Surprises from the Chromosome Front: Lessons from *Arabidopsis* on Telomeres and Telomerase

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Telomeres serve two vital functions: They act as a buffer against the end-replication problem, and they prevent chromosome ends from being recognized as double-strand DNA (dsDNA) breaks. These functions are orchestrated by the telomerase reverse transcriptase and a variety of telomere protein complexes. Here, we discuss our recent studies with *Arabidopsis thaliana* that uncovered a new and highly conserved telomere complex called CST (Cdc13/CTC1, STN1, TEN1). Formerly believed to be yeast specific, CST has now been identified as a key component of both plant and vertebrate telomeres, which is essential for genome integrity and stem cell viability. We also describe the unexpected discovery of alternative telomerase ribonucleoprotein complexes in *Arabidopsis*. Fueled by duplication and diversification of the telomerase RNA subunit and telomerase accessory proteins, these telomerase complexes act in concert to maintain genome stability. In addition to the canonical telomerase enzyme, one of two alternative telomerase ribonucleoprotein (RNP) complexes functions as a novel negative regulator of enzyme activity in response to genotoxic stress. These contributions highlight the immense potential of *Arabidopsis* in probing the depths of the chromosome end.

The study of plant telomeres dates back to Barbara McClintock's pioneering experiments in maize in the late 1930s, in which she studied the fate of broken chromosomes. McClintock discovered that a repair process was at work on dicentric chromosomes after they broke apart during mitosis. She termed this process "chromosome healing," because these chromosomes were safe from future fusion events (McClintock 1938). McClintock's observation led to the recognition of the chromosome terminus as a critical mediator of genome integrity.

Subsequently, it was discovered that the semiconservative mechanism of replicating linear, eukaryotic DNA results in a small, unreplicated segment of DNA at the chromosome terminus (Olovnikov 1971; Watson 1972). Terminal DNA attrition from the so-called "end-replication problem" would not be a concern in nongermline cells, but it had to be circumvented for the complete transfer of full-length chromosomes to offspring. In the 1980s, Elizabeth Blackburn and Carol Greider solved this riddle by identifying telomerase in the ciliated protozoan *Tetrahymena thermophila* (Greider and Blackburn 1985). Genetic and biochemical analysis followed, demonstrating that telomerase consists of a reverse transcriptase (TERT) and a template-providing RNA (TER) molecule (Greider and Blackburn 1989; Shippen-Lentz and Blackburn 1990). It has been demonstrated that telomerase-mediated maintenance of telomere tracts is highly conserved across eukaryotes and is crucial for cellular longevity. Indeed, altering the dynamics of telomere length maintenance or perturbing the specialized protein–DNA architecture that protects the chromosome terminus has profound consequences for the integrity of the entire genome (Jain and Cooper 2010; Wellinger 2010).

Telomeres typically comprise long arrays of G-rich repeats. The extreme 3′ terminus of the chromosome is single stranded and is termed the G-overhang. The G-overhang is complementary to the template region within TER and is used to prime telomere repeat synthesis by TERT, thereby solving the end-replication problem. Because the chromosome end resembles a double-strand break (DSB), it must be differentiated from damaged DNA. The task is accomplished by the unusual t-loop architecture of terminal DNA and a suite of telomere-binding proteins that guard chromosome ends and regulate telomerase access (Palm and de Lange 2008). Two distinct telomere complexes have been defined: shelterin and CST (Fig. 1A,B) (Price et al. 2010).

Vertebrate shelterin is composed of six proteins (Palm and de Lange 2008). TRF1 and TRF2 bind directly to dsDNA, whereas RAP1 localizes at telomeres through its interaction with TRF2. TIN2 acts as a bridging component between TRF/RAP1 and the G-overhang–binding heterodimer POT1/TPP1. In most species, POT1 shows high specificity toward single-stranded G-rich DNA, and this interaction is heightened by TPP1. Loss of POT1 and/or TPP1 is lethal in vertebrates and results in increased G-overhang signals and activation of a DNA-damage response (Palm and de Lange 2008). In addition to protecting the G-overhang, POT1/TPP1 recruits telomerase to the telomere through direct interactions between TPP1 and TERT (Nandakumar et al. 2012; Zhong et al. 2012).

