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Telomere maintenance and the DNA damage response: a paradoxical alliance

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Telomeres are the protective caps at the ends of linear chromosomes of eukaryotic organisms. Telomere binding proteins, including the six components of the complex known as shelterin, mediate the protective function of telomeres. They do this by suppressing many arms of the canonical DNA damage response, thereby preventing inappropriate fusion, resection and recombination of telomeres. One way this is achieved is by facilitation of DNA replication through telomeres, thus protecting against a "replication stress" response and activation of the master kinase ATR. On the other hand, DNA damage responses, including replication stress and ATR, serve a positive role at telomeres, acting as a trigger for recruitment of the telomere-elongating enzyme telomerase to counteract telomere loss. We postulate that repression of telomeric replication stress is a shared mechanism of control of telomerase recruitment and telomere length, common to several core telomere binding proteins including TRF1, POT1 and CTC1. The mechanisms by which replication stress and ATR cause recruitment of telomerase are not fully elucidated, but involve formation of nuclear actin filaments that serve as anchors for stressed telomeres. Perturbed control of telomeric replication stress by mutations in core telomere binding proteins can therefore cause the deregulation of telomere length control characteristic of diseases such as cancer and telomere biology disorders.

KEYWORDS

telomere maintenance, telomerase, shelterin, DNA damage response, replication stress, telomere replication, nuclear actin

1 Introduction

Telomeres are nucleoprotein complexes located at the ends of linear chromosomes which serve to maintain genomic integrity and ensure cellular survival. Telomeres were first identified in fruit flies and corn (McClintock, 1941; Muller, 1938) and have since been characterized in a range of eukaryotes. Human telomeres are comprised of tandem TTAGGG repeats which extend for 3–18 kb (Moyzis et al., 1988), ending in a single-stranded G-rich overhang 12–400 nucleotides long (Makarov et al., 1997; McElligott and Wellinger, 1997; Zhao et al., 2008). This overhang, which if exposed would resemble DNA damage, can strand invade the double-stranded region of the telomere to form a telomere-loop (t-loop; Figure 1A), which serves to protect the telomere from being misrecognized as a double-strand break (DSB) and activating a DNA damage response (DDR) (Doksani et al., 2013; Griffith et al., 1999; Van Ly et al., 2018).

Telomeric DNA is bound by a six protein complex named shelterin, which helps maintain telomere structure and function in many different ways (de Lange, 2005; de Lange, 2018; Figure 1). Shelterin binds to telomeres through TRF1 and TRF2 (telomere-repeat

