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Back to the future: The intimate and evolving connection between telomere-related factors and genotoxic stress

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The conversion of circular genomes to linear chromosomes during molecular evolution required the invention of telomeres. This entailed the acquisition of factors necessary to fulfill two new requirements: the need to fully replicate terminal DNA sequences and the ability to distinguish chromosome ends from damaged DNA. Here we consider the multifaceted functions of factors recruited to perpetuate and stabilize telomeres. We discuss recent theories for how telomere factors evolved from existing cellular machineries and examine their engagement in nontelomeric functions such as DNA repair, replication, and transcriptional regulation. We highlight the remarkable versatility of protection of telomeres 1 (POT1) proteins that was fueled by gene duplication and divergence events that occurred independently across several eukaryotic lineages. Finally, we consider the relationship between oxidative stress and telomeres and the enigmatic role of telomere-associated proteins in mitochondria. These findings point to an evolving and intimate connection between telomeres and cellular physiology and the strong drive to maintain chromosome integrity.

Molecular evolution is opportunistic, enabling novel cellular mechanisms to arise in response to biological challenges. One such challenge was conversion of the circular prokaryotic genome into the multiple linear DNA forms that comprise the eukaryotic genome (1). This challenge necessitated the invention of telomeres. Here we discuss the origin and evolution of telomere-related functions. Although the factors associated with chromosome ends were initially thought to be specific for this locale, in-depth analysis has revealed many such factors having noncanonical, so-called "moonlighting" roles in other transactions within the nucleus and the cytoplasm. We now appreciate that some of the moonlighting contributions may reflect ancestral functions preserved from the dawn of genome linearization, whereas others may be newly emergent.

There are several theories for how linear chromosomes evolved from their circular progenitors (2, 3), but one of the more intriguing proposals is that invasion of circular genomes by group II introns (1), via reverse splicing and reverse transcription, led to DNA linearization (4, 5) (Fig. 1). Specifically, it is posited that non-LTR[2] retrotransposons targeted to double-strand breaks (DSBs) served as "proto-telomeres" (6). The nascent chromosome ends presented two immediate challenges: the "end replication" problem and need for "end protection" (7, 8). The end replication problem occurs because the DNA replication machinery cannot fully replicate the extreme terminus of the lagging strand, which would lead to the gradual depletion of terminal DNA sequences when the genome is duplicated (9, 10). The chromosome ends may also be perceived as a DSB and must therefore be sequestered to prevent activation of the DNA damage response. Such end protection is also crucial for the avoidance of end-to-end fusions of chromosomes, which would cause improper chromosome segregation during mitosis, cell cycle arrest, genome instability, senescence, and cell death (8, 11). Most eukaryotes cope with these problems by 1) adding long arrays of noncoding DNA repeats to serve as a physical buffer to protect coding regions from attrition and 2) formation of higher-order DNA architecture that helps distinguish chromosome ends from a DSB (*i.e.* fold-back structures in yeast (12) and t-loops in other species (13, 14)).

Emergence of telomerase

To help overcome the telomere end replication problem, a group II intron likely gained the ability to use the 3′ end of linearized chromosomes as a template for reverse transcription (5). There is strong evidence that the telomerase catalytic subunit TERT evolved from a non-LTR class 2 retrotransposon (15–17) (Fig. 1). Fruit flies and silkworms maintain their chromosome ends through a telomerase-independent mechanism that employs a different class of retrotransposons (18, 19), supporting the idea that retrotransposons played an early and critical role in establishing and maintaining telomere architecture (20, 21).

The modern-day enzyme that helps solve the end replication problem is telomerase, a reverse transcriptase that compensates for incomplete replication by continually replenishing terminal DNA using a long noncoding RNA, TER, as template (22). It is possible that TER arose from a transcript derived from the progenitor group II intron (5) (Fig. 1), but TER and TERT

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[2] The abbreviations used are: LTR, long terminal repeat; DSB, double-strand break; NHEJ, nonhomologous DNA end joining; OB-fold, oligonucleotide/oligosaccharide-binding fold; ROS, reactive oxygen species; 8-oxoG, 8-oxo-guanine; Tg, thymine glycol; BER, base excision repair; MTS, mitochondrial targeting sequence.