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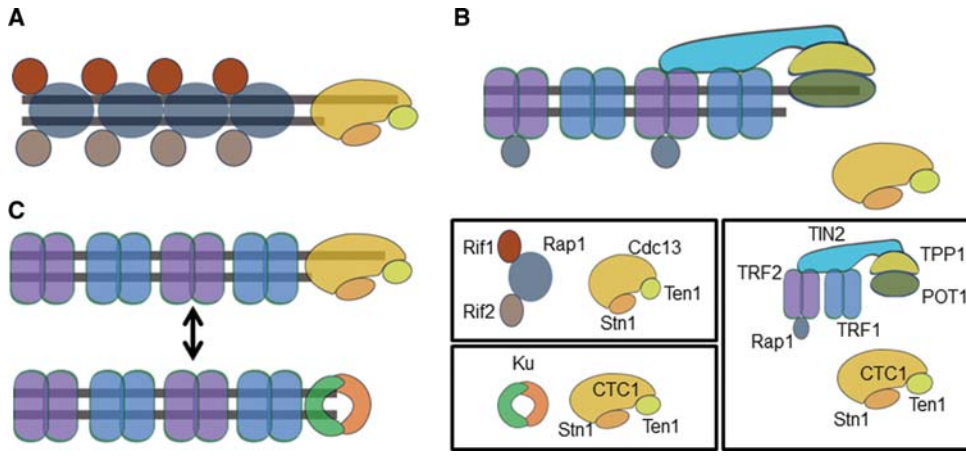

Figure 1. Telomere protein complexes in budding yeast, vertebrates, and *Arabidopsis*. (*A*) The primary telomere capping complex in budding yeast comprises the dsDNA-binding protein Rap1, which associates with two other proteins, Rif1 and Rif2. G-overhangs are bound by the heterotrimer CST (Cdc13, Stn1, Ten1). (*B*) Vertebrate telomeres associate with shelterin. Shelterin includes the dsDNA-binding proteins TRF1 and TRF2, which homodimerize; Rap1, which binds TRF2 and TIN2; and a bridge between TRF proteins and the ssDNA-binding heterodimer TPP1/POT1. Vertebrate CST (CTC1/STN1/TEN1) is proposed to engage telomeres during S phase to promote DNA replication. (*C*) *Arabidopsis* telomeres are bound by TRFL proteins but no other shelterin components. The chromosome termini are asymmetrical. The terminus with a G-overhang is likely bound by CST, whereas the blunt end is associated with the Ku heterodimer. See text for details.

The heterotrimer CST (Cdc13/CTC1, STN1, TEN1) is functionally conserved across all major eukaryotic lineages (Fig. 1A,B). In budding yeast, CST functions with the double-stranded binding protein Rap1 to stabilize chromosome ends (Giraud-Panis et al. 2010). Although yeast Rap1 is orthologous to vertebrate RAP1 (de Lange 2009), no other shelterin components have been uncovered in budding yeast. Loss of CST components results in extended G-overhangs, elongated telomeres, and activation of a DNA-damage response (Nugent et al. 1996; Grandin et al. 1997; Xu et al. 2009). In addition, CST facilitates telomerase recruitment through interactions with the telomerase accessory protein Est1 (Qi and Zakian 2000). In contrast to the situation in yeast, the vertebrate CST complex does not have a major role in telomere length regulation but is instead critical for proper G-overhang maintenance and telomere replication through interactions with DNA polymerase α primase (Miyake et al. 2009; Wang et al. 2012). Owing to the repetitive nature of telomeric DNA, it is a challenging substrate for replication machinery. CST helps to overcome this barrier by facilitating replication fork passage through the telomere region (Stewart et al. 2012). Recent data further suggest that human CST can inhibit telomerase by both sequestration of the G-overhang and physical interactions with POT1/TPP1 (Chen et al. 2012). Following replication and telomere extension, CST is necessary for C-strand fill-in along the elongated G-overhang (Wang et al. 2012).

The requirement for adequate telomere maintenance and end protection is critical for mammals, where stem cell viability and germline development must be balanced with the limited proliferation program of somatic cells to avert cancer. However, all eukaryotes share a fundamental need to fully replicate their telomeres and thus maintain genomic integrity. Owing to its remarkable tolerance of telomere dysfunction, *Arabidopsis thaliana* has proven to be an outstanding model system to address fundamental questions in telomere biology. Here, we discuss recent advances in *Arabidopsis* telomere biology. Specifically, we review the discovery of the CST complex and multiple-telomerase RNA subunits in *Arabidopsis*. These contributions reveal the surprising and distinct ways in which plants use highly conserved telomere components to promote genome stability and organismal viability.

DISCUSSION

Telomere Proteins Take Flight in Plants

The telomeres of plants and metazoans share many similarities in both the DNA repeat sequence and the mechanism of maintenance through telomerase. Because of these similarities, it was initially believed that the protein composition of telomeres would likewise be conserved (Fig. 1). This idea was bolstered by the identification of a family of 12 TRF-like (TRFL) proteins from *Arabidopsis*, six of which (class 1) bear structural similarity to the vertebrate shelterin components TRF1 and TRF2 (Karamysheva et al. 2004). Unfortunately, genetic analysis indicated significant functional redundancy within this gene family, impeding detailed functional dissection of the TRFL proteins. The plot began to thicken as BLAST searches for the shelterin components TIN1, TPP1, and RAP were unsuccessful.

