

REVIEW

DNA damage response at functional and dysfunctional telomeres

Maria Pia Longhese¹

Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, 20126 Milan, Italy

The ends of eukaryotic chromosomes have long been defined as structures that must avoid being detected as DNA breaks. They are protected from checkpoints, homologous recombination, end-to-end fusions, or other events that normally promote repair of intrachromosomal DNA breaks. This differentiation is thought to be the consequence of a unique organization of chromosomal ends into specialized nucleoprotein complexes called telomeres. However, it is becoming increasingly clear that proteins governing the DNA damage response are intimately involved in the regulation of telomeres, which undergo processing and structural changes that elicit a transient DNA damage response. This suggests that functional telomeres can be recognized as DNA breaks during a temporally limited window, indicating that the difference between a break and a telomere is less defined than previously assumed.

DNA double-strand breaks (DSBs) are among the most deleterious types of damage that can occur in the genome of eukaryotic cells, because failure to repair these lesions can lead to serious genetic instability. DNA breaks must be recognized as DNA damage to activate checkpoints and repair/recombination pathways, whose primary function is to repair the break. The natural ends of linear chromosomes must be distinguished from intrachromosomal DSBs to prevent triggering of DNA damage checkpoint and repair/recombination machineries. Here, I first summarize the features of both DSBs and telomeres and the current views on how telomerase is assembled and activated at telomeres. Then, I focus on the mechanisms protecting chromosome ends and how functional and dysfunctional telomeres can elicit a DNA damage response.

Telomere structure and homeostasis

Telomeres are specialized nucleoprotein complexes that define the physical ends of linear chromosomes. Their basic structure is conserved among eukaryotes and con-

sists of short tandem DNA repeats, which are G-rich in the strand containing the 3'-end. This strand is referred to as the G-strand, whereas the 5'-end containing the complementary strand is called the C-strand (Fig. 1). The G-strand extends beyond its complementary C-rich strand to form a single-stranded overhang, referred to as the G-tail. In mammals, the single-stranded telomeric 3' overhang invades the double-stranded telomeric DNA, base-pairing with the C-strand and displacing the G-strand. Because this strand invasion takes place at a distance from the physical end of the telomere, it generates a large duplex structure called the t-loop (Fig. 1A, top; Griffith et al. 1999). Similar structures have been reported to exist also in *Kluyveromyces lactis*, ciliates, and trypanosomes (Murti and Prescott 1999; Muñoz-Jordán et al. 2001; Cesare et al. 2007). Telomeres can also fold into G-quadruplex DNA, an unusual DNA conformation that is based on a guanine quartet (Parkinson et al. 2002).

Telomere length is maintained by a dynamic process of lengthening and shortening. Shortening can occur as a result of nucleolytic degradation and incomplete DNA replication, whereas lengthening is primarily accomplished by the action of a specialized reverse transcriptase called telomerase (Greider and Blackburn 1985) and occasionally by homologous recombination (HR) (Liu et al. 2007). Telomerase extends the 3' G-rich strand of a chromosome by reverse-transcribing the template region of its tightly associated RNA moiety. Although in vitro telomerase activity requires both its reverse transcriptase catalytic subunit (Est2 in *Saccharomyces cerevisiae* and TERT in mammals) and the RNA template (TLC1 in *S. cerevisiae* and hTR in humans), other factors are required for telomerase action in vivo. For instance, effective telomerase function in *S. cerevisiae* requires Est1 and Est3. The conventional DNA replication machinery can then fill in the C-rich strand, thus preventing any loss of DNA (for review, see Hug and Lingner 2006).

Notably, by following the elongation of a single telomere in yeast, it has been shown that telomerase does not act on every telomere in each cell cycle but instead exhibits an increasing preference for telomeres as their lengths decline, suggesting that telomeres switch between nonextendible and extendible states (Teixeira et al. 2004). The number of nucleotides added to a telomere in a single cell cycle varies between a few and >100

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¹Correspondence.

E-MAIL mariapia.longhese@unimib.it; FAX 390264483565.

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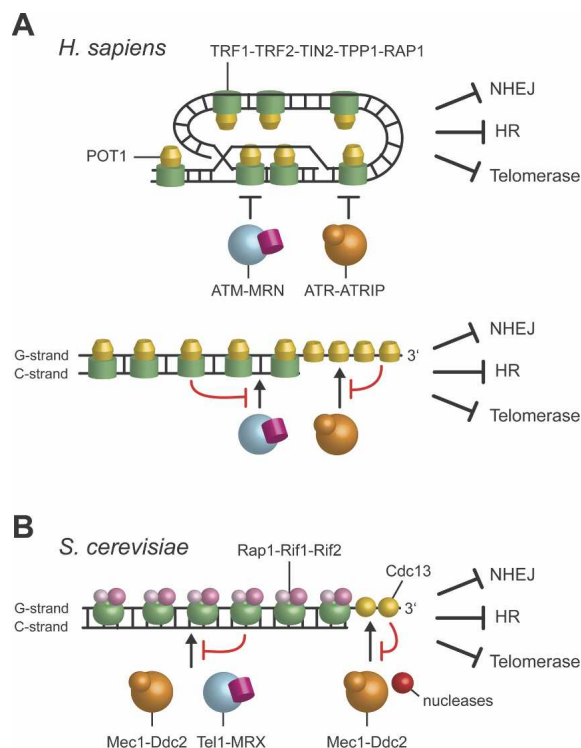


Figure 1. How chromosome end protection can be achieved. (A, top) In humans, telomeres folding into t-loops may protect the chromosome ends from NHEJ and HR. Moreover, t-loop structures may also inhibit the recruitment of telomerase and checkpoint proteins. (Bottom) For telomeres that do not adopt a t-loop conformation, the ssDNA-binding protein POT1 is thought to inhibit both ATR activation by blocking RPA recruitment to the telomeric ssDNA, and telomerase action. The dsDNA-binding protein TRF2 is proposed to prevent ATM activation. (B) In *S. cerevisiae*, Rap1 is thought to inhibit the recruitment of MRX, RPA, Mec1, and telomerase at telomeres, whereas Cdc13 binding to single-stranded telomere sequences prevents nucleases from binding, therefore inhibiting RPA recruitment and subsequent Mec1-dependent checkpoint activation.

nucleotides (nt). Telomere length affects telomerase repeat addition processivity *in vivo* (Chang et al. 2007). In fact, by examining the telomere extensions after one cell cycle, it has been shown that *S. cerevisiae* telomerase can dissociate from and reassociate to a given telomere during one round of telomere elongation. Repeat addition processivity is enhanced at extremely short telomeres, allowing cells to rapidly elongate them (Chang et al. 2007).

Although the presence of a functional telomerase is a necessary condition for telomere maintenance, it is not sufficient. ssDNA- and dsDNA-binding proteins with specificity for telomeric TG repeats are required to regulate telomerase activity and to protect chromosome ends from degradation and end-to-end joining events. In *S. cerevisiae*, Cdc13, which exhibits structural similarities to the ssDNA-binding protein Replication Protein A (RPA) (Gao et al. 2007), binds the single-stranded G-tail (Fig. 1B) and allows telomerase recruitment to the telomeric

3'-end (Nugent et al. 1996; Pennock et al. 2001; Bianchi et al. 2004). Moreover, Cdc13 limits telomerase-mediated telomere elongation in conjunction with Stn1 and Ten1, two essential Cdc13-associated proteins (Grandin et al. 1997, 2001; Chandra et al. 2001).