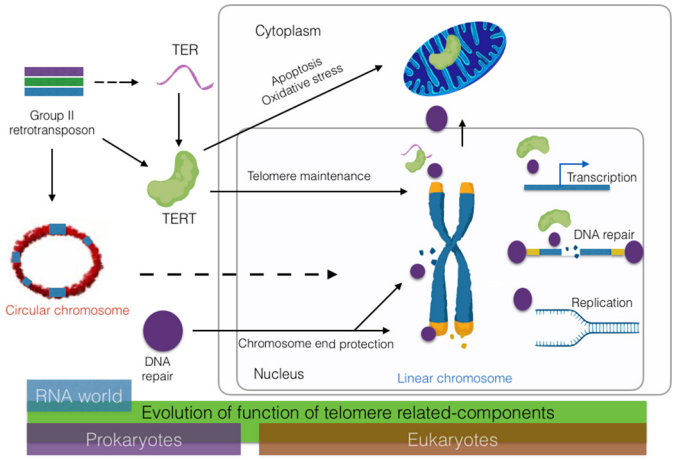

Figure 1. Model for evolution of telomerase and telomere-associated components from group II retrotransposons and DNA repair proteins. After chromosome linearization by the insertion of group II retrotransposons, telomerase and DNA repair proteins evolved roles in telomere maintenance and end protection. Telomere-associated factors also participate genome-wide in transcription, replication, and repair. Other factors function in mitochondria to modulate the response to oxidative stress. Whether the mitochondrial functions of telomere proteins reflect an ancient or newly evolved function is unknown (see text for details).

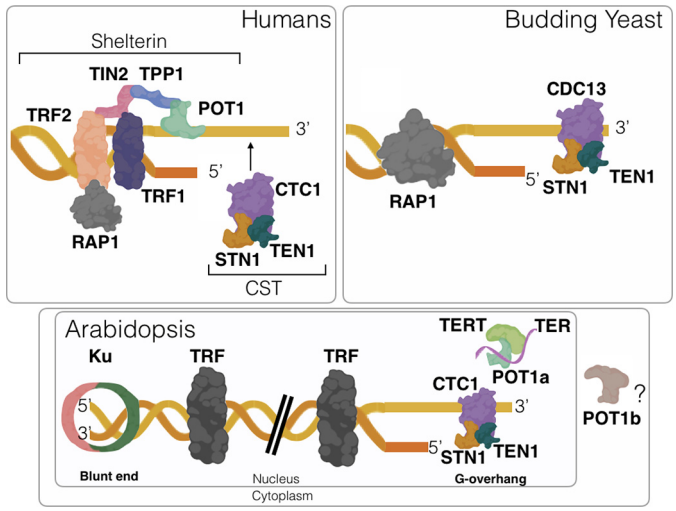

Figure 2. Models for chromosome end protection. Human telomeres are protected by the shelterin complex. CST transiently associates with the telomeric G-overhang during S phase to facilitate replication of the C-rich telomeric strand. In budding yeast, CST provides a stable, protective cap on the G-overhang, and RAP1, a shelterin component ortholog, binds the duplex region of telomeric DNA. *Arabidopsis* telomeres are asymmetrical. Ku maintains a blunt end on one chromosome terminus, whereas the other end harbors a conventional G-overhang that is bound by CST. There are two functional POT1 paralogs in *A. thaliana*. AtPOT1a is a component of the telomerase RNP, whereas AtPOT1b promotes genome stability and is proposed to reside in the cytoplasm.

are now encoded from separate loci in the genome. TERT can interact with a large array of RNAs *in vivo* (23). Ultimately, an RNA emerged with a higher affinity for TERT, a short C-rich repeat that could serve as a telomere sequence template, and a stem-loop element abutting the template that could form a functional template boundary element to allow fidelity of telomere repeat addition by TERT (24). Unlike TERT, TER is constrained by structure and not sequence (25, 26). Consequently, TER sequences diverged and expanded to give accessory proteins a foothold in the RNP complex. These new telomerase proteins enabled RNP maturation and both positive and negative regulation of the enzyme (27, 28).

Telomere-associated proteins: Origins and their role in telomere end protection

In vertebrates and fission yeast, telomere end protection is mediated by shelterin (29, 30) (Fig. 2 and Table 1). Shelterin physically caps the telomere ends, preventing the termini from being recognized as DNA damage and suffering DNA attrition via nucleolytic processing and DNA damage checkpoint activation. Shelterin is composed of TRF1/TRF2 (SpTAZ1), which binds the duplex DNA, and POT1-TPP1/SpPot1-SpTpz1, which binds the 3′ single-strand extension on the extreme terminus (termed the G-overhang). Additional proteins bridge the two DNA-binding complexes (TIN2(SpPOZ1) and RAP1). In addition to end protection, shelterin controls telomerase access and therefore contributes to telomere length regulation (29).