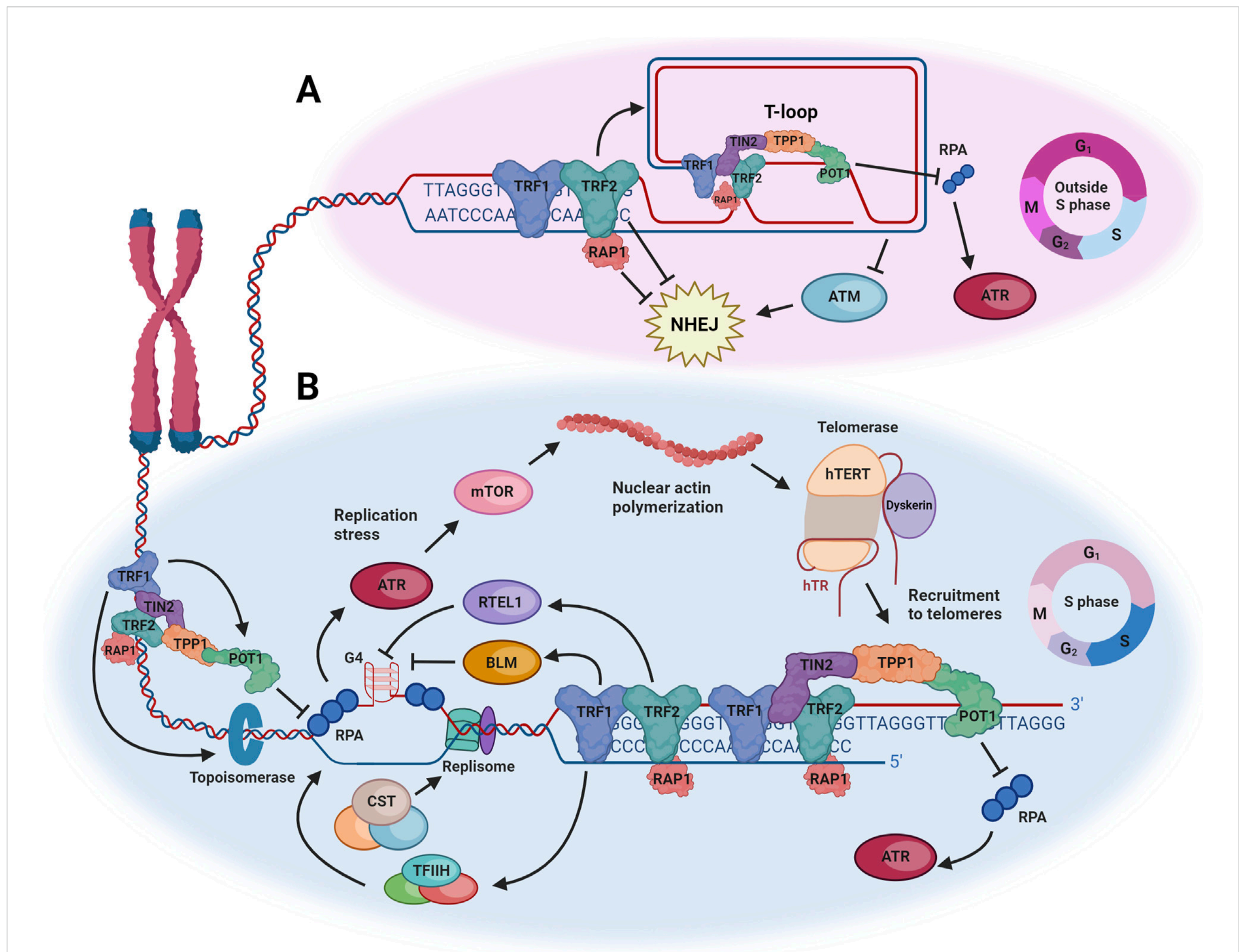

FIGURE 1
Summary of mechanisms by which the DNA damage response is repressed at telomeres or harnessed to facilitate telomerase-mediated telomere maintenance. **(A)** Shelterin protects telomeres from inappropriately activating a DNA damage response in phases of the cell cycle when the telomere can fold into a t-loop. TRF2 promotes t-loop formation, which prevents activation of ATM, and TRF2 also directly suppresses NHEJ. POT1 protects against activation of ATR. **(B)** During S phase, the t-loop is unwound to allow replication of the telomere by the canonical replication machinery, which is impeded by the repetitive sequence of telomeres, their occupation by shelterin, and propensity to form G-quadruplexes (G4). Multiple components of shelterin counteract the resulting replication stress, but any remaining stress activates ATR, which facilitates the polymerization of nuclear actin and ultimately the recruitment of telomerase to telomeres. See text for more details. Figure created with Biorender.com.

binding factor 1 and 2), which both recognize and bind to the double-stranded portion of the telomere (Bilaud et al., 1997; Broccoli et al., 1997; Chong et al., 1995). The telomere is also bound by POT1 (protection of telomere 1) which forms a heterodimer with TPP1 (TINT1/PTOP/PIP1) (Houghtaling et al., 2004; Liu et al., 2004; Ye et al., 2004b) and binds to the single-stranded overhang specifically through its oligonucleotide/oligosaccharide binding (OB) folds (Baumann and Cech, 2001; Loayza and De Lange, 2003). Rap1 is a conserved shelterin subunit and is recruited to telomeres by forming a complex with TRF2 (Li et al., 2000; Sfeir et al., 2010). The final shelterin component is TIN2 (TRF1-interacting factor 2) which bridges TRF1/2 and the TPP1-POT1 complex and helps to stabilize the latter in addition to the TRF2-Rap1 complex (Houghtaling et al., 2004; Kim et al., 1999; Liu et al., 2004; Ye and de Lange, 2004; Ye et al., 2004a). Shelterin is crucial for telomere protection, as loss of shelterin binding leads to deprotection of telomeres, unwinding of the t-loop and activation of at least seven different DDR pathways (de Lange, 2018; Sfeir and de Lange, 2012), culminating in genomic instability, cell cycle arrest and cell death. Conversely, the shelterin complex can also harness several DDR pathways to assist in maintenance of telomere length and avoidance of the cell cycle arrest and cell death that result from critically short telomeres. This article will discuss the complicated and paradoxical relationship between telomeres, the DDR, and telomere maintenance by the ribonucleoprotein enzyme telomerase, with a focus on events in human cells.