In both fission yeast and vertebrates, the POT1 (protection of telomeres 1) protein binds the single-stranded overhang on the 3' G-rich strand with high sequence specificity using two oligonucleotide/oligosaccharide-binding (OB) folds (Fig. 1A; Baumann and Cech 2001; Loayza and de Lange 2003; Lei et al. 2004). Mammalian POT1 forms the so-called shelterin complex with the telomeric-repeat-binding factor 1 (TRF1), TRF2, TRF-interacting protein 2 (TIN2), the transcriptional repressor/activator protein RAP1, and the POT1- and TIN2-organizing protein TPP1 (Liu et al. 2004; for a review, see de Lange 2005). TRF1 and TRF2 bind double-stranded telomeric repeats and anchor the complex along the length of telomeres (Fig. 1A; Chong et al. 1995; Billaud et al. 1997; Broccoli et al. 1997; Court et al. 2005). TRF1 and TRF2 recruit TIN2 (Kim et al. 1999), which can form a bridge with POT1 via TPP1 (Baumann and Cech 2001; Houghtaling et al. 2004; Liu et al. 2004; Ye et al. 2004b). TIN2 also connects TRF1 to TRF2, and this link contributes to the stabilization of TRF2 on telomeres (Houghtaling et al. 2004; Liu et al. 2004; Ye et al. 2004a). POT1 not only interacts with single-stranded telomeric DNA, but also accumulates along the duplex telomeric repeat array (Ye et al. 2004b). TRF2 also interacts directly with RAP1 and POT1 (Li et al. 2000; Yang et al. 2005).

Shelterin-related proteins are also found at telomeres in other eukaryotes. For example, POT1-like proteins are present in nearly all eukaryotes, and TRF1- and TRF2-like proteins are found in fission yeast and trypanosomes (Cooper et al. 1997; Li et al. 2005; Pitt et al. 2007), whereas Rap1 is present in fission yeast (Kanoh and Ishikawa 2001). Moreover, *S. cerevisiae* Rap1 is a highly divergent TRF ortholog, which binds double-stranded telomeric DNA together with its interacting factors Rif1 and Rif2 (Fig. 1B; Conrad et al. 1990; Hardy et al. 1992; Kyriou et al. 1992).

In both mammals and yeast, the shelterin and shelterin-like complexes negatively regulate telomerase by sequestering its DNA substrate in a closed conformation. In budding yeast, the Rap1 protein negatively regulates telomere length (Conrad et al. 1990; Marcand et al. 1997), and the Rap1-binding proteins Rif1 and Rif2 contribute to this negative regulation (Levy and Blackburn 2004). In fission yeast, loss of Taz1, a double-strand telomere-binding protein orthologous to TRF1 and TRF2, results in uncontrolled elongation of both the double- and single-stranded telomeric tracts (Cooper et al. 1997). In humans, TRF1 or TRF2 overexpression causes telomere shortening without affecting *in vitro* telomerase activity, whereas overexpression of a DNA-binding-deficient TRF1 variant results in progressive telomere elongation (van Steensel and de Lange 1997; Smogorzewska et al. 2000). In humans, reduction of TIN2 protein levels or overexpression of TIN2 mutant alleles that disrupt TIN2 interaction with TRF1 and TRF2 leads to telomere

lengthening (Kim et al. 2004; Ye et al. 2004b). Reduction of Rap1 protein levels also leads to mild telomere lengthening (Li and de Lange 2003; O'Connor et al. 2004).

A critical feature of this in *cis* negative regulation of telomerase access is the ability of shelterin to limit telomere length based on its total telomere-bound amount, which generates an inhibitory effect on telomerase, whose strength increases with increasing TG tract length (Marcand et al. 1997). Because the amount of shelterin and shelterin-like proteins bound to a telomere is proportional to the length of the TG repeat array, longer telomeres are proposed to have a greater probability of inhibiting telomerase access. Progressive telomere shortening causes the gradual loss of telomere-bound shelterin, and therefore a progressive relief of its inhibitory function on telomerase activity, thus allowing telomerase-mediated telomere elongation.

One possibility is that regulation of telomerase activity by shelterin is achieved at the level of its association with the telomere. In *S. cerevisiae*, studies of telomeric protein association to short and normal-length telomeres show that Est1 and Est2 are preferentially recruited to a shortened telomere, which is known to be a preferential substrate for telomerase (Bianchi and Shore 2007; Sabourin et al. 2007). Because Cdc13 loading to TG tracts is not regulated by telomere length (Bianchi and Shore 2007), this suggests that it is Cdc13 ability to recruit telomerase to telomeres that can be subjected to regulation by protein-bound TG tracks. In humans, regulation of telomerase activity might occur through the shelterin component POT1 (Loayza and de Lange 2003; Liu et al. 2004). In fact, overexpression of a mutant form of POT1 lacking the DNA-binding domain abrogates TRF1-mediated control of telomere length and induces rapid and extensive telomere elongation (Loayza and de Lange 2003). Moreover, in *Tetrahymena thermophila*, which contains two POT1 isoforms, POT1a removal results in telomere elongation (Jacob et al. 2007). Because in vitro association of recombinant human POT1 with telomere oligonucleotide ends inhibits telomerase binding (Kellerher et al. 2005; Lei et al. 2005), it has been proposed that POT1 limits telomerase from gaining access to the G-tail. Thus, the presence of more shelterin on longer telomeres increases the loading of POT1 on the telomeric overhang, where it blocks telomerase action.

Generation of ssDNA overhangs at both telomeres and DSBs

Although telomeric ends are apparently shielded from being recognized as DSBs, chromosome ends and accidental interruptions in duplex DNA molecules share important similarities. In fact, accidental DSBs are resected by 5'–3' exonucleases to generate 3'-ended ssDNA tails, which are channeled into different homology-dependent recombination pathways. The ends of chromosomes in humans, mice, ciliates, yeast, trypanosomes, and plants also carry ssDNA G-tails that serve as substrate for telomerase. Single-stranded G-tails of 50–100 nt are transiently detected in *S. cerevisiae* telomeres in late S phase

(Wellinger et al. 1993), while G-tails of 12–14 nt are present outside of S phase during the rest of the cell cycle (Larrivee et al. 2004). Accordingly, the Cdc13 protein is bound at *S. cerevisiae* telomeres throughout the cell cycle, with a maximal association in S phase, concomitantly with the increased amount of single-stranded telomeric DNA (Taggart et al. 2002). The RPA complex is also present at budding yeast telomeres, and its association increases during S phase (Schramke et al. 2004). In human telomeres, longer G-tails of 75–300 nt are detected throughout the cell cycle (Makarov et al. 1997; Wright et al. 1997).

These protruding G-strand overhangs play a central role in modulating telomere homeostasis, since they serve as a substrate for extension by telomerase (for a review, see Gilson and Geli 2007). While single-stranded G-tails can be generated during lagging-strand replication after removal of the last RNA primer, the chromosome end that is generated by the leading-strand polymerase is expected to be blunt-ended, hence lacking a G-tail (Lingner et al. 1995; Chai et al. 2006a). However, single-stranded G-tails can be detected at both ends of a chromosome even in the absence of telomerase (Wellinger et al. 1996; Makarov et al. 1997), suggesting the existence of an activity recessing the C-strand of newly synthesized blunt-ended molecules after completion of the leading-strand replication. Whether a 5' resection activity also processes the lagging telomere is still unknown.

The nucleolytic processing of both accidental DSBs and telomeres depends on a multifunctional highly conserved trimeric complex, known as MRN (Mre11–Rad50–Nbs1) in humans and MRX (Mre11–Rad50–Xrs1) in *S. cerevisiae*. In fact, resection of DNA ends generated in *S. cerevisiae* by the homothallic (HO) endonuclease partially depends on the MRX complex (Ivanov et al. 1994; Clerici et al. 2006). Moreover, MRX/MRN appears to be important for single-stranded G-tail generation, at least in the *de novo* telomere addition reaction (Diede and Gottschling 2001), and is required for wild-type length G-tails in both *S. cerevisiae* and humans (Larrivee et al. 2004; Chai et al. 2006b).

