1/POT1-bound states. In addition, the OB-fold domain at the N-terminus of TPP1 interacts with telomerase, suggesting a role for TPP1 in telomerase

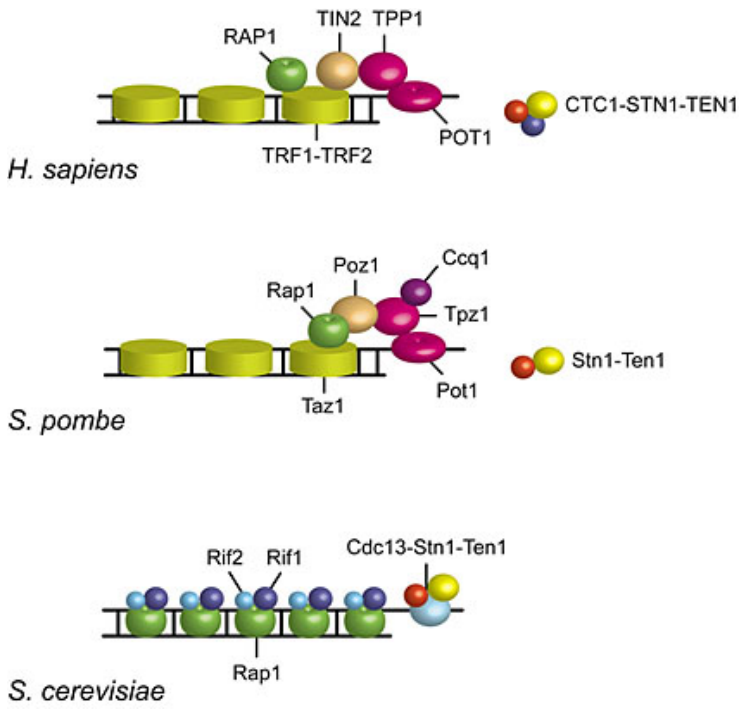

Figure 3. Telomere-specific proteins in mammalian, S. pombe and S. cerevisiae cells.

recruitment to chromosome ends (78,83). Finally, TRF2 forms a complex with RAP1, and this association is essential for RAP1 binding to telomeres (84). RAP1 contains three different domains: a Myb-like domain that may have a role in protein–protein interactions, an N-terminal BRCT motif and a C-terminal domain involved in TRF2 binding (74,85).

Shelterin-related proteins are also found at telomeres in other eukaryotes. Fission yeast telomeres are bound by a TPP1/POT1-like dimer, Tpz1/Pot1, and by a TRF-like protein, Taz1, which binds to Rap1 (Figure 3) (75,86,87). As in mammals, Taz1 recruits Rap1 (88,89), which interacts with Pot1 via a Poz1-Tpz1 bridge, thus establishing a link between the duplex and single-stranded telomeric DNA binding factors (87). Budding yeast telomeres appear to have diverged in protein composition (Figure 3). The only shelterin component structurally conserved in budding yeast is Rap1 (90-92), which contains two Myb domains with little homology to those found in TRFs proteins and interacts with the Rif1 and Rif2 proteins via its C-terminal domain (93-95). Unlike mammalian and fission yeast Rap1 that lack DNA binding activity, budding yeast Rap1 binds directly to telomeric double stranded DNA repeats.

6. SHELTERIN DYSFUNCTIONS AND THE DNA DAMAGE RESPONSE

The shelterin complex has a key role in allowing telomeres to escape the potential harmful effects of checkpoint activation, NHEJ and HR. Inhibition or deletion of individual shelterin components has revealed that this complex is required to avoid activation of the checkpoint kinases ATM and ATR. In fact, deletion of TRF2 in mouse embryonic fibroblasts or its inhibition by a dominant negative allele in human cells result in ATM-dependent DNA damage checkpoint activation (84,96,97). ATM activation under these conditions leads to recruitment of 53BP1, MDC1 and phosphorylated histone H2AX at the exposed telomeres, forming the so-called telomere dysfunction-induced foci (TIFs) (98,99). These events culminate in activation of the cell cycle regulator p53, leading to either cell cycle arrest or cell death. On the other hand, repression of ATR at telomeres does not require TRF2, but depends on POT1 (97). Conditional deletion of POT1 in mouse embryonic fibroblasts or POT1 downregulation in human cells elicit an ATR-dependent DNA damage response, as evidenced by formation of TIFs and phosphorylation of the Chk1 and Chk2 checkpoint kinases and of histone H2AX (100-103). Formation of TIFs is diminished when ATR-dependent checkpoint signalling is prevented, whereas it is unaltered in ATM-deficient cells, implicating POT1 in the repression of ATR. Thus, the two main checkpoint signalling proteins ATM and ATR appear to be independently inhibited by distinct shelterin components in mammalian cells (Figure 4A).

