Unravelling the Intricacies of Telomere Replication: A Molecular Conundrum

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ABSTRACT: Telomeres are specialized structures at the ends of linear chromosomes that protect them from degradation and fusion. Its replication is a complex process that involves both DNA polymerases and a specialized enzyme called telomerase which is a ribonucleoprotein complex that synthesizes telomeric DNA by using an internal RNA template. However, it requires auxiliary factors for complete replication. Among these, the CST complex—comprising CTC1, STN1, and TEN1—acts as a critical mediator in telomere replication. The CST complex is essential for coordinating telomerase activity and C-strand fill-in synthesis, thereby ensuring the proper elongation and processing of telomeric DNA. In certain malignant cells, the ALT pathway, a telomerase-independent mechanism, is characterized by homology-directed repair via break-induced replication and this process, facilitated by RAD52 or RAD51AP1-mediated strand invasion, extends telomeres through the Pol δ accessory subunit POLD3 or induces telomeric sister chromatid exchanges. Telomeric replication stress in ALT cells, marked by DNA damage response activation, arises from secondary structures such as G-quadruplexes and RNA-DNA hybrids formed by TERRA transcription. The Fork Protection Complex, comprising TIMELESS and TIPIN, along with the shelterin complex and replisome components, ensures replication fidelity. Certain helicases like WRN and BLM, and proteins such as PCNA, are important for resolving G4 structures, facilitating replication continuity. Telomere replication is tightly regulated by various mechanisms, such as cell cycle checkpoints, telomere length homeostasis, and telomere position effect. Dysregulation of telomere replication can lead to genomic instability, cellular senescence, and cancer, thus underscoring the importance of understanding telomere replication's molecular complexities in aging and disease.

KEYWORDS: telomere, DNA replication, G-quadruplexes, shelterin, molecular biology

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Challenges and Dynamics of Telomere Replication

Eukaryotic cells have linear chromosomes that allow the shuffling of alleles between homologous chromosomes during meiosis, which thus increases genetic diversity [1]. However, linear chromosomes also have telomeres, which are the vulnerable regions at the ends of chromosomes that consist of thousands of repeats of the sequence 5′-TTAGGG-3′, with a single-stranded 3′ overhang that can form a loop structure by invading the double-stranded repeats [2]. During replication of DNA, the inability of DNA polymerase to fully replicate the terminal regions of the lagging strand results in progressively shorter chromosomes with each cell division. This condition arises because DNA polymerases require a primer to initiate synthesis, and as replication nears the end of a chromosome, there is insufficient template for the placement of a primer for the final Okazaki fragment. As a result, this last portion of the DNA strand remains un-replicated, leading to the gradual erosion of the telomeric DNA (end replication problem). Telomeres are bound by the shelterin complex, which includes TRF1(Telomeric Repeat-binding Factor 1), TRF2 (Telomeric Repeat-binding Factor 2), POT1(Protection of telomeres protein 1), TIN2 (TERF1-interacting nuclear factor 2), RAP1, and TPP1 (also called as adrenocortical dysplasia protein homolog (ACD) protein), and protects telomeres from DNA damage responses and end joining, which causes genomic instability, cell cycle arrest, senescence, or cell death [34]. To prevent telomere erosion, shelterin recruits telomerase, a reverse transcriptase that adds repeats to the overhang using its RNA component (TERC) [5]. Telomerase catalyses the addition of telomere repeats to the 3′ end of chromosomes, which is facilitated by the enzyme's two core components: the telomerase reverse transcriptase (TERT) and the telomerase RNA component (TER) [6]. TERT utilizes TER as a template to synthesize DNA repeats that are complementary to a segment of TER, typically encompassing 1.5 to 1.8 units of the telomere repeat sequence[7]. The TER itself is structured with an alignment region that precedes a templating region, guiding the accurate addition of telomeric repeats. The architecture of TERT is