Mre11 contains four conserved N-terminal phosphoesterase motifs, and both human and budding yeast Mre11 harbor ssDNA endonuclease, 3'–5' dsDNA exonuclease, and DNA unwinding and DNA annealing activities (for a review, see Williams et al. 2007), suggesting that Mre11 nuclease activity may be responsible for end resection at both DSBs and telomeres. However, *S. cerevisiae mre11* nuclease-defective alleles cause only a mild sensitivity to ionizing radiations (IR) and do not seem to affect either resection of DNA ends generated by HO- or IR-induced sister chromatid recombination (Moreau et al. 1999). Moreover, although the nuclease-deficient *mre11* alleles block *de novo* telomere addition in G2, they allow normal telomere maintenance in cycling cells (Frank et al. 2006). This suggests that properties of MRX other than the nuclease activity are important to allow resection of both DSBs and telomeric ends. In any case, the disruption of the *S. cerevisiae* MRX complex does not completely abolish either DSB resection or

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G-tail generation (Larrivee et al. 2004; Clerici et al. 2006; Frank et al. 2006), suggesting the presence of redundant nuclease activities at both DSBs and telomeres. In *S. cerevisiae*, Exo1 may be one of these exonucleases, since the absence of MRX allows additional 5' resection of DSBs that is partially dependent on Exo1 (Llorente and Symington 2004; Clerici et al. 2006). Moreover, this exonuclease plays a critical role in C-strand resection at uncapped telomeres in yeast cells defective for Cdc13 or lacking the nonhomologous end joining (NHEJ) factor yKu70 (Maringele and Lydall 2002; Zubko et al. 2004).

In humans, shelterin appears to regulate the nucleolytic processing of the 5' C-strand. In fact, when human TRF2 is inhibited, the overall amount of single-stranded TTAGGG repeats is diminished (van Steensel et al. 1998; Celli and de Lange 2005). Remarkably, TRF2 physically interacts with Apollo, whose N-terminal domain is closely related to that of Artemis, a factor involved in V(D)J recombination and DNA repair (Lenain et al. 2006; van Overbeek and de Lange 2006). Apollo exhibits a 5'-to-3' DNA exonuclease activity in vitro, raising the possibility that it might contribute to the resection of the C-rich strand of telomeric DNA (Lenain et al. 2006).

Generation of ssDNA at both DSBs and telomeres requires the activity of cyclin-dependent kinases (CDK), whose sequential action determines cell cycle progression. In fact, inhibition of *S. cerevisiae* Cdk1 activity in G2-arrested cells prevents DSB processing and HO-induced DSB repair by HR (Ira et al. 2004; Aylon and Kupiec 2005; Jazayeri et al. 2006). Moreover, inhibition of an ATP analog-sensitive variant of Cdk1 completely blocks the addition of telomere repeats in a de novo telomere addition assay and prevents generation of G-strand overhangs at both native and uncapped *S. cerevisiae* *cdc13-1* telomeres (Frank et al. 2006; Vodenicharov and Wellinger 2006). In G1 cells, the Clb-Cdk1 complexes are kept inactive by their inhibitor Sic1. Therefore, resection at telomeres is limited to late S and G2/M, coinciding with a time frame in which the length of the G-tails increases and telomerase can elongate telomeric DNA (Wellinger et al. 1993; Marcand et al. 2000). Interestingly, Cdk1 activity is necessary but not sufficient for degradation of *S. cerevisiae* unprotected telomeres, which also requires the passage of the replication fork (Dionne and Wellinger 1998; Vodenicharov and Wellinger 2006). This suggests that the Clb-Cdk1-dependent processing activity may be brought to telomeres by the replication fork. Since HR relies on ssDNA 3' overhangs to initiate strand invasion, the dependence of 5' strand resection on active Clb-Cdk1 complexes indicates that Clb-Cdk1 activity also controls recombination at both telomeres and DSBs. The targets through which Clb-Cdk1 stimulates end resection are not known. Because the MRX complex is required to process both DSBs and telomeres, it might be a potential target of Clb-Cdk1.

Intrachromosomal DSBs elicit a DNA damage response

When an intrachromosomal DNA break occurs, it must be recognized as DNA damage to activate repair/recom-

ination pathways, whose primary function is to repair the break. DSB repair can occur by NHEJ, which is able to join two chromosomal ends with no, or minimal, base-pairing at the junction. If a DSB is not repaired by NHEJ, 5'-to-3' resection of the DSB ends generates 3'-ended ssDNA tails, which are channeled into different homology-dependent recombination pathways (for a review, see Krogh and Symington 2004).

In both yeast and human, generation of accidental DSBs triggers the activation of the DNA damage checkpoint pathway (Fig. 2A), whose primary purpose is to arrest the cell cycle in response to DNA damage, thereby coordinating cell cycle progression with DNA repair capacity (for reviews, see Longhese et al. 2006; Shiloh 2006). Key players in the DNA damage checkpoint response belong to a protein kinase family, including mammalian Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad3-related (ATR), *S. cerevisiae* Tel1 and Mec1, and *S. pombe* Tel1 and Rad3 (for reviews, see Longhese et al. 2006; Shiloh 2006). Mec1 and Rad3 are more similar to ATR, whereas Tel1 is more similar to ATM. Both yeast Tel1 and human ATM appear to bind DNA through their interaction with the MRX and MRN complexes, respectively (Nakada et al. 2003; Falck et al. 2005). Rather than using MRX/MRN, Mec1, Rad3, and ATR function in a complex with Ddc2 (Paciotti et al. 2000), Rad26 (Edwards et al. 1999), and ATRIP (Cortez et al. 2001), respectively. Mec1/ATR ability to transmit and amplify the DNA damage signals is enhanced by a proliferating cell nuclear antigen (PCNA)-like complex called Ddc1-Rad17-Mec3 in budding yeast, and Rad9-Rad1-Hus1 in both mammals and *Schizosaccharomyces pombe*, respectively (Majka et al. 2006). This complex is recruited to damaged DNA through a replication factor C (RF-C)-like complex, consisting of the four small RF-C subunits that interact with budding yeast Rad24, or its *S. pombe* and human ortholog Rad17.

Several lines of evidence indicate that the signaling event for DSB-induced checkpoint activation is the recruitment of the MRX/MRN complex to the break site (Fig. 2A), which occurs independently of any other DNA damage response protein examined so far (Lisby et al. 2004). Remarkably, MRX/MRN recognition of DSBs leads to the recruitment of Tel1/ATM (Nakada et al. 2003; Falck et al. 2005; You et al. 2005). Because Tel1/ATM signaling is up-regulated when MRX occupancy at DSBs is prolonged by the nonnull *rad50s* allele or the deletion of the *SAE2* gene (Usui et al. 2001; Lisby et al. 2004; Morales et al. 2005; Clerici et al. 2006), this indicates that the loading of MRX/MRN to the DSBs initiates checkpoint activation. Notably, the checkpoint functions of Tel1 appear more furtive than those of ATM. In fact, Tel1-deficient cells do not show obvious hypersensitivity to DNA-damaging agents and are not defective in checkpoint activation in response to a single HO-induced DSB, which depends primarily on Mec1 (Ira et al. 2004; Clerici et al. 2006; Mantiero et al. 2007). However, recent evidence indicates that Tel1 can activate the checkpoint response to DSBs independently of Mec1, although its signaling activity becomes apparent