2 DNA damage and its suppression at the telomere

Given that the structure of a telomere resembles damaged DNA, it is imperative that the DDR is suppressed to prevent unnecessary

repair of the telomere which could result in chromosome end-to-end fusions. Global DNA damage is detected largely by three phosphatidylinositol 3-kinase-related protein kinases (PIKKs): ATR (ataxia telangiectasia and Rad3-related), ATM (ataxia telangiectasia mutated) and DNA-PK (DNA-dependent protein kinase) (Blackford and Jackson, 2017). These kinases are responsible for the activation of various signaling pathways which trigger cell cycle arrest and the promotion of DNA repair mechanisms to resolve the DNA damage (Harrison and Haber, 2006). Should the extent of damage be irreparable then the kinases can instead signal the cell to undergo apoptosis or senescence (Nowsheen and Yang, 2012). ATR is activated in response to single-stranded DNA, which can occur at replication forks that are slowed or stalled in situations where DNA replication is impeded (a state known as "replication stress") (Saldivar et al., 2017; Yang et al., 2004). Conversely, both ATM and DNA-PK can be activated in response to DSBs, although they each employ different pathways to repair the damage, through homology-directed repair or non-homologous end joining (NHEJ) respectively (Davis et al., 2014; Shiloh and Ziv, 2013).

The components of shelterin both directly and indirectly suppress an unwanted telomeric DDR. Indirect suppression relies on t-loop formation, as telomeres in this closed state are unlikely to trigger a DDR (Figure 1A). This conformation would prevent the telomere end from being detected by factors which sense exposed DNA ends and activate the DDR, such as MRN (Mre11, Rad50, Nbs1) or the Ku70/80 complex (Gottlieb and Jackson, 1993; Lee and Paull, 2005). The key regulator of t-loop formation is TRF2, as its depletion from cells results in reduced t-loop formation (Doksani et al., 2013; Van Ly et al., 2018). Furthermore, TRF2 can promote strand invasion *in vitro*, as it binds to DNA and promotes formation of structures which resemble t-loops (Amiard et al., 2007; Stansel et al., 2001). As telomeres shorten, or following moderate TRF2 depletion, t-loops unfold and telomere ends become accessible to the DNA damage sensing machinery. These are termed "intermediate-state" telomeres (Cesare and Karlseder, 2012; Cesare et al., 2009), which trigger a DDR predominately through ATM (Karlseder et al., 2004; Van Ly et al., 2018), leading to cell cycle arrest and senescence. However, critically short telomeres or extreme loss of TRF2 instead results in deprotected telomeres (an "uncapped-state") which undergo ATM-dependent end-to-end chromosomal fusions, resulting in genome wide instability (Celli and de Lange, 2005; Denchi and de Lange, 2007). This is a consequence of TRF2 also directly repressing the downstream consequences of ATM activation at telomeres, via a short region named the iDDR (inhibitor of DDR) within the hinge domain of TRF2 (Okamoto et al., 2013). This motif inhibits telomeric localization of the E3 ubiquitin ligase RNF168, in turn preventing 53BP1 accumulation at telomeres and chromosomal fusion via NHEJ (Okamoto et al., 2013).