In budding yeast, instead of chromosome end protection by shelterin, the G-overhang is stably bound by the CST complex, comprised of Cdc13(CTC1), STN1, and TEN1 proteins (31) (Fig. 2 and Table 1). Notably, vertebrates also possess CST, but this complex only transiently associates with telomeres during S phase to promote telomeric DNA replication. CST is structurally related to the single-strand DNA-binding complex RPA and had likely evolved from the latter (32, 33). Interestingly, *Drosophila* lacks canonical telomere repeat arrays at its chromosome termini and yet encodes one or more proteins related to CST subunits (34, 35). Flowering plants, including *Arabidopsis*, present yet another twist on the telomere protection apparatus wherein one half of the chromosome ends harbor a G-overhang bound by CST, whereas the other half are blunt-ended and bound by Ku, which functions in the nonhomologous DNA end joining (NHEJ) pathway of DSB repair and has high affinity for DNA ends (36) (Fig. 2). The asymmetry of plant telomeres may reflect the absence of a 5′ exonuclease (*e.g.* Apollo) (37) that normally converts blunt-end telomeres created from leading-strand synthesis into termini with the typical 3′ G-overhang (38).

The shelterin and CST proteins employ one of two DNA binding motifs: the MYB domain for duplex DNA binding and the oligonucleotide/oligosaccharide-binding fold (OB-fold) for interaction with single-strand DNA. The MYB motif is common in transcription factors and may have been predisposed to function at telomeres as it is capable of binding tandemly repeated sequences (39, 40). OB-folds, on the other hand, function in a vast array of nucleic acid transactions and are found in proteins ranging from t-RNA synthetases to nucleases and RPA (41). Thus, the single-strand telomere-binding proteins have likely diverged from a common OB-fold ancestor with a role in DNA repair and/or replication (42).

Telomere protection and DNA repair

DNA damage repair pathways and telomere-associated proteins act collaboratively to promote genome integrity. Both TERT and TER have been linked to the DNA damage response (Table 1). In the presence of a DSB, human TERT relocalizes to the nucleolus (43), an outcome that would decrease the proba-

Table 1

Localization and functions of telomere-related components

Tabulated is a summary of published experimental data and *in silico* predictions for core constituents of the telomerase RNP, the CST complex, and the Shelterin complex. Different functions ascribed to POT1 paralogs from vertebrates, worms, plants, and ciliates are highlighted.

Component		Localization	Ref.	Role at telomeres	Ref.	Proposed role outside telomeres	Ref.
Telomerase							
TERT		Nucleus/nucleoplasm/nucleolus/ PML bodies/ cytosol/mitochondria/ mitochondrial nucleoid	141,142, 151,152	Reverse transcriptase using TER as a template	22,164	DNA damage response/apoptosis/cell cycle/ nuclease activity/oxidative stress response/glucose import/response to hypoxia/nitric oxide synthease activity	43,44,94, 95,141, 169, 170
TER		Vertebrate: nucleus/cajal body/cytosol; Yeast: cytosol	46,153-156	RNA template for telomeric repeats	164,165	DNA damage/cell cycle	46
CST							
STN1		Nucleus/nucleoplasm (predicted)/intermediate cytoskeleton (predicted)/ intracellular membrane (predicted)	83	Coordinates telomere replication/telomerase regulation/C-strand fill-in/protects against telomeric 5' end degradation	166	Transcriptional regulation by RNA pol II	88
TEN1		Nucleus	83,89	Coordinates telomere replication/telomerase regulation/C-strand fill-in/protects against telomeric 5' end degradation	85	Chaperone activity(Arabidopsis)/ regulation of DNA pol-alpha	88,89
CDC13/CTC1		Nucleus	82,83, 157	Coordinates telomere replication/telomerase regulation/C-strand fill-in/protects against telomeric 5' end degradation	82,83, 166	Transcription regulation RNA pol II/ replication fork restart	81,88
Shelterin							
TRF1		Nucleus/Nucleolus/PML bodies/ Cytoplasm (Predicted)	158,159	Telomere length regulation	98,99	Cell division/base excision repair	133, 171, 172
TRF2		Nucleus/PML bodies/nucleoplasm (predicted)/axon cytosol (predicted)	55,160	T-loop formation/telomere bending/represses ATM-mediated DNA damage response	98	Regulation of transcription by RNA polymerase II/cellular senescence /apoptosis/cell cycle/DNA damage/base excision repair/replication	55,73,74, 93,133
TIN2		Nucleus/mitochondria/ perinucleolar chromocenter	57, 138	Provides bridge between ds and ssDNA binding shelterin components	167	DNA damage/mitochondria oxidative phosphorylation	138
RAP1		Nucleus/cytosol	161,162	Yeast: binds ds telomeric DNA/negatively regulates telomere length Vertebrates:negative regulator of telomere length/does not bind telomeric DNA directly	90,173	Gene silencing/transcriptional regulation/oxidative stress response	60,90, 135,136
TPP1		Nucleus/cytosol	137	Connects POT1 to TIN2/recruits telomerase/ stimulates telomerase processivity	86,168	Controls POT1 localization to nucleus	137
POT1	Vertebrates	Nucleus/cytosol	133	hPOT1: binds ss telomeric DNA/represses ATR-mediated DNA damage response/telomere length regulation/telomerase recruitment/telomere capping	163	DNA duplex unwinding/meiotic synapsis/base excision repair/NHEJ/replication	62,63,133
		Nucleus	163	mPOT1a: binds ss telomeric DNA/telomere length regulation/telomere capping/represses ATR-mediated DNA damage response	102,103	None	
		Nucleus/cytosol	104	mPOT1b: binds ss telomeric DNA/telomere length regulation/telomere capping/5' end resection/represses ATR-mediated DNA damage response		Regulation of GTPase activity/regulation of phagocytosis/innate immune response	104
	Worms	Nucleus	105	CeOB1: G strand binding/telomere length regulation/telomere capping	105	None	
				CeOB2: telomere length regulation/telomere capping/C-strand binding			
				CeOB3: no known role			
			108	(MRT1): Telomere replication/necessary for telomerase-mediated telomere repeat addition	108	DNA crosslink repair/nucleotide excision repair	108
	Plants	Nucleus	113	PpPOT1: binds ss telomeric DNA/telomere length regulation/telomere capping	113	None	
		Nucleus	117	AtPOT1a:binds ss telomeric DNA/telomerase RNP component/telomerase processivity	85,117, 120,121	None	
		Cytosol (Predicted)		AtPOT1b: promotes genome stability		None	
	Ciliates	Nucleus	110	TtPOT1: binds telomeric DNA/telomere maintenance	110	Prevents activation of cell cycle checkpoint	110
				TtPOT2: facilitates de novo telomere formation?		Localizes to sites of chromosome breakage but not to telomeres	111
			109	TEBP: chromosome end protection/telomere length maintenance	109	None	
			112	RTP: telomere replication	112	Localizes with replication apparatus	112