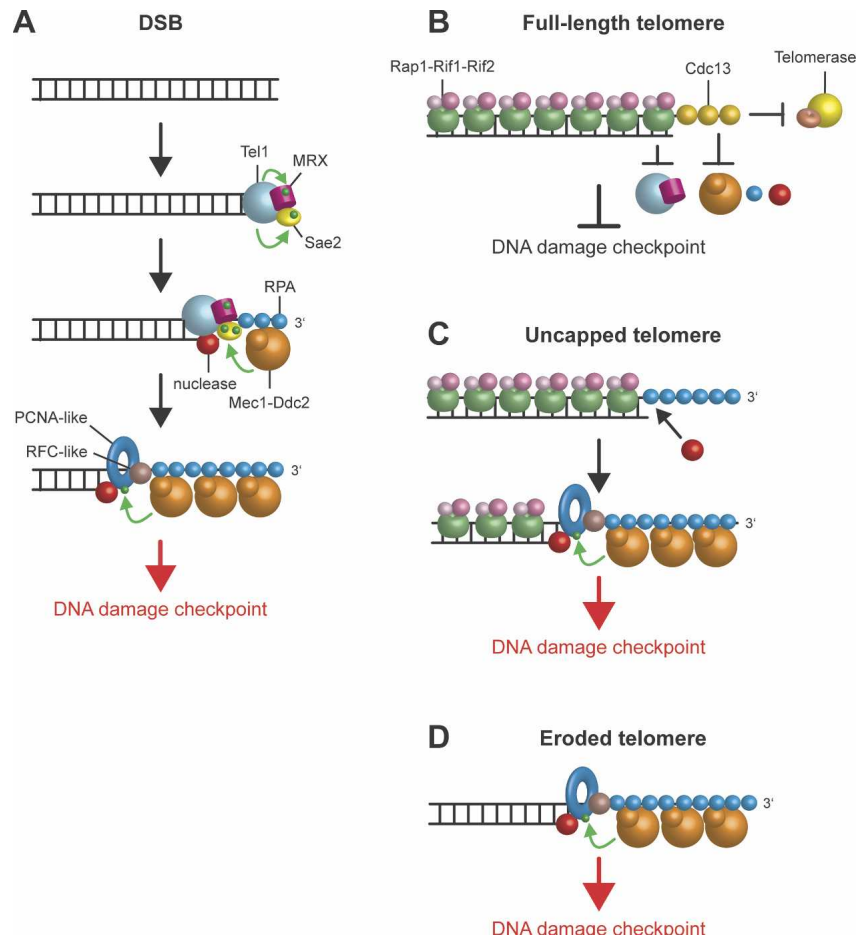


Figure 2. DNA damage response to DSBs and dysfunctional telomeres. (A) Intrachromosomal DSBs trigger a DNA damage checkpoint response. When a DSB occurs, the MRX complex and other factors localize to the unprocessed break. DSB recognition by MRX allows checkpoint activation by recruiting Tel1. Tel1 in turn phosphorylates Sae2, which is recruited to DSB ends independently of MRX. MRX, Sae2, and Tel1 contribute to resection of DSB ends by exonucleases to generate 3'-ended ssDNA tails coated by RPA, which allow the loading of Mec1-Ddc2 and subsequent Mec1-dependent checkpoint activation. Mec1 activation is also supported by independent loading of the PCNA-like Ddc1-Rad17-Mec3 complex by Rad24-RFC. (B) Full-length telomeres are protected from checkpoint activation. The presence of ssDNA- and dsDNA-binding proteins on full-length chromosomal ends inhibits recruitment of MRX, RPA, nucleases, telomerase, and checkpoint proteins. (C,D) Telomeres lose protection after loss of telomeric ssDNA- and dsDNA-binding proteins (uncapped telomere) or telomerase (eroded telomere). (C) In the absence of the ssDNA-binding protein Cdc13, telomerase recruitment is impaired, and nucleases can access the chromosome end. This leads to C-rich strand degradation and accumulation of RPA-bound ssDNA, which elicits activation of a Mec1-dependent DNA damage checkpoint response. (D) RPA-bound ssDNA accumulates at telomeres also after telomere erosion due to telomerase loss. The *S. cerevisiae* nomenclature is used. Green arrows indicate phosphorylation events.

only in the presence of multiple DSBs (Mantiero et al. 2007). Tel1 ability to sense and transduce the DSB signal is disrupted when DSB ends are subjected to 5'–3' exonucleolytic degradation (Mantiero et al. 2007), suggesting that Tel1/ATM and MRX/MRN are recruited to blunt or minimally processed DSB ends to initiate DSB signaling.

In both yeast and humans, the MRN–ATM and MRX–Tel1 complexes, once recruited to DSBs, also contribute to generate 3'-ended ssDNA (Falck et al. 2005; Adams et al. 2006; Jazayeri et al. 2006; Myers and Cortez 2006; Mantiero et al. 2007), the critical intermediate structure recognized by the Mec1–Ddc2 and human ATR–ATRIP complexes (Fig. 2A; Zou and Elledge 2003). This implies that initiation of DSB resection is a critical step for the transition from Tel1/ATM- to Mec1/ATR-dependent checkpoint activation.

Once DNA perturbations are sensed, the checkpoint signals are propagated through evolutionarily conserved protein kinases, which are called Rad53 and Chk1 in *S. cerevisiae*, and Chk2 and Chk1 in humans, respectively. Noteworthy, Rad53 and Chk1 activation is not governed by their simple interaction with Mec1 or Tel1, but requires a stepwise process. In particular, it has been pro-

posed that the Rad9 protein acts first as an adaptor to mediate the interaction between Mec1 and Rad53 (Sweeney et al. 2005), and then as a scaffold to allow Rad53 autophosphorylation and activation (Gilbert et al. 2001).

Telomere dysfunctions elicit a DNA damage response

One of the primary functions of ssDNA- and dsDNA-binding telomeric proteins is to suppress checkpoint response, repair, and recombination at telomeres (Fig. 2B). When chromosome end protection fails, dysfunctional telomeres are targeted by the DNA repair and recombination apparatus. The outcomes of such events at telomeres range from the generation of chromosomal abnormalities, general hallmarks for cancer cells in humans, to permanent cell cycle arrest and cell death. NHEJ of two telomeres creates circular or dicentric chromosomes. Furthermore, illegitimate recombination could generate aberrant telomere length and extrachromosomal telomeric circles. Indeed, telomeric circles are found in *Kluyveromyces lactis* cells with capping deficiencies (Groff-Vindman et al. 2005; Cesare et al. 2007) as well as in *S. cerevisiae*, mouse, and human cells lacking functional telomerase (Cesare and Griffith 2004;

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Wang et al. 2004; Larrivee and Wellinger 2006). Finally, HR at t-loops can lead to sudden loss of telomeric DNA (Wang et al. 2004). In fact, if the 5'-end of the telomere would pair with the displaced loop, this may generate a double Holliday Junction (dHJ), whose resolution would delete the whole loop, leaving a telomeric DNA circle and a drastically shortened telomere at the chromosome end.