TRF2 and POT1 are also required to block DNA repair and recombination activities at mammalian telomeres (Figure 4A). Conditional TRF2 deletion in mouse embryonic fibroblasts or expression of a dominant negative allele in human cells result in DNA ligase IV-dependent telomeric fusions (84,104-106). By contrast, POT1 plays a minor role in NHEJ repression, as its knockdown leads to a marginal increase in telomere fusions (101,107,108). Telomere fusion events upon loss of TRF2 are restricted to the G1 phase of the cell cycle (109), whereas both TRF2

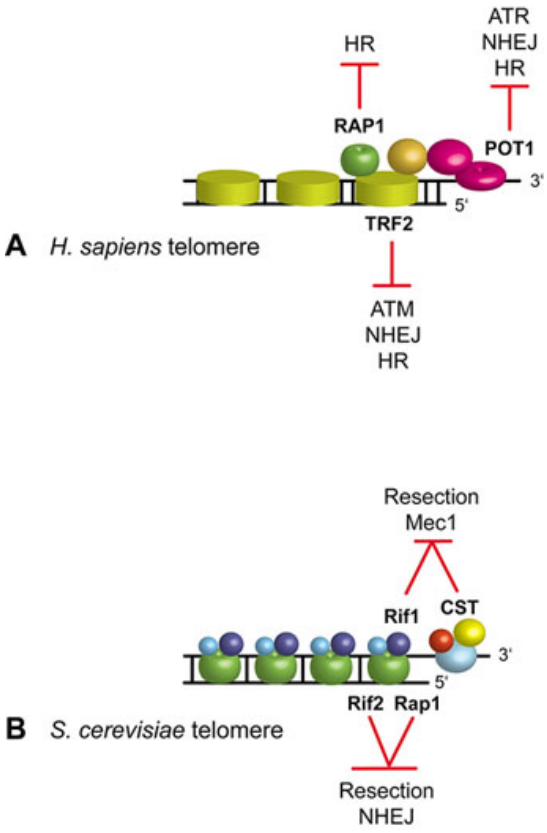

Figure 4. End-protection activities of telomeric proteins. (A) At mammalian telomeres, TRF2 represses ATM signaling and the NHEJ pathway, possibly by forming a t-loop. POT1 prevents ATR activation by inhibiting the binding of the ssDNA binding protein RPA, which is required to activate ATR. POT1 also contributes to repress NHEJ. Finally, TRF2, POT1 and RAP1 block HR at telomeres. (B) At S. cerevisiae telomeres, Rap1 and Rif2 inhibit 5'-3' resection of the telomeric DNA ends, possibly by blocking MRX access. Rap1 and Rif2 also repress the NHEJ repair pathway. Rif1 supports the function of the CST complex in preventing excessive resection at telomeric ends and Mec1-dependent checkpoint activation.

and POT1 contribute to inhibit NHEJ in G2 (101,110). Furthermore, TRF2, POT1 and RAP1 inhibit HR at telomeres (110-113). Interestingly, Ku is redundant with either TRF2 or POT1 in its ability to prevent HR at telomeres (112,114). Thus, Ku plays a dual role at telomeres: it initiates detrimental telomeric fusions as an integral part of the NHEJ machinery, but at the same time it protects telomeres from HR.

In *S. pombe*, like in mammals, the TRF-like protein Taz1 represses both NHEJ and HR at telomeres (115,116), whereas Pot1 blocks telomere nucleolytic degradation (117). The fusion events in Taz1-lacking cells appear to require the canonical NHEJ machinery. Protection from NHEJ-mediated telomeric fusions is also lost in the absence of Rap1 (118), whose recruitment at telomeres requires Taz1.

In *S. cerevisiae*, the Rap1 and Rif2 proteins inhibit both NHEJ and nucleolytic processing at telomeres (Figure 4B) (61,63,119,120). Generation of telomeric ssDNA in cells defective for Rif2 or Rap1 requires the MRX complex (61,62), suggesting that Rap1 and Rif2 prevent MRX action at telomeric ends. Rap1 and Rif2 inhibitory action on telomere processing is partially redundant with that of Ku, whose lack causes Exo1-dependent accumulation of telomeric ssDNA, as well as checkpoint-mediated cell cycle arrest at high temperatures (60,121-123). On the other hand, inactivation of Rap1 or Rif2 does not elicit a checkpoint response, suggesting that either the accumulated telomeric ssDNA is insufficient for RPA binding and Mec1 activation or that this ssDNA is still covered by Cdc13 (see below), which can inhibit the association of the checkpoint kinase Mec1 to telomeres (124).