characterized by domains which are similar to reverse transcriptases, including the reverse transcriptase domain with palm and fingers subdomains, and the carboxy-terminal element resembling a thumb[8]. These collectively contribute to the formation of a ring-like structure that is essential for the enzyme's function. TERT possesses a telomerase amino-terminal domain (TEN) linked to an RNA-binding domain (RBD) by an extensive linker region, further contributing to the enzyme's complex structure and function[9]. TER exhibits remarkable diversity across different organisms, varying significantly in length—from approximately 150 nucleotides in ciliates to over 2000 nucleotides in certain yeasts—and in structural motifs[10] and this variability is attributed to the rapid evolution of TER, a noncoding RNA that adapts to the diverse requirements of telomere maintenance[11]. In ciliates, TER is transcribed by RNA polymerase III, however in vertebrates, yeasts, and plants, it is the domain of RNA polymerase II. Such divergence has led to a variety of mechanisms for the biogenesis and processing of TER, its assembly with TERT, and its localization and recruitment to telomeres. However, despite the variabilities, TERs share conserved regions for interaction with TERT and these include the template/pseudoknot domain (t/PK), which forms a closed loop encompassing the template sequence and a pseudoknot structure, and the stem-terminus element (STE), which features a hairpin structure. The TER exhibits significant variability in regions outside the template/pseudoknot (t/PK) and stem-terminus element (STE), reflecting the diverse evolutionary adaptations of TER across different organisms. In ciliates, for instance, the La-related protein group 7 (LARP7) protein, specifically p65 in *Tetrahymena thermophila*, plays a crucial role in the protection of the 3′-end and in the assembly of TER with TERT [12]. Human TER, on the other hand, features a specialized H/ACA small Cajal body-specific RNA (scaRNA) domain that interacts with the H/ACA small Cajal body ribonucleoproteins (scaRNPs), facilitating the complex's assembly and function. Yeasts, particularly fission and budding yeasts, have larger TERs that associate with a distinct array of proteins, including Sm and LSm proteins, and in the case of budding

yeast, the Pop1–Pop6–Pop7 subcomplex derived from mitochondrial ribonuclease P[13].

Structural studies of these associations have been limited, with notable exceptions such as the crystal structure of the *Saccharomyces cerevisiae* Ku70/80–TER hairpin[14]. In humans, the core ribonucleoprotein (RNP) of telomerase binds to telomere-associated proteins that either enhance its processivity, such as TPP1 and POT1 (components of the shelterin complex), or inhibit telomerase activity and assist in recruiting DNA polymerase α-primase for the synthesis of the C-strand, as seen with the CTC1–STN1–TEN1 (CST) complex[15 16 17]. These interactions are needed for the functional regulation of telomerase at telomeres, which also promotes the lagging-strand synthesis[18]. The shelterin complex is made up of six proteins that come together at the ends of chromosomes, known as telomeres. These proteins are TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 (Figure 1). TRF1 and TRF2 start the process by attaching to the telomere's DNA sequence, which is rich in the repeating units of TTAGGG. Once they are in place, they then help bring in the other four proteins[19]. Both TRF1 and TRF2 have a role in controlling the length of the telomeres by limiting the action of telomerase and POT1 also helps in this process by binding to the single-stranded part of the telomere DNA, which is important for stopping telomerase from working in that area. If any part of the shelterin complex doesn't work properly, it can cause the cell to mistakenly think the telomeres are damaged DNA. This can lead to the activation of the cell's repair systems, like ATM or ATR, and result in the cell stopping its division cycle and the chromosomes becoming unstable. When scientists look closely at how much of each shelterin protein is present in the cell compared to the number of places where TRF1 or TRF2 can bind, they found some interesting things. For example, there isn't an excess of TRF1 or TRF2 compared to their binding sites: in fact, there's about twice as much TRF2 as TRF1, suggesting that TRF1 and TRF2 might have different roles. Also, there are smaller groups within the shelterin complex, like TRF1-TIN2-TPP1/POT1 and TRF2-RAP1, which indicates that these proteins might work together in a more specific manner. The amount

of POT1 that gets attached to the telomeres seems to be limited by how much TPP1 is available, and TPP1 only binds to TIN2. This indicates that the balance of these proteins is important for the whole complex to work correctly. Other proteins that join the complex might be brought in by TRF1, TRF2, or POT1, and these proteins could vary a lot in how much they are present at the telomeres.