In yeast, mice, and humans, telomeres that are stripped of the protective shelterin complex evoke a DNA damage response and may undergo repair attempts by HR or NHEJ. In fact, POT1 lack of function results in rapid loss of telomeric DNA, telomere fusions, aberrant HR, chromosome segregation defects, increase in the amount of G-strand overhangs, and cell death in both fission yeast and vertebrates (Baumann and Cech 2001; Veldman et al. 2004; Hockemeyer et al. 2005; Yang et al. 2005; Churikov et al. 2006; He et al. 2006; Wu et al. 2006). In addition, budding yeast Rap1 loss results in frequent fusions between telomeres by NHEJ (Pardo and Marcand 2005), and ligase IV-dependent chromosome fusions accumulate in *S. pombe* cells lacking Rap1 or Taz1 (Ferreira and Cooper 2001; Miller et al. 2005). In mammals, TRF2 protects telomeres by repressing both HR and NHEJ. In fact, in mice, the frequency of exchanges between sister telomeres increases when both TRF2 and Ku70 are absent (Celli et al. 2006). Moreover, a dramatic increase in telomere–telomere fusions has been associated with expression of a dominant-negative allele of TRF2 (van Steensel et al. 1998). Finally, a conditional knockout of TRF2 in mouse embryonic fibroblasts reveals an even more dramatic phenotype with virtually all telomeres engaged in end-to-end fusions (Celli and de Lange 2005). In both yeast and mammals, cells lacking DNA ligase IV or Ku70 display reduced ability to fuse telomeres after shelterin inhibition, indicating that these fusion events require the same factors as normal NHEJ (Ferreira and Cooper 2001; Smogorzewska et al. 2002; Mieczkowski et al. 2003; Celli and de Lange 2005). Because TRF2 can promote t-loop formation in vitro (Stansel et al. 2001), one possibility is that TRF2 blocks NHEJ by hiding the telomere ends in the t-loop structure that could block end-loading of Ku (Celli et al. 2006). However, human telomeres become fusogenic only upon loss of 12.8 double-stranded TTAGGG repeats (Capper et al. 2007), suggesting that telomere protection is still intact even on telomeres that are too short to form t-loops.

In mammals, NHEJ at telomeres requires the removal of the long telomeric 3' overhangs by the nucleotide excision repair endonuclease ERCC1/XPF, which cuts DNA duplexes adjacent to a 3' ssDNA flap (Zhu et al. 2003). The absence of ERCC1/XPF in cells that have uncapped telomeres due to TRF2 inhibition leads to the generation of the so-called telomeric DNA-containing double minute chromosomes (TDMs), circular extra-chromosomal elements, appearing as two closely positioned dots in metaphase. Presumably, these TDMs are generated through the recombination between the end of telomeres and interstitial telomere-related TTAGGG-like sequences, thus generating a terminally deleted

chromosome and a circular product containing telomeric DNA (Zhu et al. 2003).

The lack of shelterin also leads to activation of the DNA damage checkpoint (Fig. 2C; for a review, see Viscardi et al. 2005). In *S. cerevisiae*, inactivation of the telomere end-binding protein Cdc13 leads to C-rich strand degradation and accumulation of very long ssDNA regions that extend into the nontelomeric DNA (Fig. 2C; Garvik et al. 1995; Zubko et al. 2004). This hallmark of telomere dysfunction activates a DNA damage checkpoint preventing anaphase entry, which requires Mec1, Ddc2, Rad9, Rad24, Ddc1, Mec3, Rad17, Rad53, and Chk1 (Garvik et al. 1995; Lydall and Weinert 1995).

In mammals, the loss of TRF2 activates the ATM-dependent pathway, leading to p53 up-regulation and p21-mediated G1/S arrest (Karlseder et al. 1999; Celli and de Lange 2005). After inhibition of TRF2 or when telomeres become critically short, 53BP1, γ -H2AX, the MRN complex, Rif1, and phosphorylated ATR and ATM accumulate at telomeres, thus forming the so-called telomere dysfunction-induced foci (TIFs) (Takai et al. 2003). TIFs are also generated when the shelterin components TIN2 or POT1 are inhibited (Kim et al. 2004; Hockemeyer et al. 2005). The consequences of TRF2 loss are exacerbated when the expression of its interacting protein Apollo is inhibited, suggesting that TRF2 works together with Apollo to protect chromosome termini (Lenain et al. 2006; van Overbeek and de Lange 2006).

A DNA damage response is also elicited in response to telomere erosion by inhibition of telomere replication (Fig. 2D). In *S. cerevisiae*, this response is mediated by Mec1–Ddc2 and by their accessory factors Rad24 and Ddc1–Rad17–Mec3 complex (Ijpma and Greider 2003). In contrast to what is observed in response to *cdc13*-induced damage, where checkpoint activation is entirely Rad9-dependent, both Rad9 and Mrc1 are required to activate the checkpoint in telomerase-deficient cells (Ijpma and Greider 2003; Grandin and Charbonneau 2007). Because telomeric chromatin has the ability to form various unusual structures (heterochromatin-like, t-loop, and G-quadruplex structures), replication forks experience a physiological pausing and/or stalling whenever they reach telomeres (Ivessa et al. 2002; Makovets et al. 2004; Verdun and Karlseder 2006). Because Mrc1 mediates the response to replication blocks after treatment with the DNA synthesis inhibitor hydroxyurea (HU) (Alcasabas et al. 2001), inhibition of telomere replication might be recorded as a DNA replication stress, thus explaining Mrc1 activation. Mrc1 also contributes to protect uncapped telomeres from Exo1-dependent degradation, suggesting that telomere capping is intimately linked with DNA replication (Tsolou and Lydall 2007).

Potential mechanisms for checkpoint inhibition at functional telomeres

It is well known that accumulation of ssDNA at DSBs above a certain threshold invokes an ATR/Mec1-dependent DNA damage response (Lee et al. 1998; Zou and

Elledge 2003). Although ssDNA overhangs are present at chromosome ends, budding yeast telomeres transiently acquire long (50–100 bases) single-stranded G-tails only during a short time window in late S phase (Wellinger et al. 1993). Thus, one possibility, at least in *S. cerevisiae*, is that ssDNA in the G-tails does not persist long enough to be detected by the checkpoint machinery. Mammalian telomeres have long single-stranded telomeric ends (Makarov et al. 1997). One elegant solution to establishing an inaccessible state of a telomere in mammals is the remodeling of linear DNA into t-loops, which can conceal the chromosome ends from being recognized by the DNA damage checkpoint (Fig. 1A, top). Although their role in t-loop formation has not yet been tested in vivo, shelterin components have DNA remodeling activities that are relevant for t-loop formation. In particular, purified TRF2 can remodel an artificial telomeric substrate into loops in vitro (Stansel et al. 2001). Interestingly, it has been recently shown that TRF2 generates positive supercoiling and condenses DNA (Amiard et al. 2007). This topological activity correlates with the ability to stimulate strand invasion, suggesting that TRF2 complexes, by constraining DNA around themselves in a right-handed conformation, can induce untwisting of the neighboring DNA, thereby favoring strand invasion (Amiard et al. 2007).

However, given that both *S. cerevisiae* and *S. pombe* telomeres are presumably too short to generate t-loops and that it is presently unclear whether all telomeres or only a subset of them adopt the t-loop conformation in other organisms, alternative mechanisms should exist to prevent telomeric single-stranded overhangs from eliciting a DNA damage response. The finding that inhibition of POT1 triggers an ATR-dependent checkpoint response (Lazzerini Denchi and de Lange 2007) suggests that POT1 inhibits ATR activation by blocking the recruitment of RPA to the single-stranded telomeric DNA (Fig. 1A, bottom; Lei et al. 2004, 2005; Kelleher et al. 2005). Similarly, the analysis of the mechanism by which *S. cerevisiae* TG repeats inhibit checkpoint signaling from the adjacent DSB has shown that Cdc13 binding to TG sequences at a newly formed DSB prevents Exo1 from binding to the DNA end, therefore inhibiting Exo1-dependent degradation and RPA recruitment (Hirano and Sugimoto 2007). As a result, the TG sequences attenuate the Mec1-dependent checkpoint (Fig. 1B).