On the other hand, ATR activation resulting from an exposed single-stranded telomeric overhang is thought to be primarily suppressed by POT1, since POT1 depletion activates ATR and results in ATR-dependent DNA damage signaling at telomeres (Denchi and de Lange, 2007; Figures 1A, B). This is dependent on the ability of POT1 to bind to telomeres, as preventing its recruitment via TPP1 or TIN2 inhibition also results in ATR activation (Hockemeyer et al., 2007; Takai et al., 2011). POT1 binds to single-stranded telomeric repeats, including the 3′telomeric overhang, preventing binding of RPA (replication protein A) (Gong and de Lange, 2010), which coats single-stranded DNA and is a key factor in the activation of ATR (Zou and Elledge, 2003).

3 Shelterin suppression of the DDR resulting from telomere replication

While the structural similarity of exposed telomeres and damaged DNA suggests that telomere-associated proteins would strive to keep DDR proteins away, paradoxically this does not always appear to occur. In fact, many DDR proteins are recruited to telomeres, not just in cancer cells, but also in normal somatic cells. For example, ATR localizes to telomeres during S phase in normal human fibroblasts (Verdun and Karlseder, 2006), and is necessary to protect telomere fidelity in both human and mouse cells (McNees et al., 2010; Pennarun et al., 2010). One possible explanation for this phenomenon is that telomeres are difficult regions for the DNA replication machinery to efficiently and correctly traverse, as repetitive DNA sequences (termed "fragile sites") are highly prone to stalling replication machinery at the replication fork. Replication fork pausing or stalling has been observed in telomeres from yeast to humans (Fouche et al., 2006; Ivessa et al., 2002; Makovets et al., 2004; Sfeir et al., 2009), and may be further compounded at telomeres by shelterin binding also impeding DNA replication (Ohki and Ishikawa, 2004). Additionally, given their G-rich composition, telomeres readily form G-quadruplexes (G4s), DNA secondary structures which can also impede replisome progression (Bochman et al., 2012; Bryan, 2020). The inhibition of replication fork progression requires activation of processes to stabilize, repair and ultimately restart the replication fork. This response is regulated by ATR, which is activated by RPA-coated single-stranded DNA at the stalled replication fork (Shechter et al., 2004). Excessive replication stress at telomeres which cannot be resolved results in a "fragile telomere" phenotype: telomeres with breaks which appear as elongated or multiple signals at the ends of metaphase chromosomes (Sfeir et al., 2009). Given this, it is imperative that replication of the telomere is regulated to ensure its completion to prevent significant loss of telomeric DNA.

To prevent the generation of replication stress, shelterin helps to promote efficient telomere replication [reviewed in Bonnell et al. (2021); Higa et al. (2017a); Figure 1B]. The promotion of telomere replication by shelterin proteins is conserved across evolution, since Taz1, the fission yeast orthologue of TRF1, also has this capability (Miller et al., 2006). Mammalian TRF1 specifically mitigates lagging strand replication stress by promoting the recruitment of BLM (Bloom syndrome) (Martinez et al., 2009; Sfeir et al., 2009; Zimmermann et al., 2014), a RecQ family helicase which is capable of unwinding G4s which would impede replisome progression (Drosopoulos et al., 2015; Sun et al., 1998). A similar RecQ helicase, WRN (Werner syndrome), is also important for telomere replication as its depletion results in large telomeric deletions (Crabbe et al., 2004). WRN is capable of unwinding G4s (Drosopoulos et al., 2015; Mohaghegh et al., 2001), co-localizes at stalled replication forks with ATR and PCNA

(proliferating cell nuclear antigen), a DNA clamp component of the replisome (Constantinou et al., 2000; Rodrı´guez-López et al., 2003), and is capable of maintaining telomeric overhangs *in vitro* in a DNA-PK-dependent manner (Kusumoto-Matsuo et al., 2010). Furthermore, both BLM and WRN have been shown to interact with TRF1, TRF2 and POT1 *in vitro*, which in most cases stimulates their helicase activity (Lillard-Wetherell et al., 2004; Opresko et al., 2005; Opresko et al. 2004; Opresko et al. 2002). The specific ability of TRF1 to recruit helicases to telomeres during S phase is modulated by post-translational modification of TRF1, including phosphorylation and poly-ADP-ribosylation (Li et al., 2018; Maresca et al., 2023).