POT1, on the other hand, is highly conserved in plants, and like its vertebrate counterparts, it is crucial for chromosome end protection in the moss *Physcomitrella patens* (Shakirov et al. 2010). Intriguingly, *A. thaliana* encodes two full-length POT1 proteins (POT1a and POT1b) as well as a smaller, truncated POT1 (POT1c) (Rossignol et al.

2007). Both AtPOT1a and AtPOT1b retain the requisite secondary structure elements of vertebrate and fission yeast POT1 proteins, having two OB folds and a carboxy-terminal protein interaction domain (Baumann and Cech 2001; Surovtseva et al. 2007). AtPOT1c encodes for little more than a single OB fold (A Nelson and D Shippen, unpubl.).

Unexpectedly, we discovered that AtPOT1 proteins do not behave like the moss POT1. None of the three AtPOT1 paralogs specifically bind telomeric DNA in vitro (Shakirov et al. 2005, 2010). This phenomenon is not a quirk of *Arabidopsis*, because biochemical analysis of POT1 proteins across the plant kingdom revealed only two examples of DNA binding by one of two POT1 paralogs from *Zea mays* and the single POT1 protein from green algae (Shakirov et al. 2009a,b). In addition, genetic analysis indicated that AtPOT1a is not required for chromosome end protection. Instead, its removal resulted in an ever shorter telomere (EST) phenotype, first described for budding yeast mutants that lack a key component of telomerase (Lundblad and Szostak 1989). The EST phenotype of *pot1a* null mutants mimicked a *tert* null (Surovtseva et al. 2007), where the progressive loss of telomeric DNA leads to worsening genome instability with each successive plant generation (Riha et al. 2001). It was subsequently shown that AtPOT1a is required for optimal telomerase activity (Surovtseva et al. 2007), and it physically associates with telomerase via direct binding to TER1, one of the telomerase RNA subunits (Cifuentes-Rojas et al. 2011). In other organisms, POT1 proteins bind telomeric DNA through two amino-terminal OB-fold domains (Baumann and Cech 2001). The corresponding domains are used by AtPOT1a to bind TER1 (Cifuentes-Rojas et al. 2011).

Less is known about AtPOT1b, but evidence suggests that it has a critical role in chromosome end protection. Overexpression of a truncated isoform of AtPOT1b results in severe genome instability typical of uncapped telomeres, including dramatic telomere shortening and telomere fusions (Shakirov et al. 2005). The function of AtPOT1b became more enigmatic when it was discovered that AtPOT1b physically associates with the noncanonical telomerase RNA subunit TER2 (Cifuentes-Rojas et al. 2012). As with POT1b, POT1c retains at least a portion of the chromosome-end-protection role of ancestral POT1 proteins (A Nelson and D Shippen, unpubl.). Altogether, these findings indicate that POT1 proteins are evolving rapidly and, in addition, their function in a significant spectrum of the plant kingdom is likely to be mediated through interactions with telomerase.

Refining the Model for Telomere Stabilization in Eukaryotes: The Identification of CST in *Arabidopsis*

The lack of a full complement of shelterin components in plants left no clear candidates to bind and protect the 3′ G-overhang. In 2007, the identification of STN1 in *Schizosaccharomyces pombe* (Martin et al. 2007) fueled the discovery of essential telomere proteins in *Arabidopsis*. A PSI-BLAST search using the *S. pombe* STN1 sequence as a query revealed the *A. thaliana* STN1 ortholog (Song et al. 2008). AtSTN1 encodes a single OB-fold containing protein that is less than half the size of its vertebrate and yeast orthologs. STN1 colocalizes with telomeres, and loss of STN1 results in immediate and catastrophic telomere dysfunction. Plants deficient in STN1 display rapid erosion of telomere tracts, telomere fusions, and defects in cell division. These anomalies are accompanied by gross extension of the G-overhang and extrachromosomal telomere circles, indicative of inappropriate telomere recombination. Thus, AtSTN1 is critical for telomere integrity. The discovery of STN1 in *Arabidopsis* was important for two reasons. First, it emphasized that this core component of CST, which was formerly believed to be unique to fungi, was conserved across Eukarya. Second, it provided a window into the mechanism of telomere stabilization in plants. However, the two other CST components TEN1 and CDC1 could not be detected in the *Arabidopsis* genome.