In any case, because even unprocessed DSBs can elicit a DNA damage response that depends primarily on MRX/MRN and ATM/Tel1 (Mantiero et al. 2007), protection of single-stranded telomeric overhangs may be insufficient to prevent a DNA damage response at telomeres. Consistent with this hypothesis, it has been shown that neither the presence of long 3'-ended single-stranded overhangs nor t-loop formation is essential to prevent NHEJ-mediated ligation of human telomeric ends in vitro (Bae and Baumann 2007). Instead, a tandem array of 12 telomeric repeats is sufficient to impede illegitimate repair at nearby DNA ends (Bae and Baumann 2007). This suggests that full-length telomeres have acquired a structure that may physically hide the telomeric

ends from DNA repair/recombination/checkpoint activities and telomerase. The existence of an anti-checkpoint activity has been demonstrated in *S. cerevisiae*, by virtue of its capacity to inhibit checkpoint activation from a DSB through adjacent telomeric sequences (Michelson et al. 2005). Although its precise molecular nature is unknown, the effect of this anti-checkpoint is exerted only in *cis*, suggesting that telomeres create a local protein environment that inhibits checkpoint signaling.

The telomeric anti-checkpoint activity may reside on shelterin, whose lack in both yeast and humans elicits a DNA damage response. Long arrays of Rap1 at full-length *S. cerevisiae* telomeres protect them against nucleolytic attack and activation of the DNA damage checkpoint. In fact, by examining events at DNA breaks generated adjacent to either natural telomeric sequences or arrays of Rap1-binding sites that vary in length, it has been shown that Rap1 binding at long TG tracts inhibits recruitment of both Mre11 and Cdc13 (Negrini et al. 2007). Moreover, it prevents exonucleolytic resection and binding of both RPA and Mec1 to the telomeric ends (Fig. 1B; Negrini et al. 2007). In humans, attenuation of checkpoint signaling at telomeres may be achieved through the action of the shelterin component TRF2 (Fig. 1A, bottom), whose loss activates a checkpoint response that is primarily dependent on ATM (Lazzerini Denchi and de Lange 2007). Consistent with this hypothesis, biochemical fractionation and reconstitution experiments have shown that tandem array-dependent telomere protection is mediated by a complex containing human TRF2 and RAP1 (Bae and Baumann 2007).

How TRF2 prevents ATM activation is not yet clear. Because TRF2 binds to a region in ATM that contains Ser1981 (Karlseder et al. 2004), which in turn undergoes DNA damage-induced autophosphorylation in *trans* leading to ATM activation (Bakkenist and Kastan 2003), it has been suggested that TRF2 may block this activation. Because the abundance of shelterin at telomeres depends on the length of the duplex telomeric repeat array, the diminished loading of TRF2 and POT1 at critically short telomeres may relieve inhibition of the ATM and ATR kinases, resulting in checkpoint-mediated cell cycle arrest and inappropriate DNA repair at telomeres (Lazzerini Denchi and de Lange 2007).

Interestingly, removal of POT1 leads to an ATR-dependent DNA damage response in *Atm*^{-/-} cells, suggesting that ATM is not required for the activation of ATR under these conditions (Lazzerini Denchi and de Lange 2007). This finding contrasts with the situation at irradiation- and HO-induced DSBs, where ATM and Tel1 signaling can promote activation of ATR and Mec1, respectively, possibly by stimulating the formation of ssDNA at the broken ends (Jazayeri et al. 2006; Mantiero et al. 2007).

DNA repair/recombination and DNA damage checkpoint proteins are involved in telomere homeostasis

Although one of the primary functions of telomeric proteins is to suppress both repair/recombination and

checkpoint pathways, DNA repair/recombination and checkpoint proteins are found at functional telomeres each cell cycle and assist the telomere in assuming its proper structure and function.

DNA repair/recombination proteins at telomeres

Both human Ku70–Ku80 and ERCC1/XPF interact with components of the shelterin complex (Song et al. 2000; Chai et al. 2002; Zhu et al. 2003), suggesting that shelterin cooperates with them to regulate telomere length and protection. Consistent with this hypothesis, the Ku70–Ku80 heterodimer is involved in maintaining telomere length in *S. cerevisiae*, *S. pombe*, and humans, and this function appears to be distinct from its role in NHEJ (for a review, see Fisher and Zakian 2005). Yeast *yku* mutants have critically short telomeres with long G-tails that persist throughout the cell cycle (Gravel et al. 1998; Polotnianka et al. 1998). Because both the telomere length defects and the generation of long G-tails in a *ykuΔ* mutant are suppressed by *EXO1* disruption (Maringele and Lydall 2002; Bertuch and Lundblad 2004), the lack of telomere protection toward Exo1 and/or other nucleases in *yku* mutants may explain the generation of the observed constitutive overhangs. Notably, yKu proteins interact specifically with a stem-loop portion of *TLC1* RNA in yeast cells (Peterson et al. 2001; Stellwagen et al. 2003), and this interaction is essential for Est2 telomere association in G1 (Fisher et al. 2004), indicating that Ku promotes telomere addition by targeting telomerase to chromosomal ends.

Although telomeres prevent chromosome fusions by HR, the latter provides a telomerase-independent mechanism for maintaining telomeric repeats in some budding yeast mutants, in a few experimentally immortalized human cells, and in a fraction of cancer cells (Lundblad and Blackburn 1993; Muntoni and Reddel 2005). In fact, when *S. cerevisiae* telomeres become critically short in the absence of telomerase, cells experience progressive erosion of telomeric repeats and cease to divide (Lundblad and Szostak 1989). However, rare survivors emerge that use HR to maintain their telomeres (Lundblad and Blackburn 1993). Based on different genetic requirements and telomeric DNA rearrangements, two pathways for survivor generation have been identified. Both pathways require Rad52, but generation of type I survivors relies on proteins belonging to the Rad51 epistasis group, while type II survivor generation uses a Rad50-dependent amplification of the TG repeats (for a review, see Lundblad 2002). A characterization of telomeric DNA in telomerase- and capping-independent budding yeast survivors has revealed the presence of extrachromosomal circular DNA molecules that are probably recombination products (Larrivee and Wellinger 2006). Because HR relies on ssDNA 3' overhangs to initiate strand invasion, initiation of survivor pathways should depend on generation of single-stranded G-tail overhangs. Consistent with this hypothesis, the exonuclease Exo1 is implicated in generating type I and type II yeast survivors (Maringele and Lydall 2004).

DNA damage checkpoint proteins at telomeres

Telomere structure and function depend on proteins known to be required for DNA damage checkpoint activation. In both yeast and human, the Tel1/ATM checkpoint kinase is found at telomeres during S phase in an MRX/MRN-dependent manner (Verdun and Karlseder 2006; Hector et al. 2007; Sabourin et al. 2007). Tel1/ATM contributes to maintain telomere length. In fact, ATM inhibition by caffeine treatment or MRN elimination leads to extended telomere exposure and telomere fusions in human cells (Bi et al. 2004; Ciapponi et al. 2004; Verdun et al. 2005; Foster et al. 2006). Moreover, both *S. cerevisiae* telomerase and Tel1 are needed to prevent telomeres from fusing to DSBs by NHEJ (Chan and Blackburn 2003). Tel1 inactivation in the absence of telomerase also increases the rate of gross chromosomal rearrangements, which are represented by NHEJ-mediated translocations and chromosome fusions (Pennaneach and Kolodner 2004). Finally, the lack of Tel1/ATM in *S. cerevisiae*, *S. pombe*, and human cells causes telomere shortening (Metcalf et al. 1996; Dahlen et al. 1998; Matsuura et al. 1999; Ritchie et al. 1999). The telomere length defects of *mre11Δ* and *tel1Δ* mutants are presumably due to a reduced frequency of telomerase-mediated elongation. In fact, *S. cerevisiae* cells lacking either Mre11 or Tel1 exhibit a reduced recruitment to short telomeres of both the telomerase catalytic subunit Est2 and its accessory protein Est1 (Goudsouzian et al. 2006), indicating that Tel1 and MRX promote telomerase recruitment at telomeres (Fig. 3A). Recent data also implicate Tel1 in increasing telomerase repeat addition processivity at critically short telomeres (Chang et al. 2007).