In 2024, Takai et al. reported a new study which revealed an additional challenge in telomere replication, specifically the incomplete synthesis of the lagging strand's C-rich telomeric repeats. This issue is addressed through fill-in synthesis by the CST–Polα-primase[20]. Experimental evidence suggests that priming for lagging-strand replication is absent at the 3′ overhang, halting synthesis approximately 150 nucleotides away from the template's end. Cells deficient in CST–Polα-primase exhibit significant shortening of lagging-end telomeres, losing 50–60 nucleotides of CCCTAA repeats with each cell division. Leading-end telomeres also experience shortening, approximately 100 nucleotides per division, likely due to resection forming 3′ overhangs. The observed overall reduction in C-strand length without CST–Polα-primase correlates with the sum of incomplete lagging-strand replication and leading-end resection. The findings suggest that standard DNA replication presents dual challenges at telomere ends, necessitating telomerase for G-strand maintenance and CST–Polα-primase for C-strand upkeep.

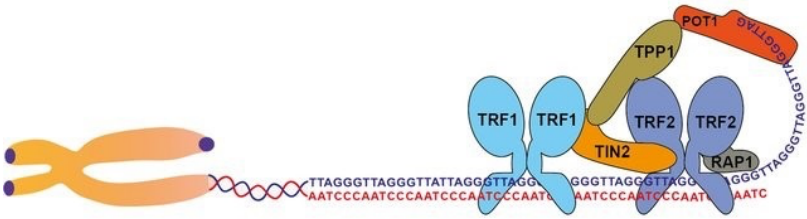

Figure 1. The role of shelterin in protecting and regulating telomeres. This is a diagram of how the shelterin complex attaches to the telomeric DNA. The shelterin components TRF1 and TRF2 form dimers that bind to specific regions of the telomeric DNA.

Image credit: Doksani Y. (2019). The Response to DNA Damage at Telomeric Repeats and Its Consequences for Telomere Function. Genes, 10(4), 318. https://doi.org/10.3390/genes10040318

ALT Telomere Shortening Mechanism

Cancer cells can bypass telomere shortening by activating telomerase or using the alternative lengthening of telomeres (ALT) mechanism[21]. ALT maintenance mechanism is frequently observed in certain malignancies, notably osteosarcomas and glioblastomas, where targeted therapies remain elusive. In cells utilizing ALT, telomeres exhibit spontaneous telomere dysfunction-induced foci (TIFs) and often coalesce with promyelocytic leukemia (PML) nuclear bodies to form ALT-associated PML bodies (APBs). These APBs are hypothesized to be the sites of telomere elongation within ALT cells and are characterized by the presence of DNA damage response (DDR) proteins. The integrity of ALT telomeres is thus compromised, leading to their mobilization and aggregation, a response to telomeric damage that can be mitigated through homology-directed repair (HDR), specifically via break-induced replication (BIR) which is initiated by RAD52 or RAD51AP1-mediated strand invasion. The outcome of BIR at ALT telomeres can manifest as either an extension of telomere length, reliant on the Polδ accessory subunit POLD3, or as telomeric sister chromatid exchanges (T-SCEs), which are typically unproductive. The aberrant repair activities in ALT cells give rise to distinctive telomeric structures,