bility of *de novo* telomere formation at sites of DNA damage. In addition, human cells lacking TERT fail to mount an effective DNA damage response to ionizing radiation (44). Intriguingly, these cells also display altered chromatin structure and fragmented chromosomes, suggesting that TERT plays a role in chromatin reorganization (45). Human TER (hTR) has been proposed to play a TERT-independent role in the response to DNA damage. Inhibition of hTR causes rapid arrest of cell growth, whereas increased hTR, which occurs in response to DNA damage induced by UV light, inhibits the DNA damage

checkpoint kinase ATR (46). In contrast, loss of TR in mice does not trigger phenotypes distinct from those of mTERT mutants, suggesting that the core RNA and protein components of telomerase act in the same pathways (47, 48).

Shelterin proteins also modulate the DNA damage response (Table 1). TRF2, for instance, prevents ATM-mediated DNA damage signaling at telomeres (49) and also helps recruit various DNA damage response and repair factors, such as ERCC1, Apollo, the MRE11-RAD50-NBS1 complex, helicases BLM and WRN, Ku, and PARP1/2 (50) (Table 1). The recruitment of these factors facilitates telomeric DNA replication, promotes the formation of a single-strand overhang on the chromosome terminus, and ensures that telomeres are properly sequestered to prevent inappropriate recombination or activation of a DNA damage response (51, 52). TRF2 can also associate with DSBs within the body of the chromosome as part of the early response to DNA damage (53, 54). As such, the ability of TRF2 to engage the machineries concerned with the DNA damage response and DNA repair likely promotes genome stability on a global scale. Interestingly, both TRF1 and TRF2 are modified by MMS21, a SUMO ligase involved in DNA repair and recombination. This modification is associated with alternative lengthening of telomeres (ALT) (55), a mechanism germane for telomere maintenance in cancer cells that lack telomerase (56).

Like TRF2, TIN2 and RAP1 associate with chromosome locales other than the telomeres. TIN2 accumulates at nontelomeric regions (57) associated with HP1 (58), a heterochromatin mark that has been implicated in the DNA damage response (59). Moreover, in human cells, RAP1 interacts with noncoding interstitial TTTAGGG repeats present on some chromosomes, raising the possibility that RAP1 helps prevent fragility and recombination at these sites (60).

POT1 has also been implicated in the DNA damage response (Table 1) (Fig. 3). The association of POT1 with the telomeric G-overhang prevents activation of an ATR-mediated DNA damage response (61), and recent studies indicate that human POT1 increases the fidelity of NHEJ at nontelomeric sites (62). Intriguingly, the C terminus of hPOT1 bears structural similarity to a Holliday junction resolvase domain (63), supporting the notion that POT1 affects other facets of DNA metabolism beyond telomere biology.