RTEL1 (regulator of telomere elongation helicase 1) is another important regulator of telomeric replication, as it is capable of unwinding G4s and t-loops to promote replisome progression (Vannier et al., 2012; Vannier et al., 2013). RTEL1 is recruited to telomeres in S phase by TRF2 and interacts with PCNA (Sarek et al., 2015; Vannier et al., 2013). Loss of RTEL1, or inhibition of its interaction with PCNA or TRF2, results in replication fork stalling, fragile telomeres and t-loop cleavage (Sarek et al., 2015; Vannier et al., 2012; Vannier et al., 2013). These observations suggest that RTEL1 is capable of unwinding t-loops to facilitate complete replication of telomeres, which is further supported by the ability of RTEL1 to unwind t-loop-like structures *in vitro* (Youds et al., 2010).

Another way in which TRF1 and TRF2 help telomeres avoid replication stress is by the recruitment of topoisomerase IIα and the nuclease Apollo to relieve topological stress at telomeres (d'Alcontres et al., 2014; Ye et al., 2010). Furthermore, TRF1-mediated recruitment of the transcription factor and nucleotide excision repair complex TFIIH is required to suppress the fragile telomere phenotype (Yang et al., 2022). If replication fork stalling does occur, TRF1 also helps mitigate the resulting ATR-mediated DDR by virtue of its tethering TIN2/POT1/TPP1 to the telomere, which may be able to displace RPA from exposed single-stranded DNA at replication forks (Zimmermann et al., 2014). The shelterin complex thus employs a full armory of defenses against the many ways in which telomeres could provoke unwanted DDRs, particularly during DNA replication.

4 Replication stress is a trigger for telomere maintenance by telomerase

Shelterin proteins also ensure complete telomere maintenance through their role in recruiting the telomere lengthening enzyme telomerase. Telomerase is an evolutionarily conserved ribonucleoprotein complex (Greider and Blackburn, 1985; Morin, 1989), containing two major components: the catalytic protein subunit telomerase reverse transcriptase (hTERT in humans; Nakamura et al., 1997), and the telomerase RNA subunit (hTR in humans; Feng et al., 1995), which provides the template for *de novo* telomere synthesis. Most normal human somatic cells lack telomerase expression (Nakamura et al., 1997), and hence experience telomere shortening over successive population doublings (Harley et al., 1990), due to the inability of the conventional DNA replication machinery to copy the extreme ends of linear DNA molecules (Lingner et al., 1995; Takai et al., 2024). Approximately 85% of cancer cells overcome telomere shortening via activation of telomerase; however, telomerase is also active within highly proliferative cells, including stem and germline cells [reviewed in Bryan and Cohen (2023); Roake and Artandi (2020)]. Following synthesis of a telomeric repeat, telomerase translocates along the newly synthesized repeat to continue extending the 3′overhang of the same telomere (Greider, 1991; Wu et al., 2017). Following this, the complementary C-rich telomeric strand is filled in by the CST-Polα/primase complex [reviewed in Cai and de Lange (2023)].

Telomerase activity relies upon its successful recruitment to telomeres; this process is very tightly regulated and typically only occurs during S phase (Diede and Gottschling, 1999; Jady et al., 2006; Marcand et al., 2000; Tomlinson et al., 2006). The timing of recruitment appears to be regulated over the cell cycle, at least partially, by human TRF1 or its fission yeast orthologue Taz1, as their depletion results in accumulation of telomerase at telomeres outside S phase (Dehe et al., 2012; Tong et al., 2015). Stable recruitment of human telomerase is dependent on its interaction with TPP1, specifically an N-terminal patch termed the TEL patch within the TPP1 OB fold (Grill et al., 2018; Nandakumar et al., 2012; Zhong et al., 2012). The interaction between POT1 and TPP1 is also essential for telomerase processivity (Latrick and Cech, 2010; Wang et al., 2007), while the depletion of TPP1 and TIN2 (which bridges TPP1 and TRF1/2) results in reduced telomerase presence at telomeres (Abreu et al., 2010). The interaction of hTERT with TPP1 relies upon the TEN domain within hTERT (Schmidt et al., 2014; Stern et al., 2012), and a region within the reverse transcriptase domain (Padmanaban et al., 2023).