such as fragmented DNA and extrachromosomal circles, including C-circles, which are partially single-stranded telomeric DNA circles rich in cytosine and the presence and abundance of C-circles serve as a hallmark for the identification of ALT activity, reflecting the recombinogenic nature of ALT telomeres that tend to be elongated and heterogeneous in length (Figure 2). The fragility of telomeres stems from inherent replication challenges, making them susceptible to DNA replication stress, which is notably heightened in ALT cells, which triggers DDR pathways that culminate in BIR-mediated telomere extension. The replication difficulties are partly due to the propensity of telomeric sequences to adopt complex secondary structures, such as G-quadruplexes (G4s) and RNA-DNA hybrids formed by the transcription of telomeric repeat-containing RNA (TERRA) from subtelomeric regions, which are more prevalent in ALT cells compared to non-ALT cells. ALT telomeres are also characterized by an abundance of telomeric variant degenerate repeat sequences that disrupt shelterin binding and attract recombinogenic factors. The mutations in histone chaperones ATRX and DAXX, which are implicated in the ALT pathway, lead to altered telomeric heterochromatin, contributing to increased sister chromatid cohesion and the manifestation of ALT phenotypes like APBs, C-circles, T-SCEs, and telomere elongation. ATRX and DAXX are responsible for depositing the histone variant H3.3 at telomeres and promoting the deposition of macroH2A1.2 during replication stress to enhance telomere stability. The depletion of ASF1 isoforms, ASF1a and ASF1b, in cells with elongated telomeres induces ALT phenotypes, suggest that ASF1 silencing triggers ALT through the induction of telomeric replication stress. Telomeres are thus the challenging regions for DNA replication, as they present multiple obstacles for the replication machinery and replication forks often slow down and stall near the telomeric chromatin, and may collapse if not resolved, leading to double-strand breaks and homologous recombination[22]. This can result in telomere loss or aberrations, which can be detected by FISH on metaphase chromosomes[23]. These abnormal structures may also reflect telomere entanglement or incomplete replication. Telomere replication is therefore a source of stress that threatens

telomere integrity and stability. The timing of telomere replication is also crucial for telomere homeostasis and telomerase regulation. In mammalian cells, telomeres replicate throughout the S phase, whereas in yeasts, they replicate at the end of the S phase[24]. However, short telomeres or global replication perturbations can advance the replication of telomeres, altering the telomere length equilibrium[25].

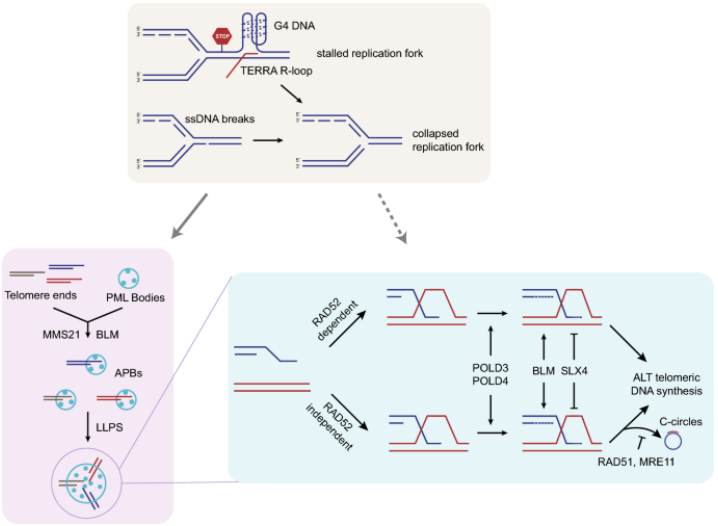

Figure 2. An overview of the ALT pathways. Telomere replication stress, potentially a catalyst for ALT activation, arises from the build-up of R-loops, G-quadruplexes, and single-strand DNA breaks. This accumulation can disrupt replication, leading to fork collapse and single-ended double-strand breaks (DSBs). APBs, rich in DNA repair and replication factors, may enhance ALT activity. Within APBs, BIR commences due to telomeric DSBs. ALT processes involve both RAD52-dependent and independent BIR pathways. POLD3/POLD4-dependent conservative replication during BIR is facilitated by BLM and hindered by SLX4. Conversely, RAD52-independent BIR generates C-circles and is restrained by RAD51 and MRE11.