Unlike Rif2 and Rap1, Rif1 is not involved in preventing telomeric fusions by NHEJ (120) and plays a very minor role in protecting telomeres from degradation (61). Instead, Rif1 is functionally connected with the protein complex CST (Figure 4B) (125), which is formed by Cdc13, Stn1 and Ten1, and binds to telomeric ssDNA overhangs (reviewed in 126). The three CST subunits, each of which contains one or more OB-fold domains, bear a structural resemblance to the three components of the RPA complex (127), suggesting that CST is a telomere-specific version of RPA. CST is required for telomere integrity, as loss of Cdc13, Stn1 or Ten1 function results in telomere degradation and checkpoint activation (128-133). Interestingly, deletion of *RIF1* in *cst* mutants increases generation of telomeric ssDNA that causes activation of the DNA damage checkpoint (125), indicating that Rif1 plays a unique role in supporting the capping function of CST. Whether functional connections between shelterin and CST exist also in other organisms remains to be determined.

7. HOW DOES SHELTERIN HIDE CHROMOSOME ENDS?

How TRF2 inhibits ATM activation at telomeres is unknown. One possibility is that TRF2 capping function depends on its ability to determine a proper terminal DNA end-structure. In mammals, the single-stranded telomeric 3' overhang invades the duplex part of the telomere, forming a structure called t-loop (134). Based on the observation that purified TRF2 has the ability to generate t-loop-like structures *in vitro*, TRF2 has been proposed to promote t-loop formation (135,136). Thus, as t-loops sequester the telomeric ends within a base-paired structure, TRF2-mediated t-loop formation would provide a mechanism to hide telomeres from NHEJ and ATM activation. Alternatively, or in addition, TRF2 might directly inhibit the ATM kinase, as TRF2 overexpression results in downregulation of ATM activation in response to DSBs at nontelomeric sites (137). Notably, t-loops are unlikely to occur at *S. cerevisiae* telomeres that are short (300 bp) and have short G-strand overhangs for most of the cell cycle. However, given that the ATM yeast ortholog, Tel1, has a very minor role in eliciting a DSB-induced checkpoint compared to Mec1 (22), the advantages that might be offered by t-loops are minimal in *S. cerevisiae*.

In the t-loop structure, the displaced D-loop contains a short segment of ssDNA that might activate ATR. Thus, t-loop formation does not explain how telomeres block activation of ATR, which is triggered by generation of ssDNA covered by RPA. ATR repression at telomeres does not require TRF2, but it depends on POT1 and TPP1. Based on the finding that POT1 is present on the telomeric ssDNA overhangs, it has been proposed that POT1/TTP1 prevents the binding of RPA to these overhangs (97). This competition may also play an important role in the repression of HR, which requires binding of RPA and other DNA recombination proteins to ssDNA.

The *S. cerevisiae* telomeric single-stranded overhangs are bound by Cdc13 in association with the RPA-like proteins Stn1 and Ten1 (126,127). It has been shown that Cdc13 binding to the single-stranded telomeric G tails attenuates Mec1 association with these DNA ends (124). Thus, as proposed for ATR inhibition by POT1, Cdc13 might prevent Mec1 activation by blocking RPA binding to telomeric ssDNA. In any case, it is well known that ssDNA accumulation at DSBs invokes an ATR/Mec1-dependent DNA damage response when it exceeds a certain threshold (138,139). Thus, one way to ensure that telomeres do not activate the DNA damage response would be to reduce the amount of ssDNA by resisting to nuclease attack. In *S. cerevisiae*, Rap1 and Rif2 have been found to limit MRX-dependent generation of telomeric ssDNA (61). The finding that MRX association at telomeres is enhanced in Rif2- or Rap1-defective mutants (61,140) suggests that Rap1 and Rif2 directly or indirectly inhibit MRX recruitment onto telomeric ends.

As discussed above, the Rif1 component of the budding yeast shelterin-like complex has a unique function in supporting the capping activity of the CST complex (125). CST-like complexes exist also in *S. pombe*, plants and mammals (141-144). Indeed, the yeast CST complex genetically and physically interacts with the polymerase alpha-primase complex (145,146) that is essential for lagging strand replication. Furthermore, the human CST-like complex increases polymerase alpha-primase processivity (147,148). As also Rif1 was found to functionally interact with the polymerase alpha-primase complex (125), one possibility is that Rif1 might participate together with CST in coupling telomerase-dependent telomere elongation to the priming of telomeric C strand synthesis.