Ku harbors two subunits (Ku70 and Ku80) and is a core component of the NHEJ pathway (64). Within the context of telomere biology, Ku facilitates telomere protection and telomeric DNA replication (36, 65, 66). Recent studies in budding yeast provide clues for how the DNA repair and telomere protection functions of Ku might be parsed at chromosome termini. Ku harbors two solvent-exposed α-helices on opposite sides of the heterodimer. The surface facing the telomere end is necessary for NHEJ, whereas the inward facing helix is required for telomeric heterochromatin formation (67). In addition to discrete structural boundaries, separation of function can be influenced by cell cycle regulation. For example, the cell cycle regulator CYREN was recently shown to interact with Ku and block NHEJ at telomeres during the S and G_2 cell cycle phases (68). Ebrahimi and Cooper (69) have postulated that localization of telomeres within different regions of the nucleus influences a broad range of cellular processes, including meiotic recombination, chromosome segregation, and gene expression. Hence, in a broader sense, both temporal and spatial regulation of telomeres impact cellular physiology.

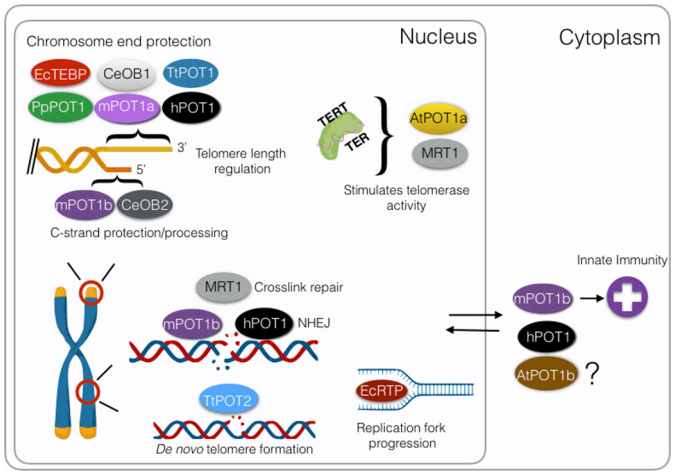

Figure 3. Diverse functions of POT1. Many POT1 orthologs bind single-stranded G-rich telomeric DNA, serving to control telomere length and to protect chromosome ends from eliciting the DNA damage response. Other POT1 proteins are tailored to engage the telomeric C-strand and its replication machinery. There are also examples of POT1 proteins that do not stably engage the chromosome terminus, but rather function to stimulate telomerase activity or to facilitate DNA repair. In addition, several POT1 proteins have been shown to accumulate in the cytoplasm or are predicted to reside here. Cytoplasmic mouse POT1b (*mPOT1b*) is proposed to promote an innate immunity response. Shown are the *A. thaliana* POT1a (*AtPOT1a*) and POT1b (*AtPOT1b*); *C. elegans* POT1 proteins CeOB1, CeOB2, and MRT1; human POT1 (*hPOT1*), mouse POT1a (*mPOT1a*), and POT1b (*mPOT1b*); *P. patens* POT1 (*PpPOT1*); and *T. thermophila* POT1 (*TtPOT1*) and POT2 (*TtPOT2*).

A role for telomere-associated proteins in DNA replication and transcription

Given that telomere accessory factors have likely evolved from factors that function in DNA repair, DNA replication, and transcription, it is not surprising that some of the telomere-associated factors also function in the aforementioned processes. Because of the highly repetitive nature of G-rich telomeric DNA and its propensity to form higher-order structures, such as the G-quartet, auxiliary factors are needed to ensure timely and proper replication through telomeric tracts. Notably, both POT1 and TRF2 stimulate the helicase activity of WRN (70, 71), and POT1 has been found to promote G-quartet unwinding by the WRN and BLM helicases (72) (Table 1). TRF2 has been proposed to assist in telomeric replication, and it does so by inducing positive supercoiling in DNA that favors enhanced access by DNA topoisomerases and the Apollo nuclease, enzymes critical for replication (73, 74). Furthermore, TRF2 is also hypothesized to assist in the assembly of the pre-replication complex during telomere replication (75, 76).

The primary function of the CST heterotrimer appears to be in telomere replication (Table 1). Originally identified as a DNA Pol α accessory factor (77), the vertebrate CST complex was subsequently shown to stimulate synthesis of the telomeric C-strand after telomerase extends the G-strand (78–80). CST plays a crucial role in the restart of stalled replication forks at nontelomeric sites (81), and CST mutations lead to genome-wide instability (82, 83). Vertebrate CST only transiently engages telomeres (84), but in budding yeast and in *Arabidopsis*

thaliana, CST is a constitutive component of telomeres that facilitates both replication of the C-rich telomere strand by Pol α/primase and the G-rich strand by stimulating telomerase activity (82, 84, 85). Hence, some of the POT1-TPP1 functions within the context of shelterin (86) may be fulfilled by CST. Indeed, Lue (87) has provided a compelling argument that POT1-TPP1 evolved from CST. The multifunctional nature of CST is further evidenced by the involvement of components of the yeast complex in transcriptional regulation through interactions with RNA polymerase II and the elongation factor Spt5. The interactions of CST with the transcription machinery are thought to help mitigate the consequences of RNA polymerase II collision with replication forks (88). In addition, studies in *Arabidopsis* have revealed that the CST component TEN1 possesses protein chaperone activity that is activated in response to heat stress (89) (Table 1).