Image credits: Zhang, J. M., & Zou, L. (2020). Alternative lengthening of telomeres: from molecular mechanisms to therapeutic outlooks. Cell & bioscience, 10, 30. https://doi.org/10.1186/s13578-020-00391-6

Unwinding of G4 Structures for Telomere Replication

The obstacles that slows down the replication fork include heterochromatin, T-loop, TERRA, RNA:DNA hybrids, and nuclear envelope attachment. One of the most challenging obstacles is the G4 structure, which is formed by four guanines stacking together in a planar arrangement. G4 can occur in the single-stranded G-rich lagging strand template during replication or transcription, and can block the fork or cause it to break, which leads to chromosome instability and telomere loss[26]. To prevent this, cells have several strategies to overcome the telomere replication problem, such as helicases, nucleases, and fork protection complex (FPC) which is part of the replisome and ensures proper fork pausing and passage[27]which safeguards the stability and proper functioning of the replisome during DNA replication. This complex is composed of the core components TIMELESS (TIM) and TIPIN, which are known as Swi1 and Swi3 in the yeast species *S. pombe*, and as Tof1 and Csm3 in *S. cerevisiae*. Alongside these, the proteins AND-1 and CLASPIN (CLSPN) serve as auxiliary factors that enhance the replisome's stability, facilitating smooth progression of the replication fork. The FPC functions as a scaffold, bridging the CMG helicase and the polymerase, which prevents the separation of their activities, which could otherwise lead to replication inefficiency. By maintaining a tight linkage between these two components, the FPC ensures that the replication machinery progresses efficiently along the DNA strand.

During times of replication stress, the FPC takes on an additional role by bolstering the ATR-CHK1 checkpoint signaling pathway, by promoting the interaction of TIM-TIPIN and CLSPN with RPA-coated single-stranded DNA at replication forks that have stalled[28]. This interaction is crucial for allowing CLSPN to facilitate the phosphorylation of CHK1 by ATR, a key step in the checkpoint signaling process. TIM and TIPIN are known to form a stable heterodimer, and interestingly, they do not possess any enzymatic activity. This lack of enzymatic function indicates that their role is primarily structural, contributing to the integrity of the replisome. The importance of the FPC is underscored by the fact that its loss leads to significant issues in DNA replication and overall genome stability, highlighting the necessity of the FPC's structural support in

maintaining the stability of the replication fork. Furthermore, studies have shown that TIM is upregulated in various types of cancers and this upregulation suggests that an increase in FPC activity could potentially counteract the replication stress encountered in tumor cells, which often results from the activation of oncogenes. The shelterin complex also helps to promote efficient telomere replication and prevent fork stalling and collapse[29]. Thus, both replisome and shelterin cooperate to maintain telomere stability. To prevent the interference of G4 structures with telomere replication, several helicases and single-strand DNA binding proteins (SSB) are recruited to unwind G4. For example, WRN and BLM, which are 3′–5′-directed helicases from the RecQ family that are mutated in Werner's and Bloom's syndromes, respectively[30]. WRN may be involved in G4 resolution at telomeres by interacting with replication protein A complex (RPA), PCNA, Pol δ, and TRF2[31]. RPA is known for its ability to attach to single-stranded DNA and initiate the unwinding of G4 structures. Despite not being classified as a helicase, research conducted in laboratory settings has demonstrated that RPA can disassemble G4 formations without the need for ATP. RPA can bind and unfold G4 structures by itself, or recruit other helicases through physical interactions[32]. It is posited such that HERC2, a HECT E3 ligase, is instrumental in this interaction, particularly during the S-phase of the cell cycle, by interacting with BLM, WRN, and RPA complexes. This interaction is crucial for the suppression of G-quadruplex DNA, as depletion of HERC2 has been shown to dissociate RPA from BLM and WRN complexes, leading to increased formation of G4 structures. Phosphorylation-dependent association of WRN with RPA is essential for the recovery of replication forks stalled at secondary DNA structures and when WRN fails to bind RPA, fork recovery is impaired, resulting in the accumulation of single-stranded DNA gaps, which are exacerbated by the structure-specific nuclease MRE11. Telomeric proteins, such as POT1 and the shelterin components TRF1 and TRF2, may also prevent G4 formation by binding to telomeric tails or acting as scaffolds for replication factors[33]. The proliferating cell nuclear antigen (PCNA) may coordinate this network by recruiting different factors to the