Resolution of this conundrum came from a serendipitous discovery. While analyzing an ethylmethane sulfonate (EMS) mutagenesis line with a mutation within AtPOT1c, Surovtseva et al. (2009) identified plants with a severe morphological phenotype, indicative of profound telomere dysfunction. Curiously, the phenotype did not segregate with the POT1c point mutation. Map-based cloning revealed the defect to be a premature stop codon in a large and uncharacterized gene that became known as conserved telomere maintenance component 1 (*CTC1*). Loss of CTC1 resulted in heterogeneous, very short telomeres, extensive telomere fusions, increased G-overhangs, and extrachromosomal telomere circles. Collaborative studies with Carolyn Price (Surovtseva et al. 2009) and independent work by Fuyuki Ishikawa and colleagues (Miyake et al. 2009) showed that CTC1 was conserved in humans. CTC1 interacts with STN1 in plants as well as vertebrates and is necessary for telomere integrity (Miyake et al. 2009; Surovtseva et al. 2009). Although not a sequence homolog of yeast Cdc13, CTC1 is predicted to harbor multiple OB-fold domains, like Cdc13 (Mitton-Fry et al. 2002). The biochemical properties and function of CTC1 argue that CTC1 is a functional homolog of Cdc13.

The third component of CST, TEN1 was identified alongside CTC1 and STN1 in humans (Miyake et al. 2009), raising the possibility that this component was also present in *Arabidopsis*. Using hTEN1 as the query in a BLAST returned a gene encoding for another single OB-fold-containing protein structurally similar to human and fission yeast TEN1 (KA Leehy et al., in prep.). As in other organisms, TEN1 interacts strongly with STN1 in vitro. Characterization of AtTEN1 accelerated when Hashimura and Ueguchi (2011) reported an *Arabidopsis* mutant from an EMS screen that failed to maintain stem cell populations and was hypersensitive to DNA damage. This gene, *meristem disorganization 1* (*mdo1*), encodes TEN1. The growth defects of *mdo1-1* mutants were remarkably similar to those of *stn1* and *ctc1* mutants.

Analysis of telomeres in the mutants revealed short, highly heterogeneous tracts, a high incidence of telomere fusions, and increased G-overhangs (KA Leehy et al., in prep.). Thus, as in other systems, each member of the CST complex is critical for maintaining telomere integrity.

In *Arabidopsis*, the absence of any one of the CST components leads to an inability to maintain apical meristems, physiologically manifesting in fused stems, aberrant organ development, and decreased fertility (Song et al. 2008; Surovtseva et al. 2009; KA Leehy et al., in prep.). Similarly, mutations within human CTC1 are the causative factor in certain types of Dyskeratosis congenita and Coats plus, severe genetic disorders characterized by premature death within stem cell niches (Levy et al. 2010; Anderson et al. 2012; Keller et al. 2012). The high incidence of chromosomal fusions in *Arabidopsis* CST mutants argues that genome instability underlies stem cell death and, in addition, a mechanism is in place to eliminate genetically defective cells. Genetic analysis from our laboratory and the White/Gallego laboratory revealed that this protective mechanism is dependent on the DNA-damage-response kinase ATR (Amiard et al. 2011; Boltz et al. 2012). Removal of ATR from plants lacking CTC1 temporarily rescues the morphological phenotypes associated with *ctc1* mutants (Boltz et al. 2012). Hence, ATR may cull stem cells with severe telomere dysfunction owing to a lack of CST so that only those with intact genomes can proceed through cell division.

Although many functions of CST are conserved, mutations in the human complex are not as detrimental as in plants, perhaps because of functional overlap with the shelterin complex (Price et al. 2010). Current models for vertebrate CST propose that its major role is in promoting replication of telomeric DNA. Notably, hCTC1 and hSTN1 were originally identified as components of a DNA Polα/primase stimulatory complex (Goulian et al. 1990). In yeast, CST recruits Polα/primase to the C-strand of telomeres following telomere elongation by telomerase (Bianchi and Shore 2008). Like vertebrates, *Arabidopsis* CST may also mediate telomere replication via interactions with the catalytic subunit of Polα (ICU2) (Price et al. 2010). The outcomes associated with CST mutation in *Arabidopsis* can be explained by defects in telomere replication. Replication fork stalling in CST mutants would result in rapid shortening of telomeres, potentially resulting in critically short telomeres within a few cell divisions. Moreover, the inability to completely fill-in the telomeric C-strand would result in elongated G-overhangs and activation of an ATR-dependent DNA damage response (Boltz et al. 2012).