Both *S. cerevisiae* and *S. pombe* cells rely on Mec1/Rad3 to maintain short but stable telomeres in the absence of Tel1, suggesting that the function of Mec1/ATR at telomeres is redundant with or masked by Tel1 and MRX in normal cells. Concomitant inactivation of both Tel1/ATM and Mec1/ATR kinases in *S. cerevisiae* and *S. pombe* leads to chromosome self-circularization and complete loss of telomeric sequences, similar to that seen in cells lacking active telomerase (Naito et al. 1998; Ritchie et al. 1999; Nakamura et al. 2002). Moreover, as observed with *TEL1* inactivation, the lack of *RAD50* in a *mec1* mutant leads to telomere erosion (Ritchie et al. 1999; Ritchie and Petes 2000).

Tel1/ATM (and possibly Mec1/ATR) may affect multiple aspects of telomere homeostasis through phosphorylation of telomeric targets. Recent work in *S. cerevisiae* has identified serine residues in Cdc13 that are phosphorylated by Tel1 and Mec1 (Tseng et al. 2006) in a domain of the protein known to be involved in telomerase recruitment (Pennock et al. 2001; Bianchi et al. 2004). This suggests that Tel1-dependent phosphorylation is responsible for rendering Cdc13 competent for interaction with Est1 preferentially at short telomeres (Fig. 3A). Because short telomeres are preferentially elongated by telomerase (Teixeira et al. 2004) and Tel1 mainly associates with short telomeres and mediates telomerase binding to them (Hector et al. 2007; Sabourin

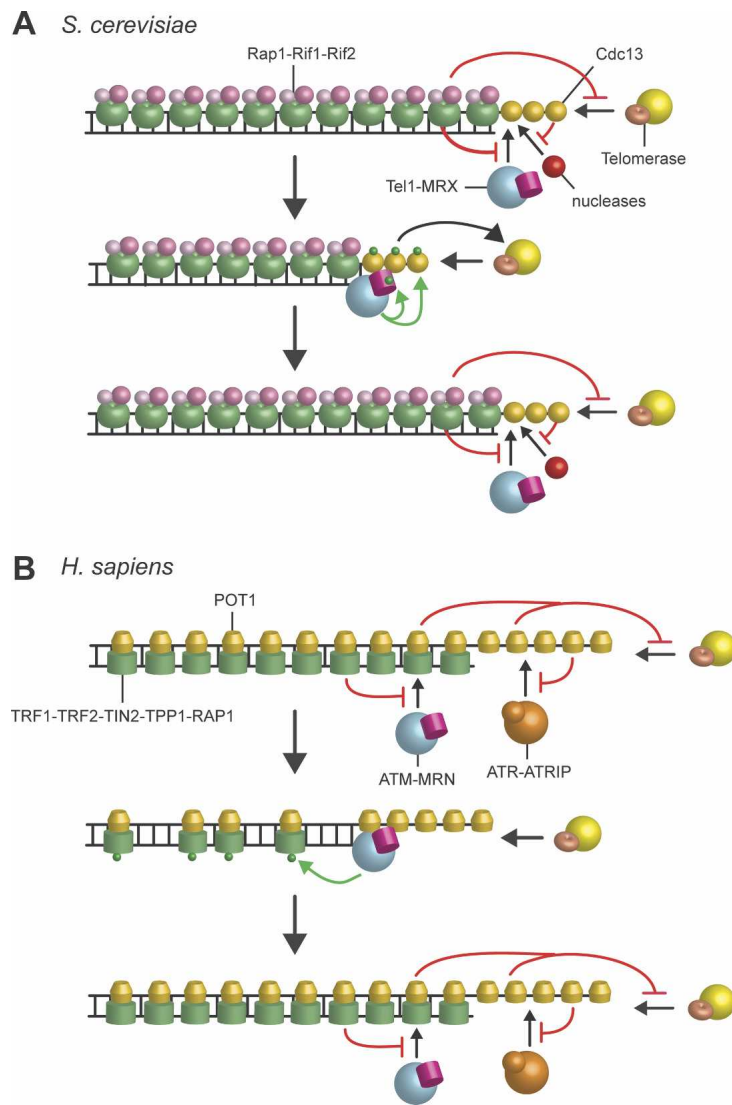


Figure 3. Contribution of DNA damage checkpoint proteins to telomere homeostasis regulation. (A) In *S. cerevisiae*, the access at full-length telomeres of telomerase, Tel1-MRX, and nucleases is inhibited. As telomere length declines, MRX and Tel1 promote the recruitment of telomerase at telomeres by phosphorylating the ssDNA-binding protein Cdc13, which mediates telomerase recruitment. (B) In humans, MRN and ATM promote telomerase-dependent telomere elongation by phosphorylating TRF1 on shortened telomeres. Phosphorylated TRF1 dissociates from telomeres, thus promoting telomerase access. In both yeast and humans, the subsequent telomere elongation increases the loading on telomeres of Rap1-Rif1-Rif2 and shelterin proteins, respectively, which can in turn block Tel1/ATM from acting, thus resulting in telomere reprotection. Green arrows indicate phosphorylation events.

et al. 2007), these data point to telomere length-dependent binding of Tel1 to telomeres as a critical step in the regulation of telomerase association with telomeres in S phase. In any case, it is likely that other substrates exist and their phosphorylation may mediate the different aspects of Tel1 function.

Both human TRF1 and TRF2 undergo ATM-dependent phosphorylation in response to ionizing radiation (Kishi et al. 2001; Tanaka et al. 2005). Moreover, ATM- and MRN-mediated TRF1 phosphorylation appears to negatively regulate TRF1 association with telomeres (Wu et al. 2007). This suggests that MRN promotes telomerase-dependent telomere elongation by engaging ATM at telomeres, which in turn phosphorylates TRF1. Phosphorylated TRF1 dissociates from telomeres, thus promoting telomerase access to the ends of telomeres (Fig. 3B).

The connections between telomeres and the DNA damage checkpoint are not limited to Mec1/ATR and Tel1/ATM. Telomere length maintenance in *S. cerevisiae* is also influenced by the checkpoint kinase Rad53

and by the Rad17-Mec3-Ddc1 complex (Longhese et al. 2000). Notably, this role for Rad17-Mec3-Ddc1 is evolutionarily conserved. *Caenorhabditis elegans* strains lacking HUS-1 or MRT-2, the functional orthologs of *S. cerevisiae* Mec3 and Rad17, respectively, display progressive telomere shortening and loss of germline immortality (Ahmed and Hodgkin 2000; Hofmann et al. 2002), and mutations in a similar set of genes affect telomere length in fission yeast (Dahlen et al. 1998; Nakamura et al. 2002). Moreover, the human Rad9-Hus1-Rad1 complex is constitutively associated with telomeres in both human and mouse cells, and the lack of Hus1 leads to a dramatic telomere shortening (Francia et al. 2006).