replisome[34]. PCNA associates with a cohort of factors that collectively regulate the resolution of G4 structures and ensure the continuation of replication. Among these, the interaction of PCNA with DNA polymerase δ/ε is crucial, as it facilitates the processivity of the polymerase during DNA synthesis. PCNA also engages with replication factor C (RFC) and DNA ligase 1 (Lig1), which are instrumental in the re-synthesis of new DNA fragments post-G4 resolution. Moreover PCNA's association with Flap Endonuclease 1 (Fen1) is essential for the removal of RNA primers and the subsequent joining of Okazaki fragments during lagging-strand synthesis. The interplay between PCNA and the FANCJ helicase is also worth mentioning; FANCJ is implicated in the unwinding of G4 structures, thereby facilitating the progression of the replication fork. This dynamic collaboration between PCNA and its partners not only mitigates the replication stress induced by G4 structures but also maintains the integrity of the genome. PCNA forms a ring-shaped clamp around DNA, which allows it to slide along the DNA strand. This sliding clamp function is essential because it helps DNA polymerases to stay attached to the DNA during replication, increasing their ability to add nucleotides efficiently (this process is known as processivity).

BLM may collaborate with TRF1, which has the FxLxP motif for BLM binding which recruit BLM to remove G4 and avoid telomere fragility[35]. Another helicase that can resolve G4 with a 5′–3′ polarity is RTEL1, which is essential for DNA replication and recombination. RTEL1 may be associated with the replisome by its PIP box domain that binds PCNA. BLM and RTEL1 have different roles, as their deficiency causes additive telomere fragility[36]. Therefore, helicases that are linked to the replisome or shelterin can unwind G4 and ensure telomere replication. The Pif1 helicase family is widespread in eukaryotes and has various roles in DNA metabolism, including G4 unwinding. In yeast, there are two Pif1 family members: ScPif1 and Rrm3. ScPif1 is a potent G4 unwinder that inhibits telomerase by displacing its RNA component from telomeric ends whereas Rrm3 travels with the replication fork and helps replicate telomeric repeats[37 38]. In humans and mice, PIF1 also

unwinds G4 and interacts with TERT[39]. In fission yeast, Pfh1 is essential for replicating difficult regions and resolving G4 at telomeres[40 41]. Another protein that may process G4 at telomeres is DNA2, a 5′–3′ helicase/nuclease that cleaves G4 in vitro and co-immunoprecipitates with TRF1–TRF2[42].

Overcoming Replication Challenges at Telomeres

The T-loop is a structure formed by the invasion of the telomeric 3′ overhang into the double-stranded part of the telomere, creating a D-loop. This protects the telomere from degradation, but also poses a challenge for DNA replication. To avoid replication fork collision and allow telomerase access, the T-loop needs to be disassembled in a timely manner. This is done by RTEL1, a helicase that participates in this process by interacting with the shelterin protein TRF2, that binds to the T-loop base[43]. RTEL1 also associates with the replisome through PCNA to promote replication[44]. How RTEL1 coordinates its interactions with PCNA and TRF2 throughout the cell cycle is a bit unclear, as well as how it distinguishes between different replication barriers such as G4, T-loops, or others (Figure 3). Helicases, such as WRN, BLM, and RECQL4, may also be involved in T-loop resolution[45]. If RTEL1 fails, the SLX1–SLX4 nucleases resolve the T-loop inappropriately, causing telomere instability[46]. TRF2 also recruits Apollo, a 5′-exonuclease that prevents topological stress at the T-loop base[47]. The regulation of T-loop resolution likely depends on a complex network of post-translational modifications, involving the shelterin proteins. TERRA is a type of non-coding RNA that is transcribed from the subtelomeric regions to the TTAGGG repeats at the ends of eukaryotic chromosomes[48]. TERRA can form RNA:DNA hybrids with the telomeric DNA, displacing the G-rich strand and creating R-loops.[4950] This R-loop can interfere with the replication of telomeric repeats and cause telomere fragility and genomic instability.[51] To prevent this, TERRA levels are regulated during the cell cycle, peaking at G1–S and declining from S to G2.[52 53] Moreover, several factors are involved in resolving TERRA R-loops, such as RNase H, which degrades the RNA strand, ATRX,

which is a chromatin remodeler that may recognize or modify G4 structures, and UPF1, which is a helicase that participates in telomere replication.[54] [55] [56] [57] These mechanisms ensure that TERRA does not impair the completion of leading-strand telomere replication and maintain telomere integrity.