Because plants lack many of the core components of shelterin, CST is a prime candidate to fill the role of telomere sentinel. Recent studies by Riha and colleagues support this conclusion, but also reveal that chromosome end protection is more interesting and complex in *Arabidopsis* than anticipated. Unlike all other eukaryotes studied, only half of the telomeres in *Arabidopsis* contain a G-overhang: The other half are blunt ended (Kazda et al. 2012). CST is hypothesized to bind telomeres with G-overhangs, whereas the heterodimer Ku (Ku70/80) protects blunt ends (Fig. 1C). Blunt-ended chromosomes likely represent a natural DNA replication intermediate that in other organisms is fully processed to create symmetrical G-overhangs on both sides of the chromosome (Nelson and Shippen 2012). The presence of asymmetrical chromosome ends would lead to a slower rate of telomere depletion, because nucleolytic processing to create a 3′ overhang occurs on only half of the chromosome ends. For plants, this feature would be advantageous because it would extend the proliferative capacity of stem cell niches and other cells lacking telomerase, thereby increasing the ability to adapt to changing environmental conditions (Nelson and Shippen 2012).

Finally, recent data suggest that TEN1 engages in off-telomere functions that are distinct from the other two members of CST (KA Leehy et al., in prep.). Plants lacking *TEN1* exhibit a significantly higher incidence of telomere fusions than in *stn1* or *ctc1* mutants. Even more intriguing is the observation that *ten1* mutants have elevated levels of telomerase enzyme activity caused by an increase in telomere repeat addition processivity. Although it is unknown how TEN1 negatively regulates telomerase, the finding that human CST can suppress telomerase activity by physical sequestration of the G-overhang (Chen et al. 2012) underscores the potential for a conserved, but still enigmatic, role for CST in telomerase regulation.

Three's Company: The Emergence of Multiple, Divergent Telomerase RNP Complexes in *Arabidopsis*

The core subunits of human telomerase consist of TERT, TER, and the RNP maturation complex dyskerin (Cohen et al. 2007). Each of these components is essential for telomerase function in plants. AtTERT, at ~130 kD, is similar in size to hTERT and, like its human counterpart, is expressed in actively dividing cell populations including young seedlings, flowers, and cell culture but not in vegetative tissues (Fitzgerald et al. 1999). As discussed earlier, plants lacking TERT exhibit a progressive telomere-shortening phenotype and ultimately arrest at a terminal vegetative state by the 10th generation of the deficiency (Riha et al. 2001). Likewise, dyskerin is necessary for telomere maintenance in plants (Kannan et al. 2008), and analysis of dyskerin immunoprecipitations confirmed an interaction with the telomerase RNP (Cifuentes-Rojas et al. 2012).

In contrast to the core protein subunits of telomerase, TER is highly divergent. The discovery of *Arabidopsis* TER came about through biochemical purification of telomerase from cell culture (Cifuentes-Rojas et al. 2011). The defining feature of TER is a sequence complementary to the telomere repeat. Strikingly, sequence analysis of RNAs that copurified with *Arabidopsis* telomerase revealed not one, but two distinct RNAs, each with the same 11-nucleotide telomere template domain. These RNAs, termed TER1 (748 nucleotides) and TER2 (784

nucleotides), share a ~220-nucleotide region of high similarity but outside this region cannot be aligned with any confidence (Fig. 2A).

Gene duplication is common in plants, but it was unprecedented for an organism to encode two telomerase RNAs. *Arabidopsis* was not finished surprising us, however, because primer extension revealed a third TER isoform, termed $TER2_S$ (219 nucleotides). $TER2_S$ corresponds to a spliced and truncated version of TER2 in which the 529-nucleotide intervening nonconserved region is removed, the two conserved regions are precisely joined, and 36 nucleotides are deleted from the RNA 3′ end (Fig. 2A) (Cifuentes-Rojas et al. 2012). How these processing events occur in vivo is still unclear. All three TER isoforms can associate with TERT to reconstitute enzyme activity in vitro (Cifuentes-Rojas et al. 2011). However, TERT shows a hierarchy of binding with TER2 > TER1>>$TER2_S$ (Cifuentes-Rojas et al. 2012). Under standard growth conditions, the levels of TER1 and $TER2_S$ are similar and peak in highly proliferating cell populations. In contrast, TER2 is expressed at a much lower level.