8. REGULATION OF TELOMERE LENGTH BY SHELTERIN

Shelterin has an inhibitory effect on telomere length. Although telomeres vary considerably in length between organisms, the average length is kept within a narrow species-specific range, indicating that the telomerase enzyme is regulated *in cis* at individual telomeres. It was proposed that telomere length regulation is achieved through a negative feedback loop, where the telomeric DNA is bound by an inhibitor of telomerase in an amount proportional to telomere length (149). According to this model, elongation of a telomere by telomerase leads to an increased amount of the inhibitor, thus decreasing the probability of further elongation of this telomere by telomerase.

Some data in both yeast and mammals indicate that the shelterin proteins can be used to "size" the length of a telomere. In *S. cerevisiae*, the Rap1 protein negatively regulates telomere length (91,149), and the Rap1-binding proteins Rif1 and Rif2 contribute to this negative regulation (150). In fission yeast, loss of Taz1 results in uncontrolled telomere elongation (86). In humans, TRF1 or TRF2 overexpression causes telomere shortening, whereas a dominant negative form of TRF1 that removes the endogenous TRF1 from telomeres induces progressive telomere elongation (151,152). Furthermore, reduction of TIN2 or Rap1 protein levels also leads to telomere lengthening (78,81,153,154). POT1 is thought to have a key function in telomerase inhibition, because it is the only shelterin component that binds the telomeric single-stranded overhangs, which are necessary for telomerase action. In fact, extensive telomere elongation is observed when either a mutant form of POT1 lacking the DNA-binding domain is overexpressed or POT1 loading at telomeres is diminished (78,155). Because *in vitro* association of recombinant human POT1 with telomere oligonucleotide ends inhibits telomerase binding (156,157), it has been proposed that telomerase competes with POT1 for binding to the telomeric 3' single-stranded G-overhangs. Based on this model, depletion of the other shelterin components may lead to telomere overelongation, because their removal decreases POT1 recruitment to telomeres, thereby rendering the chromosome ends accessible to telomerase. On the other hand, POT1 might also promote telomerase action, especially when it is bound to TPP1 that is known to physically interact with telomerase (79,83).

The molecular details of shelterin-mediated regulation of telomerase activity are still largely unknown. Some insights into this topic are provided by data in *S. cerevisiae*, where telomerase binds and preferentially elongates short telomeres (53). In current models, telomerase is targeted to short telomeres by the Tel1 checkpoint kinase, which has been shown to be essential for the increased association of telomerase to short telomeres during late S/G2 (48-52). The MRX complex, once bound to short telomeres, enhances the localization of Tel1, which in turn promotes telomerase recruitment and activity possibly through phosphorylation events. Using a system that induces a specific chromosome break adjacent to telomere repeats, it has been shown that Rif2 inhibits MRX-dependent loading of Tel1 onto telomeric ends (140). Furthermore, Rif2 directly binds to the Xrs2 C-terminus, which has been implicated in Tel1 binding, and inhibits Tel1-Xrs2 interaction *in vitro*. These data support a model in which Rif2 counteracts telomerase action by competing with Tel1 for binding to the MRX complex. Indeed, Tel1 no longer binds preferentially to short telomeres in cells lacking Rif2, suggesting that reduced Rif2 content is a signal that marks short telomeres for preferential Tel1 binding and telomerase-mediated elongation (158).

9. SUMMARY AND PERSPECTIVES

Considerable progress has been made in unraveling the molecular mechanism that distinguish telomeres from DSBs. Work carried out in different experimental systems are converging to a scenario where protection of telomeric ends depends on a bulk of proteins (shelterin) with specificity to telomeric DNA. These proteins allow chromosome ends to escape the potential harmful effects of checkpoint activation, chromosome end-to-end fusions or sequences exchanges that involve two telomeres or a telomere and another part of the genome.

Notably, mounting evidence in recent years, suggests a potential role of shelterin components in cancer and aging (reviewed in 159). Studies using mice that were genetically modified for various shelterin components suggest a role for these proteins in cancer susceptibility and aging-related pathologies even in the presence of normal telomerase activity (reviewed in 160). Furthermore, expression of TRF1, TRF2, TIN2 and POT1 is altered in some human tumors and mutations in TIN2, TRF2 and TRF1 have been linked to some cases of Dyskeratosis congenita and aplastic anemia (reviewed in 160). Thus, a more thorough understanding of the molecular mechanisms that allows these proteins to maintain telomere identity is important to gain a full characterization of telomere regulation and to devise strategies for mitigating the impact of telomere dysfunction on cancer, aging and disease.

10. ACKNOWLEDGEMENTS

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Abbreviations: DDR: DNA damage response, DSB: double-strand break, ssDNA: single-strand DNA, HR: homologous recombination, NHEJ: non homologous-end joining, Cdk: Cyclin-dependent kinase

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