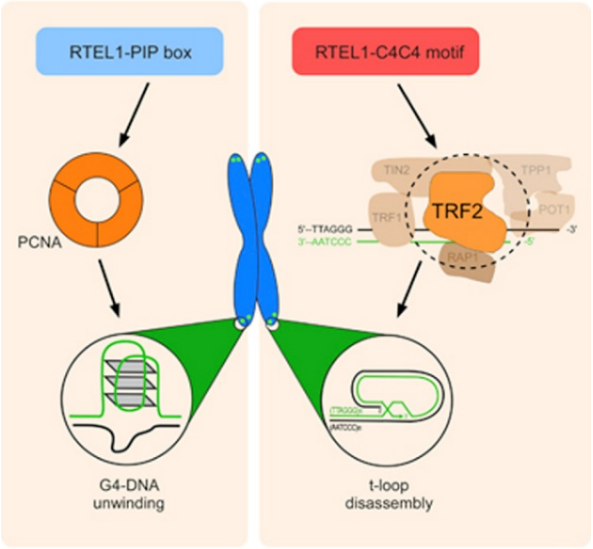

Figure 3. The image depicts two critical processes in telomere maintenance: G4-DNA unwinding and T-loop disassembly. The RTEL1-PIP box interacts with PCNA, shown as an orange circle, to unwind G4-DNA structures, which are green helical representations of DNA. On the other side, the RTEL1-C4C4 motif is associated with TRF2, indicated by an orange dashed circle, along with proteins TIN2, TPP1, POT1, and RAP1, to facilitate the disassembly of T-loops in telomeres. These processes are essential for the replication and protection of chromosome ends, ensuring genomic stability.

Image credits: Sarek, Grzegorz et al. "TRF2 recruits RTEL1 to telomeres in S phase to promote t-loop unwinding." Molecular cell vol. 57,4 (2015): 622-635. doi:10.1016/j.molcel.2014.12.024

TERRA also has many positive roles in telomere biology, such as regulating telomere length, replication, protection, chromatin structure, and mobility[58]. Therefore, TERRA levels and R-loop

formation must be tightly controlled to avoid replication–transcription conflicts. Several proteins can degrade or displace TERRA, such as Pif1 and FEN1 helicases, but the coordination and regulation of these mechanisms are not fully understood[59]. Telomeres also form a compact chromatin structure that protects them from DNA damage response, but also poses a barrier to the replication fork. TRF2 binds to telomeric DNA, modulates the topological state of telomeres and cooperates with Apollo and topoisomerase 2α to remove superhelical constraints[60]. Telomere anchoring is another source of topological stress that needs to be resolved during replication. The nuclear envelope (NE) and the nuclear matrix (NM) are two structures that constrain the localization and movement of telomeres, the ends of chromosomes. This structure called as telomere bouquet is a well-studied structure observed across a multitude of organisms during meiosis, where telomeres converge at the NE to facilitate homologous chromosome pairing, synapsis, and recombination. This conserved phenomenon, essential for the successful completion of meiosis, is controlled by the LINC complex—a fusion of KASH and SUN domain-containing trans-membrane proteins localized at the NE. The LINC complex serves as a bridge, connecting the NE to the cytoskeleton, thus generating the mechanical forces necessary for chromosomal movement. SUN domain proteins within this complex provide anchoring points for telomeres at the NE, ensuring their proper positioning. The shelterin complex, particularly TRF1 localizing at telomeres, assist in their tethering. A specialized "linker" system, comprising proteins such as TERB1, TERB2, and MAJIN in mice, forms a structural and functional bridge between the LINC and shelterin complexes, facilitating the bouquet's integrity[61]. The cyclin-dependent kinase CDK2, along with its activators SPDYA and Cyclin E, is also crucial for the formation of the telomere bouquet. Knockout studies in mice have demonstrated that the absence of CDK2, SPDYA, or Cyclin E results in the disassociation of telomeres from the NE, indicating a disruption in the LINC-linker-shelterin nexus. CDK2's kinase activity is modulated by the binding of typical cyclins, such as Cyclin E and A, and the atypical cyclin SPDYA, which leads to the