TER1 is encoded on chromosome 1 with the 3′ end of the RNA embedded within the *RAD52* gene (Cifuentes-Rojas et al. 2011; Samach et al. 2011), whereas TER2 is encoded on chromosome 5, with its 5′ end embedded, but in the opposite orientation to an uncharacterized gene designated *TAD3* (Fig. 2B). Strikingly, thermal asymmetric untranslated–polymerase chain reaction (TAIL-PCR) identified only a single *TER* locus in other Brassicaceae species, which was flanked on one side by *RAD52* and on the other side by *TAD3* (Beilstein et al. 2012). Even more surprising was the observation that the putative templating domain at some of these loci harbors nucleotide substitutions that would render the TER nonfunctional or cause synthesis of altered telomere repeats. Given the near ubiquitous presence of the TTTAGGG telomere repeat sequence within the plant kingdom, these findings raise the interesting possibility that these species encode other TER molecules from alternative loci.

The *Arabidopsis* Telomerase RNPs

TER1 functions as a canonical templating RNA for telomerase in vivo (Cifuentes-Rojas et al. 2011). Depletion of TER1 results in telomere shortening, and expression of TER1 with a mutated template in plants leads to incorporation of the corresponding mutant telomere repeats on chromosome ends. As mentioned earlier, TER1 interacts with POT1a. Although not a sequence ortholog, AtPOT1a functions are remarkably similar to budding yeast Est1, which binds to the telomerase RNA and serves as a bridge between the telomerase core and CST (Fig. 3A,C) (Qi and Zakian 2000). Preliminary data indicate that AtPOT1a also interacts with CST components (K Renfrew and D Shippen, unpubl.). Thus, *A. thaliana* commandeered a highly conserved telomere-binding protein and moved it onto the telomerase RNP where it may be used to recruit telomerase to the chromosome end (Fig. 3B,C).

Unlike TER1, TER2 does not direct telomere repeat incorporation and therefore does not have a major role in telomere length maintenance (Cifuentes-Rojas et al. 2011). Instead, TER2 functions as a novel negative regulator for telomerase enzyme activity. A null mutation in TER2 increases telomerase activity, whereas overexpression of TER2 diminishes telomerase activity (Cifuentes-Rojas et al. 2012). Because TERT binds TER2 with higher affinity than TER1, TER2 may act as a competitive inhibitor that sequesters TERT in a nonproductive complex (see below). The distinct function of TER2 may also be a reflection of its unique RNP accessory proteins. In addition to TERT, in vitro binding studies and in vivo pull-down experiments show that TER2 does not associate with POT1a. Rather, TER2 binds POT1b and the Ku70/80 heterodimer (Cifuentes-Rojas et al. 2012). $TER2_S$ forms a subcomplex containing POT1b and, to a lesser extent, Ku. Dyskerin is associated with the TER2 RNP but not the $TER2_S$ RNP (Fig. 3B). This latter finding was anticipated because the predicted binding region on TER2 for dyskerin lies within the 3′ region that is eliminated in $TER2_S$.

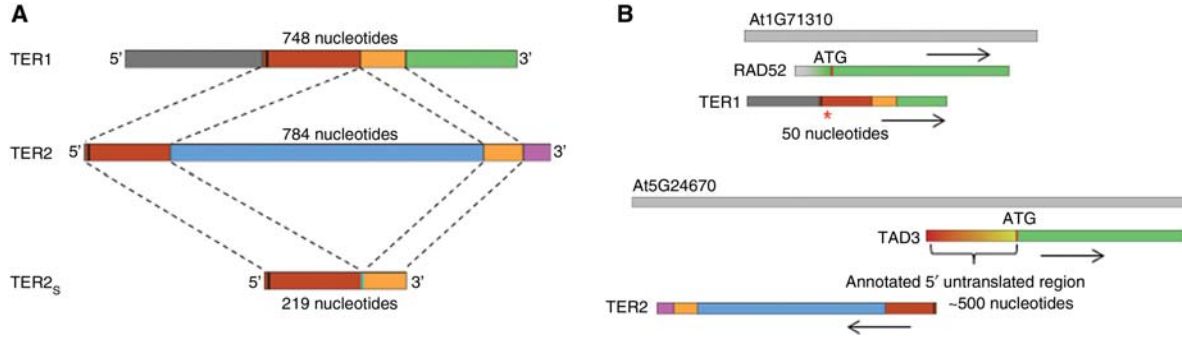

Figure 2. Multiple TERs in *A. thaliana*. (*A*) Depiction of the three *Arabidopsis* TER isoforms, with regions of high similarity (>90%) illustrated with dashed lines. $TER2_S$ is generated by the removal of a 529 intervening sequence (blue) and the 3′ 36-nucleotide (purple). (*B*) Schematic diagram of the *TER1* (*top*) and *TER2* (*bottom*) loci. *TER1* is oriented in the same direction as *RAD52*. The template region of TER1 is located 50 nucleotides 5′ from the ATG of RAD52 (red asterisk). *TER1* terminates near the intron 2/exon 3 junction of *RAD52*. TER2 is oriented in the opposite direction of *TAD3*. The template region of TER2 lies within the extreme 5′ untranslated region of the adjacent gene, ~500 nucleotides from the ATG.