Besides CST, other telomere-associated proteins also influence transcriptional regulation (Table 1). Yeast RAP1 was originally described as a transcriptional regulator at many promoters (90, 91). Human RAP1 modulates NF-κB expression (92), whereas interaction of TRF2 with the promoter of the cyclin-dependent kinase CDKN1a affects its expression (93). TERT has also been reported to enhance the expression of genes such as cyclin D1 (94) and NF-κB (95).

Gene duplication: Refining the landscape of telomere protein function

Gene duplication has fueled protein evolution, including telomere proteins. The duplication event giving rise to vertebrate TRF1 and TRF2 dates back 540 million years ago (96), at the beginning of the Chordate lineage (97). The conserved C-terminal MYB domain of TRF1/2 facilitates telomeric DNA engagement, whereas divergent N-terminal domains (98) are important for telomeric DNA length regulation (primarily accomplished by TRF1) (99) or chromosome end protection (TRF2) (100) (Table 1). The *Candida* clade possesses two copies of the gene that encodes the Cdc13 component of CST (101). The two paralogous proteins, Cdc13A and Cdc13B, are significantly smaller than their counterparts in budding yeast and have overlapping but nonredundant functions in telomere length regulation.

One of the most fascinating outcomes of gene duplication is seen with POT1 (Fig. 3) (Table 1). Here, independent gene duplication events occurred repeatedly throughout evolution. Although humans have a single POT1 protein, mice possess two POT1 paralogs, mPOT1a and mPOT1b, that share 72% sequence similarity (102). Recent studies suggest that both mPOT1a and mPOT1b attenuate ATR signaling at chromosome ends (103). However, mPOT1b uniquely contributes to the regulation of 5′ end resection to form the 3′ G-overhang (103) and may also play a cytosolic role in the innate immunity response (104).

The POT1 isoforms in worms and ciliated protozoa exhibit more profound functional divergence. *Caenorhabditis elegans* encodes four single OB-fold proteins with structural similarity to the OB-folds of mammalian POT1 (105). CeOB1 binds the telomeric G-rich strand, whereas CeOB2 engages the complementary C-rich strand. Mutation in either of these *CeOB* genes leads to telomere elongation, providing evidence that their encoded proteins serve as a negative regulator of telomerase (106, 107). The function for CeOB3 is unknown; however, CeOB4 (MRT1) was originally identified in a screen for genes required for germ line mortality as a result of telomere shortening (108). CeOB4 is required for telomerase activity *in vivo*. Intriguingly, CeOB4 also bears a SNM1 family nuclease domain and has been implicated in both DNA cross-link and nucleotide excision repair (108).

In the ciliates *Euplotes crassus* and *Tetrahymena thermophila*, there are two POT1 paralogs (109, 110). The *Tetrahymena TtPOT1*-encoded protein is essential for telomere length maintenance and prevents checkpoint activation much like the vertebrate POT1 proteins (110). However, TtPOT2 protein does not associate with chromosome ends, but instead localizes to internal sites in macronuclear chromosomes that are destined for developmentally programmed cleavage and *de novo* telomere formation (111). In *E. crassus*, the telomere end-binding protein caps chromosome ends (109). Replication telomere protein, the other POT1-like protein, is not associated with telomeres, but rather co-localizes with the replication apparatus as it moves through the macronuclear genome (112). This remarkable observation underscores the strong connection between telomere proteins and the DNA replication machinery.