phosphorylation of T160 on the T-loop, thereby activating CDK2. Intriguingly, SPDYA can activate CDK2 independent of T-loop phosphorylation. Studies suggest that CDK2 phosphorylates SUN1, hinting at a potential role for CDK2 in fortifying the LINC-linker bond, a hypothesis that beckons further investigation to fully comprehend the molecular choreography underpinning the telomere bouquet's assembly and function[62].Telomeres are attached to the NE on one side of the nucleus and centromeres on the other in yeast cells[63]and this attachment is mediated by different proteins, such as Esc1–Sir4–Rap1 and yKu–Mps3 in budding yeast, and Bqt4 and Rap1 in fission yeast. Fft3, a chromatin remodeler, also contributes to this anchoring[64]. Human telomeres, however, are distributed throughout the nucleus and interact with the NM via shelterin and lamins[65]. Only some telomeres are found at the NE. To replicate telomeres, these topological constraints have to be overcome by detaching telomeres from the NE or NM. This is a potential research topic for the future.

Conclusion

The replication of telomeres, the ends of chromosomes, is a challenging process that requires overcoming several obstacles which includes secondary structures (G4 and T-loops), transcription, and topological constraints due to compaction and anchoring of telomeric chromatin which can cause replication stress and fork stalling at telomeres. Shelterin, a complex of telomere-binding proteins, protects telomeres from replication stress by modulating two distinct pathways. TRF1 prevents fork stalling and ATR activation in S phase, while TRF2 resolves supercoiling generated by fork progression. The coordination and regulation of these pathways, as well as the molecular interactions between shelterin and the replisome, are not fully understood and require further investigation. Post-translational modifications of TRF1 and TRF2 may also play a key role in this process[66]. Telomere replication also influences telomere length maintenance by telomerase enzyme. Two models have been proposed to explain how telomerase elongates short telomeres preferentially

and both of them involve the association of telomerase with the replication fork and the dissociation of telomerase due to natural barriers at telomeres which elucidate the fact that telomere replication and elongation are tightly linked processes[67]. The first model, often referred to as the "Replication Fork Model", posits that telomerase is recruited to the telomere during the replication process. As the replication fork progresses, it exposes the single-stranded 3' overhang of the telomere, which is the substrate for telomerase action. Telomerase, with its intrinsic RNA template, extends the overhang by adding telomeric repeats. This model suggests that the shorter the telomere, the more accessible it is to telomerase, as longer telomeres may have more complex secondary structures or bound proteins that hinder telomerase access. Once the lagging strand synthesis approaches completion, telomerase is displaced due to the physical barrier posed by the approaching replication machinery or the binding of protective shelterin complex proteins, which cap the telomere ends.

The second model, known as the "Telomere Positioning Model", proposes that telomerase elongation activity is regulated by the spatial positioning of telomeres within the nucleus. According to this model, telomeres are organized in such a way that shorter telomeres are preferentially located in regions of the nucleus that are enriched with telomerase. This spatial arrangement facilitates easier access of telomerase to shorter telomeres. The dissociation of telomerase in this context is thought to be influenced by the completion of telomere replication and the re-establishment of higher-order telomere structures, which may sequester the elongated telomeres away from the telomerase-rich nuclear regions. Both models underscore the concept that telomere length homeostasis is a highly regulated process, ensuring that telomeres are maintained at an optimal length to protect chromosome ends from degradation and to prevent the activation of DNA damage responses. The preferential elongation of short telomeres by telomerase is a thus key mechanism, allowing cells to sustain chromosomal integrity over successive rounds of replication. It is thus truly a quintessential molecular conundrum.

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