It has become clear that in addition to their role in ensuring accurate telomere replication, DDR proteins are also important regulators of telomerase recruitment to the telomere. In particular, the telomeric replication stress response can be considered an evolutionarily conserved trigger for bringing telomerase to those telomeres experiencing stress. The requirement for an ATR-mediated DDR for telomerase recruitment to telomeres was initially demonstrated in the fission yeast *Schizosaccharomyces pombe*, as depletion of Rad3 (the fission yeast homolog of ATR) negatively impacts telomerase recruitment in this organism (Moser et al., 2011; Moser et al., 2009; Yamazaki et al., 2012). In humans, patients with ATR mutations have poor telomere maintenance, resulting in a range of telomere abnormalities (Pennarun et al., 2010); depletion or chemical inhibition of ATR also results in reduced telomerase recruitment to telomeres in human cells (Tong et al., 2015). This may involve the 9-1-1 (Rad9-Rad1-Hus1) checkpoint complex, which is needed for full catalytic activation of ATR at single-stranded DNA (Majka et al., 2006), and also shows an association with active human telomerase (Francia et al., 2006). Inversely, induction of replication stress can promote the recruitment of telomerase and therefore its ability to lengthen telomeres. Chemical induction of replication stress using the DNA polymerase inhibitor aphidicolin results in telomere lengthening (Sfeir et al., 2009) and an ATR-dependent increased accumulation of telomerase at telomeres (Tong et al., 2015). This regulation of telomerase recruitment to telomeres by ATR appears to be highly specific to natural chromosome ends, as contrastingly it has recently been demonstrated that ATR inhibits *de novo* telomere formation by telomerase at resected DSBs (Kinzig et al., 2024).

Induction of interstitial DSBs, using either Cas9 or I-SceI, resulted in telomerase-mediated *de novo* telomere formation which was significantly upregulated after inhibition of ATR, but not ATM (Kinzig et al., 2024).

As described briefly above, components of shelterin have long been known to play important roles in regulating telomere length by modulation of the access of telomerase to telomeres [reviewed in Smogorzewska and de Lange (2004)]. There have been various models proposed to provide conceptual frameworks for how this achieved; for example, the "protein counting model" postulates that the overall abundance of shelterin proteins at telomeres provides a negative regulatory signal for telomerase recruitment and/or extension (Marcand et al., 1997; Smogorzewska and de Lange, 2004). An alternative model proposes that telomerase travels along the telomere with the replication fork, and the chances of it reaching the end of the telomere are impacted by its encounters with shelterin proteins and the distance from subtelomeric origins of replication (Greider, 2016). Here, we would like to instead argue that the role of shelterin proteins in controlling telomere replication stress, discussed above, is a major component of their ability to also regulate telomerase recruitment to telomeres (Figure 1B).

As discussed above, a major function of TRF1 at telomeres is to recruit and coordinate other proteins to reduce telomeric replication stress, and TRF1 is also a major negative regulator of telomerase-mediated telomere lengthening (Smogorzewska et al., 2000). TRF1 levels at human telomeres decrease during S phase (Maresca et al., 2023; Verdun et al., 2005); this decrease is partially regulated by ATM, since experimental depletion of ATM results in accumulation of TRF1 at telomeres (Wu et al., 2007). Phosphorylation of S367 on TRF1 by ATM results in telomere elongation, due to removal of TRF1 from the telomere (McKerlie et al., 2012). This phosphorylation event also promotes telomerase recruitment to the telomere, as a phospho-null mutation of TRF1 S367 diminishes telomerase recruitment (Tong et al., 2015). Concordantly, experimental depletion or inhibition of ATM results in telomere shortening (Wu et al., 2007), and reduced recruitment of telomerase (Lee et al., 2015; Tong et al., 2015), and cells from patients with mutations in ATM have short telomeres (Metcalfe et al., 1996). Together, these data support a model in which partial depletion of TRF1 during S phase triggers telomere replication stress that results in increased telomerase recruitment. This model is supported by data from *S. pombe*, showing that deletion of the TRF1 orthologue Taz1 results in deregulated arrival of replicative polymerases at telomeres, resulting in an extended period in which both RPA and TERT are simultaneously and aberrantly recruited to telomeres, implicating a replication stress-induced ATR response in telomerase recruitment (Chang et al., 2013).