The plant kingdom is replete with large gene families, arising from both localized gene duplication and whole-genome duplication. It is therefore noteworthy that most *POT1* genes in plants are not duplicated. The *POT1* gene in the early diverging land plant *Physcomitrella patens* retains the ancestral functions of binding single-stranded G-rich telomeric DNA and protecting chromosome ends from fusion (113). However, at least two independent *POT1* duplications occurred in higher plants, one in the grasses and the other in the Brassicaceae family to which *A. thaliana* belongs (114). There are three POT1 paralogs in *A. thaliana*, AtPOT1a, AtPOT1b, and AtPOT1c (114, 115). AtPOT1a and AtPOT1b exhibit only 52% sequence similarity. AtPOT1a resembles the mammalian shelterin component TPP1 (86, 116) in that it physically associates with the telomerase RNP and stimulates its repeat addition processivity (84, 117). However, unlike TPP1 (118), AtPOT1a accumulates at telomeres only in S phase (117), indicating that it is not a stable component of the end protection complex. Initially, AtPOT1a was not thought to bind telomeric DNA (119), but a recent study showed that the first OB-fold of AtPOT1a has single-strand telomeric DNA-binding activity (120). Strikingly, the AtPOT1a lineage, but not AtPOT1b, has been subjected to positive selection from an ancestral POT1 protein, leading to enhanced interaction with CST (114). Hence, AtPOT1a appears to have been evolved to be specialized for telomere maintenance through CST interaction. A role for AtPOT1b in telomere biology is not clear. It cannot complement the *pot1a* mutant (114) and cannot bind telomeric DNA *in vitro* (120). However, overexpression of the AtPOT1b C-terminal domain leads to massive chromosome fusion (121). Whereas this finding implicates AtPOT1b in chromosome end protection, AtPOT1b probably does so in a manner distinct from the single-copy POT1 proteins from vertebrates and fission yeast. The

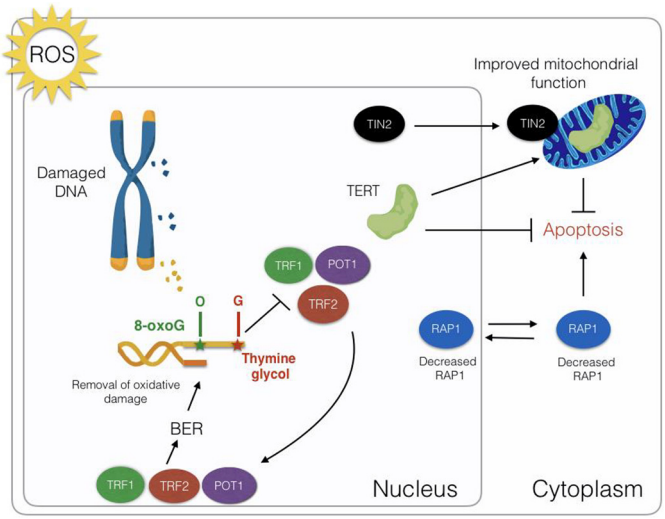

Figure 4. Impact of oxidative stress on telomeres and telomere-associated proteins in mammals. Telomeres are a hot spot for oxidative damage causing base modifications including thymine to thymine glycol and guanine to 8-oxoG. These lesions interfere with DNA binding by TRF1, TRF2, and POT1. These same proteins stimulate BER at telomeres and perhaps elsewhere in the genome, enabling the removal of damaged bases from the DNA. Oxidative DNA damage decreases the abundance of both cytoplasmic and nuclear RAP1, which in turn triggers apoptosis. Conversely, oxidative stress leads to the accumulation in mitochondria of TERT and TIN2, which promote mitochondrial functions that protect against apoptosis.

third *POT1* gene in *A. thaliana*, *AtPOT1c*, arose only 5 million years ago as a partial duplication of the *AtPOT1a* locus. The insertion of a transposon into the promoter of *AtPOT1c* rendered this gene silent almost immediately after its genesis (122). This finding, coupled with the remarkable functional divergence associated with POT1 paralogs across eukarya, argues that *POT1* dosage affects the fitness of organisms, and one or more of the duplicated copies must diverge quickly or be silenced.

Telomere proteins and their role in the genome-wide response to oxidative stress

The majority of DNA lesions in mammalian and plant cells can be attributed to oxidative damage (123, 124), and recent data indicate that several shelterin components safeguard the genome against this assault (Fig. 4). Reactive oxygen species (ROS) modifies DNA bases, most commonly resulting in 8-oxoguanine (8-oxoG) and thymine glycol (Tg) (125). If not repaired, 8-oxoG induces GC-TA transversion mutations as well as single-strand or double-strand breaks, leading to genomic instability (126). Tg is the most prevalent oxidative product of thymine, responsible for 10–20% of ionizing radiation-induced genomic damage (127). Due to their high G-T content, telomeres are a hot spot for oxidative damage (128–130).

Base excision repair (BER) is the most important pathway for removing 8-oxoG and Tg lesions (131). Mice lacking the glycosylase NTH1, which removes Tg via BER, exhibit increased telomere fragility (132). Intriguingly, TRF1, TRF2, and POT1 stimulate BER after oxidative damage (133). Interestingly, 8-OxoG and Tg modifications inhibit telomeric DNA binding by TRF1, TRF2, and POT1 *in vitro* (134). These observations suggest a feedback loop wherein oxidative damage at telomeres leads to the expulsion of the aforementioned telomere proteins, which then become available to assist in the BER-mediated repair of damaged telomeric bases, so as to enable the re-engagement of shelterin at the chromosome terminus (133) (Fig. 4). Because TRF1 and TRF2 can associate with other genomic locales, they may exert a broader impact in the response to oxidative stress.