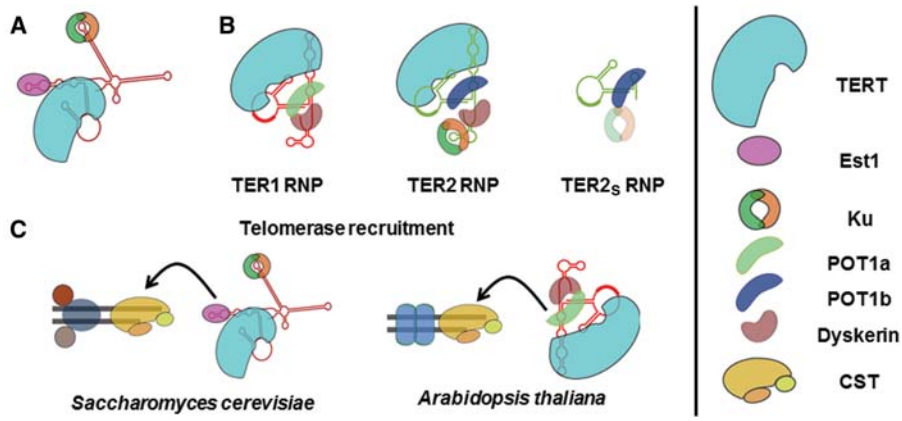

Figure 3. Telomerase RNPs of budding yeast and *A. thaliana*. (*A*) The core components of yeast telomerase include TERT and Tlc1 (TER). Accessory factors Est1 and Ku bind to Tlc1. Other accessory factors include Est3 and Sm proteins (not pictured). (*B*) Three telomerase RNP complexes in *Arabidopsis*. TER1 and TER2 associate with TERT and dyskerin in vivo. Accessory factors include POT1a, which binds TER1; POT1b, which binds TER2 and $TER2_S$; and Ku, which binds TER2 and, to a lesser extent, $TER2_S$. (*C*) Model for telomerase recruitment in yeast and *Arabidopsis*. Telomerase is proposed to dock onto the telomere via interactions between Est1 and CST in yeast and POT1a and CST in plants.

Just as the TER1 accessory factor POT1a works in concert with TER1 and TERT to promote telomere maintenance, the binding partners of TER2 negatively regulate telomerase enzyme activity and its action on chromosome ends. Plants null for POT1b display increased levels of telomerase activity (A Nelson and D Shippen, in prep.). Although plants lacking Ku have wild-type levels of telomerase enzyme activity, their telomeres are grossly extended; therefore, Ku is a potent negative regulator of telomere length (Riha et al. 2002). In contrast, the interaction of Ku with yeast telomerase promotes telomere synthesis (Fisher et al. 2004). Thus, Ku's interaction with the TER2 RNP provides another illustration of how *Arabidopsis* devised a new use for a very old telomere protein.

Necessity as the Mother of Invention: DNA Damage and the Down-Regulation of Telomerase

It was not immediately clear why *Arabidopsis* would need to negatively regulate telomerase. *Arabidopsis* mutants that constitutively express telomerase in all organs show no obvious phenotypic affects under standard growth conditions (Ren et al. 2004). One setting where telomerase inhibition could be important is following the introduction of DSBs. To promote faithful chromosome repair, telomerase must be blocked from acting on sites of DNA damage (Fig. 4A,B). In support of this model, we discovered that telomerase activity decreased significantly in seedlings treated with the radiomimetic drug zeocin (Boltz et al. 2012). Strikingly, TER2, but not TER1 or $TER2_S$, is induced under these conditions (Cifuentes-Rojas et al. 2012). This response is specific to the introduction of DSBs: It is not observed in seedlings treated with HU, which induces replication fork stalling, or in *ctc1* mutants, whose dysfunctional telomeres activate an ATR-mediated DNA-damage response (Cifuentes-Rojas et al. 2012). Importantly, the repression of telomerase in response to DSBs is dependent on TER2.

We suspect that TER2-mediated repression of telomerase is only one of the ways that *Arabidopsis* promotes genome stability following genotoxic stress. De novo telomere formation (DNTF) can have catastrophic consequences on the genome, owing to the loss of centromere-distal DNA (Fig. 4A). We recently developed a system to study DNTF in *Arabidopsis* using transfer DNAs to introduce a small telomeric seed sequence that can be either integrated into the body of the chromosome or serve as a telomerase-binding site for new telomere formation.