This may also be an explanation for the telomere lengthening induced by mutant versions of POT1. The first hints that POT1 is also involved in facilitating telomere replication came from the identification of familial or somatic mutations in patients with cancer; these mutations induced fragile telomeres (Calvete et al., 2015; Ramsay et al., 2013; Shi et al., 2014), and were directly shown to result in increased fork stalling in a DNA combing replication assay (Pinzaru et al., 2016). Expression of POT1 lacking its OB fold (POT1-ΔOB), rendering it incapable of binding the telomeric overhang, also results in telomeric replication stress (Pinzaru et al., 2020), which ultimately leads to increased telomerase recruitment (Laprade et al., 2020) and telomere lengthening (Loayza and De Lange, 2003; Tong et al., 2015). Consistently, the cancers that carry mutations in the POT1 OB fold also have long telomeres (Calvete et al., 2015; Ramsay et al., 2013; Robles-Espinoza et al., 2014; Shi et al., 2014). This has also been observed in mice, which possess two POT1 homologs with separation of function; the major role of POT1a is to suppress the DDR at telomeres, while POT1b also regulates telomeric overhang length (He et al., 2006; Hockemeyer et al., 2006; Wu et al., 2006). When POT1b is depleted from mouse cells, their telomeres initially shorten, triggering a DDR and an increase in ATR-mediated telomerase recruitment to telomeres (Gu et al., 2021; Takasugi et al., 2023). These cells ultimately develop ultralong telomeres as a result of the increased telomerase presence at telomeres (Takasugi et al., 2023). While physical sequestration of the 3′ telomeric overhang may contribute to the negative regulation of telomerase by POT1, it is likely that its role in preventing replication stress also contributes to the same outcome.

Another protein complex that both helps to overcome replication stress at telomeres and participates in telomere length control is the CST complex (consisting of proteins CTC1, STN1 and TEN1 in humans) (Olson and Wuttke, 2024). Human CST promotes replication restart after fork stalling; it does this through promotion of latent origin firing (Stewart et al., 2012), recruitment of the replication stress-response protein RAD51 (Chastain et al., 2016) and activation of the ATR pathway [Ackerson et al. (2020); reviewed in Olson and Wuttke (2024)]. In addition, human CST depletion causes a telomerase-dependent increase in telomere length (Chen et al., 2012). A mutation in CTC1 that leads to telomere elongation also causes an increase in the recruitment of telomerase to telomeres (Gu et al., 2018). While this may be at least partially explained by the inhibitory effect of wild-type CST on telomerase binding to its DNA substrate (Chen et al., 2012; Zaug et al., 2021), resulting in dissociation of telomerase from the telomere, it is also possible that some CTC1 mutations increase telomerase recruitment by increasing telomeric replication stress. Given that mutations in CST components cause the telomere biology disorder Coats Plus, and that these patients do not always show the telomere shortening typical of telomere biology disorders (Revy et al., 2023), further understanding of the interplay between CST, replication stress and telomerase recruitment may shed light on the telomere dysfunction underlying their disease.

5 Mechanisms and outcomes of replication stress-induced telomerase recruitment

Although the ways in which ATR and the replication stress response result in telomerase recruitment are incompletely understood, recent evidence implicates nuclear actin as one downstream element of this pathway. A large body of work in recent years has identified that nuclear filamentous actin (F-actin) is a key regulator of the DDR. Actin rapidly polymerizes within the nucleus in response to replication stress and DSBs; under replication stress conditions this polymerization is dependent on ATR, whose activity is required for downstream phosphorylation of another PIKK family kinase, mTOR (Lamm et al., 2020), in turn

regulating F-actin through the Wiskott-Aldrich syndrome protein (WASP) family (Mok et al., 2015). F-actin facilitates the DDR by re-localization of damaged DNA to the nuclear periphery for fork restart or DNA repair (Belin et al., 2015; Caridi et al., 2018; Lamm et al., 2020; Palumbieri et al., 2023; Ryu et al., 2015). Stressed telomeres are processed by the same pathway; they have also been shown to move toward the periphery under conditions of replication stress in an F-actin-dependent manner (Lamm et al., 2020; Pinzaru et al., 2020). It has also been previously demonstrated that telomeres located closer to the nuclear periphery are more likely to be later replicating (Arnoult et al., 2010); however, it is unclear whether this timing is solely due to their position within the nucleus, or if these are stressed telomeres.