Functional telomeres can generate temporally limited DNA damage responses

It is well established that the natural chromosome ends need to be protected from inappropriate repair/recombi-

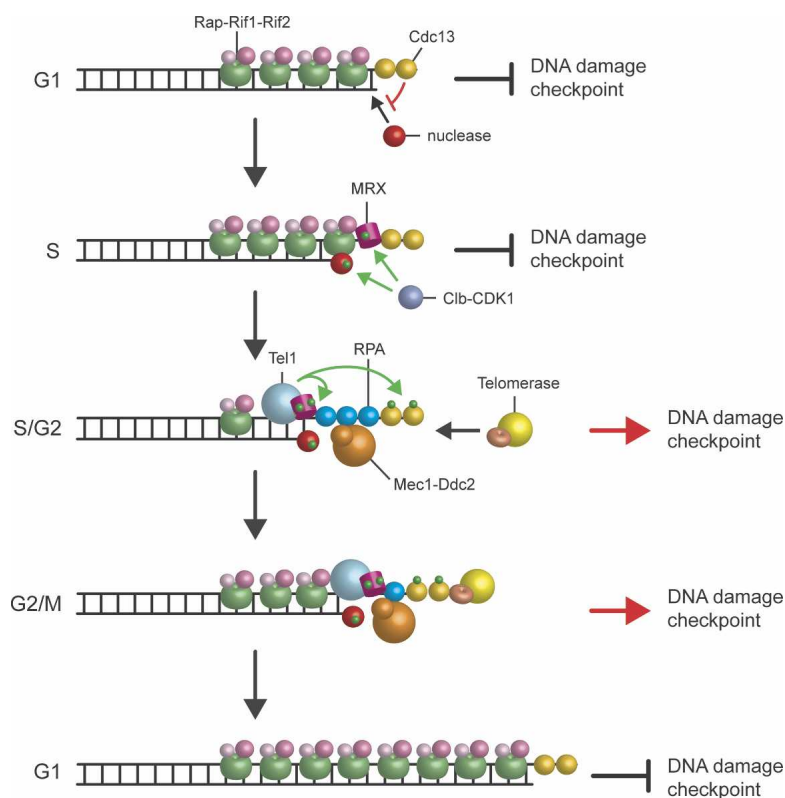
nation and checkpoint events, so it seems paradoxical that several proteins involved in DNA repair and checkpoints localize at telomeres and are required to maintain telomere length. This implies that the difference between a DNA break and a telomere is less pronounced than previously assumed, suggesting a temporally limited window where telomeres are detected as DSBs. Because t-loop formation and shelterin capping function can be temporarily lost after the passage of a replication fork, and native telomeres are susceptible to Clb-CDK1-dependent nucleolytic processing in late S/G2 (Frank et al. 2006; Vodenicharov and Wellinger 2006), functional telomeres may be recognized as DNA breaks during and/or soon after their replication.

It has been recently shown that *S. cerevisiae* telomeres behave similarly to intrachromosomal DSBs when they are suitable for elongation (Fig. 4; Viscardi et al. 2003, 2007). In fact, a prolonged expansion of either a single or multiple shortened telomeres causes the activation of an MRX-dependent checkpoint, suggesting that telomeres can be perceived and treated as DSBs in a window of time during their replication. MRX binding at short telomeres is the signaling event for checkpoint activation and is sufficient to activate the checkpoint independently of telomere elongation (Viscardi et al. 2007). These results imply that only telomeres that become susceptible to be bound by MRX, and therefore suitable for elongation, can be recognized as DSBs by the checkpoint machinery. Indeed, MRX is recruited to native telomeres in late S phase (Zhu et al. 2000; Takata et al. 2005), and only telomeres with short TG tracts are avidly bound by

MRX, as well as by the telomerase enzyme (Negrini et al. 2007; Viscardi et al. 2007), suggesting that under unperturbed conditions, only S-phase telomeres are potentially detectable as DSBs by the checkpoint machinery. However, the yeast telomerase enzyme only acts on short telomeres within one cell cycle, and the rate of telomere elongation appears limited to a few base pairs per generation (Teixeira et al. 2004). This limitation may prevent unscheduled checkpoint activation during an unperturbed S phase.

Generation of transient checkpoint signals at S-phase telomeres appears to be conserved in humans, where functional telomeres have been shown to undergo structural changes that elicit a DNA damage response during or after DNA replication (Verdun et al. 2005; Verdun and Karlseder 2006). By studying the association of checkpoint and repair proteins at elongating telomeres in primary human fibroblasts, it has been shown that a DNA damage response depending primarily on ATR is elicited before telomere replication is completed. After replication, telomeres attract the ATM and the HR machineries (Verdun and Karlseder 2006). Both ATR and ATM recruitment follow the localization of MRN. Because ATR recognizes ssDNA potentially due to replication fork pausing and/or stalling, whereas ATM is thought to recognize DSBs, this suggests that there are at least two distinct DNA damage signals triggered at telomeres during and after replication, respectively. Notably, degradation of MRN, as well as inhibition of ATM, leads to telomere dysfunction, suggesting that a localized DNA damage response at telomeres after replication is essen-

Figure 4. A model for the generation of transient DNA damage signals at functional telomeres. During the G1 cell cycle phase, neither short nor full-length telomeres are susceptible to be elongated by telomerase. They are also inert for processing events by nucleases. After completion of DNA replication in late S phase and during the ensuing G2 phase, telomeres become susceptible to Clb-CDK1-dependent nucleolytic processing, which can generate RPA-coated ssDNA. During this time, telomeres share many features with DSBs. Telomeres with short TG tracts become preferentially suitable to be processed and bound by MRX. RPA-coated ssDNA generation and telomere-bound MRX can activate a transient Mec1- and Tel1-dependent checkpoint, which in turn promotes telomere elongation by phosphorylating Cdc13. Telomere elongation increases the amount of proteins bound to TG tracts, and this change blocks telomerase and Mec1/Tel1 recruitment. A functional cap could be reassembled in the next G1, when Clb-CDK1 activity is low. Green arrows indicate phosphorylation events.



tial to re-establish chromosome end structure (Verdun et al. 2005). Once telomeric proteins assemble at single- and double-stranded ends, they may inhibit MRN-ATM, thus enforcing telomere identity.

Conclusions

In summary, our understanding of the molecular mechanisms ensuring chromosome end maintenance and identity has substantially increased in the past decade. ssDNA- and dsDNA-binding proteins (shelterin) shape chromosome ends and ensure their identity. These proteins regulate telomerase recruitment and protect the chromosome ends from being sensed as DSBs by the DNA damage checkpoint and repair/recombination machineries. However, the difference between telomeres and DSBs are less defined than previously assumed. In fact, proteins involved in DNA repair and DNA damage checkpoint are thought to assist shelterin functions. At the same time, shelterin must control both the actions and the persistence of these proteins at telomeric ends, because some of them have the potentiality to destroy them.

These insights also raise many new questions. For example, what aspects of shelterin functions might be assisted by ATM/ATR and MRN/MRX remains to be determined, and the identification of the telomeric components targeted by ATM/ATR and MRN/MRX could provide many insights into this question. Another major challenge will be to establish the role of the checkpoint proteins in ensuring telomere identity/maintenance, and the molecular mechanism controlling telomerase accessibility to the telomere. Moreover, apart from the identification of additional DNA repair/checkpoint factors acting at normal and/or dysfunctional telomeres, an important open question is the nature of the telomere signals that trigger a checkpoint response. Due to the critical role of both DNA damage checkpoints and telomere homeostasis in maintaining genetic stability and in counteracting cancer development, the knowledge of their interconnections is essential for our understanding of these key cellular controls.

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Maria Pia Longhese

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