Interestingly, in diploid *Arabidopsis*, the frequency of DNTF is one order of magnitude higher than in yeast (11% vs. $<$1%) (Kramer and Haber 1993; Nelson et al. 2011) and more closely resembles mammalian cancer cells (40%–60%; Nigg 2001). The rate of DNTF increases to $\sim$50% in tetraploid *Arabidopsis*, the genetic redundancy buffering against loss of individual chromosome arms (Nelson et al. 2011). This powerful DNTF system should help to shed light on the role of TER2 and other negative regulatory molecules in modulating telomerase action at DNA breaks.

It is conceivable that telomerase regulation by long noncoding RNAs such as TER2 will be another conserved facet of telomere biology. On the basis of the TER2 paradigm, such regulatory RNAs would not have to share sequence similarity with the canonical TER; they would simply have to compete for the same RNA-binding site on TERT. TERT could then swap among different RNA scaffolds depending on cellular conditions (Fig. 4C). Notably, human TERT is reported to associate with numerous RNAs besides the canonical TER (Maida et al. 2009). In addition, human TERT engages in a variety of nontelomere functions (e.g., promotion of cell proliferation and anti-apoptosis) (Parkinson et al. 2008). Thus, the potential for dynamic RNA-mediated control of TERT on and off the telomere, and in response to changing environmental conditions, is feasible.

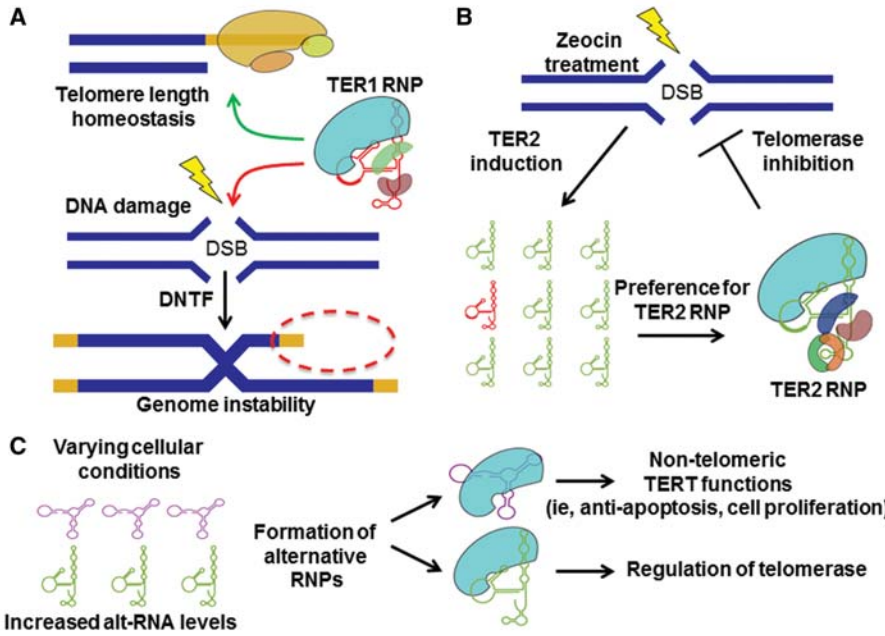

Figure 4. Telomerase regulation in response to genotoxic stress. (*A*) Telomerase may synthesize telomere repeats at natural chromosome ends (green arrow) or at a DSB (red arrow) to instigate de novo telomere formation (DNTF). DNTF can cause the lethal loss of genetic material (red circle). (*B*) In *Arabidopsis*, TER2 (green) is induced in response to zeocin. Increased TER2 may outcompete TER1 (red) for TERT binding, favoring the formation of TER2 RNP and causing decreased telomerase activity. The reduction in telomerase activity would enhance the fidelity of DSB repair and reduce the probability of DNTF. (*C*) Other changes in the environmental conditions could potentially induce alternative TER2-like molecules (purple and green) that modulate TERT interactions on and off the telomere.

CONCLUSIONS

Arabidopsis thaliana serves as both a perplexing and exciting model for telomere biology. Some telomere components have played evolutionary musical chairs, whereas the conservation of function among other telomere complexes (e.g., CST) provides a paradigm for understanding how telomere proteins promote stem cell function in plants and humans. The duplication of TER fueled the emergence of alternative RNP complexes as well as novel regulatory mechanisms that act in concert with the canonical telomerase enzyme to promote genome integrity. The unexpected lessons that *Arabidopsis* has taught us so far indicate that it will continue to reveal fascinating and fundamental new insights into telomeres and their synthesis by telomerase.

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