Telomerase recruitment to telomeres also requires the function of nuclear F-actin; inhibition of actin polymerization, or regulators of its polymerization or function, results in decreased telomerase recruitment under endogenous conditions in human cell lines, as well as abrogating replication stress-mediated recruitment (Harman et al., 2024). Furthermore, nuclear F-actin serves as a direct site for telomerase recruitment to stressed telomeres, which reside along these actin fibers (Harman et al., 2024). The requirement for nuclear F-actin in this process, as well as its role in telomere replication, provides a further link which connects telomerase activity to telomere replication and the DDR.

Another mechanism by which the replication stress response may increase telomerase recruitment involves the known role of ATR in promoting firing of "dormant replication origins" (Ge and Blow, 2010) within a region under replicative stress, which allows replication in problematic regions to be efficiently completed. Replication of telomeres in human cells occurs across S phase and largely originates from origins within subtelomeric regions, proceeding unidirectionally through the telomere (Drosopoulos et al., 2015; Drosopoulos et al., 2012; Higa et al., 2017a). While this appears to account for most telomeric replication, replication origins have been observed within telomeric regions (Drosopoulos et al., 2012). These origins appear to be dormant, as they are fired following replication stress in a TRF2-dependent manner (Drosopoulos et al., 2020). This likely occurs via the recruitment of ORC proteins by TRF2 (Deng et al., 2007; Higa et al., 2017b; Tatsumi et al., 2008).

There is evidence that recruitment of telomerase to telomeres needs the passage of the replication fork. In budding yeast, telomerase cannot extend an artificial minichromosome unless it contains an origin of replication (Marcand et al., 2000), and generation of a 3′ telomeric overhang (the substrate of telomerase) does not happen without passage of a replication fork (Dionne and Wellinger, 1998). In human cells, telomerase acts after the conventional replication machinery has finished replicating the rest of the telomere (Hirai et al., 2012; Zhao et al., 2009). Therefore, it is possible that increased firing of dormant replication origins within telomeres during replication stress, mediated by ATR, increases the likelihood of the replication machinery reaching the end of the telomere, triggering telomerase recruitment.

The conservation of the link between replication stress and telomerase recruitment to telomeres implies that telomerase serves a vital cellular role after such stress. Indeed, there is an increasing amount of evidence that yeast need telomerase to survive replication stress, even when global telomere length has not decreased (Chang et al., 2009; Jay et al., 2016; Noel and Wellinger, 2011; Xie et al., 2015). Replication stress can cause sudden loss of telomeres (Crabbe et al., 2004; Zhang et al., 2017), so it is likely that telomerase is needed to counteract this potentially lethal event. If the requirement for telomerase to survive replication stress extends to human cells, this could open up ways to rapidly target cancer cells by combining replication stress-inducing chemotherapeutic agents with telomerase inhibition.

6 Conclusion

Telomeres impose a difficult balancing act that cells must perform to maintain their proliferative capabilities and genome integrity while also avoiding aberrant telomere overextension. Cells possess a tightly controlled regulatory system which impedes the DDR from inappropriately recognizing telomeres and causing telomere loss. Concurrently, the DDR is utilized to ensure that telomeres are correctly replicated and that their length is not shortened as a byproduct of replication stress, whether exogenous or endogenous. The interactions between telomere binding proteins and the telomeric replication stress response are emerging as major factors in the maintenance of telomere lengths within defined limits, which can be perturbed in diseases such as cancer and telomere biology disorders. Further elucidation of this interplay is therefore likely to increase understanding of the mechanisms underlying these diseases.

Data availability statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Author contributions

AH: Visualization, Writing–original draft, Writing–review and editing. TB: Conceptualization, Funding acquisition, Visualization, Writing–review and editing.

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Conflict of interest

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