Recent data reveal an intriguing response of RAP1 to oxidative stress and other types of DNA damage (135). RAP1 levels decrease in the nucleus and the cytoplasm in response to ROS. Diminished levels of cytoplasmic RAP1 appear to promote apoptosis in aging cells (92) (Fig. 4). Notably, in yeast, shortening of telomeres due to senescence releases RAP1, which then becomes associated with extratelomeric sites. Release of RAP1 from telomeres correlates with the down-regulation of genes encoding core histones and the translational apparatus and up-regulation of genes responsive to senescence (136) (Table 1).

Genome protection from a distance: The role of telomere proteins outside the nucleus

The role of telomere proteins in the response to oxidative stress correlates with cytoplasmic activities, but the molecular mechanisms that govern telomere protein function outside the nucleus are largely unexplored. In addition to RAP1, several other telomere-related proteins accumulate in the cytoplasm (Table 1). POT1, TPP1, and trace amounts of TIN2 shuttle in and out of the nucleus and can be detected as subcomplexes in the cytoplasm (137). TTP1 bears a nuclear export signal that is crucial for modulating the levels of the TTP1-POT1 complex within the nucleus. Abrogation of TPP1 nuclear export causes overelongation of telomeres and activates the DNA damage response (137).

TIN2 possesses a mitochondrial targeting sequence (MTS) that enables its transport into the mitochondria, where it is post-translationally modified (138) (Fig. 4). Interestingly, in cells lacking TIN2, glycolysis is inhibited, and ROS production is elevated along with ATP and oxygen consumption. Strikingly, these phenotypes do not correlate with telomeric abnormalities (138), indicating that TIN2's mitochondria-related functions are distinct from its role at telomeres.

TERT proteins from vertebrates and plants also harbor a MTS (Table 1). Extracts prepared from mitochondria are enriched in telomerase activity (139). In addition, TERT is associated with the outer mitochondrial membrane translocators TOM20 and TOM40 (140, 141) as well as tFAM, HSP60, tim23, and a variety of mitochondrial RNAs (23, 142). Notably, oxidative stress triggers hTERT export from the nucleus to mitochondria, and elevated levels of hTERT in this compartment correlate with stabilization of mitochondrial DNA, reduced ROS, increased mitochondrial membrane potential, and enhanced mitochondrial function (143–145) (Fig. 4). There are also reports that mitochondrial TERT not only associates with non-TER RNAs but also possesses noncanonical enzyme activities. These include an RNA-dependent RNA polymerase activity that is implicated in the production of siRNA (23) and reverse transcriptase activity using mitochondrial tRNA as a template (142). The biological relevance of this latter activity is unknown.

TERT in the mitochondria has been proposed to stimulate mitochondrial DNA replication and repair (142). Compared with WT mice, the RNA expression profiles of *tert* mutants monitored for four consecutive generations (G1–G4) reveal statistically significant changes in the expression of both mitochondrial and nuclear encoded genes required for oxidative phosphorylation, mitochondrial function, and antioxidant defense (146). Similar results were obtained *A. thaliana tert* mutants of generations G2 and G7 (147). Interestingly, yeast and ciliate TERT proteins lack an MTS (139), raising the possibility that the mitochondrial function of TERT is not conserved in these species or that these TERT proteins are transported into mitochondria via a different mechanism.

Conclusions and future directions

With the advent of linear chromosomes, factors involved in different facets of DNA metabolism were coopted to solve the telomere end protection and end replication problems. Some of these factors retain their functions in DNA replication, DNA repair, and transcriptional regulation. Telomeric DNA is a magnet for oxidative damage, and hence in the drive to maintain genome integrity, telomere proteins may have gained the capacity to protect chromosome ends from this assault by promoting BER proximally, or at a distance by affecting mitochondrial function. Alternatively, some noncanonical functions of telomere proteins may have an older origin. Mitochondria, which possess group II introns (148) and proteins structurally similar to the ancestral OB-folds of RPA (149), emerged 1.45 billion years ago (150). Thus, the building blocks for some of the modern-day telomere proteins and their functions in the oxidative stress response may reflect a mitochondrial ancestry. Finally, the ancient and emerging functions of telomere proteins have been linked to gene duplication events. In particular, *POT1* gene duplications that occurred across evolution have given rise to multifaceted roles of telomere proteins in chromosome biology and their integration into the broader context of cellular physiology.

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