Essay

Unravelling the Intricacies of Telomere Replication: A Molecular Conundrum

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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. Telomere replication is a complex process that involves both DNA polymerases and a specialized enzyme called telomerase. Telomerase is a ribonucleoprotein complex that synthesizes telomeric DNA by using an internal RNA template. However, telomerase alone cannot fully replicate the telomeric DNA, and requires the cooperation of other factors, such as shelterin, CST, and DNA repair proteins. Moreover, 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. Therefore, understanding the molecular details of telomere replication is crucial for elucidating the role of telomeres in aging and disease.

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Challenges and Dynamics of Telomere Replication

Eukaryotic cells have linear chromosomes that enable the shuffling of alleles between homologous chromosomes during meiosis, which increases genetic diversity [1]. However, linear chromosomes also have telomeres, which are 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 [3]. Telomeres are bound by the shelterin complex, which includes TRF1, TRF2, POT1, TIN2, RAP1, and TPP1, and protects telomeres from DNA damage responses and end joining, which can cause genomic instability, cell cycle arrest, senescence, or cell death [3]. Telomeres are shortened by the end replication problem, which occurs during the leading-strand synthesis and removes some of the telomeric repeats [2,4]. To prevent telomere erosion, shelterin recruits telomerase, a reverse transcriptase that adds repeats to the overhang using its RNA component (TERC) [5]. Telomerase is regulated by the CST complex, which also promotes the lagging-strand synthesis [6]. Cancer cells can bypass telomere shortening by activating telomerase or using the ALT mechanism [7]. Telomeres are challenging regions for DNA replication, as they present multiple obstacles for the replication machinery. 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 [2,8]. This can result in telomere loss or aberrations, which can be detected by FISH on metaphase chromosomes [9]. 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 [2]. In mammalian cells, telomeres replicate throughout the S phase, whereas in yeasts, they replicate at the end of the S phase [10]. However, short telomeres or global replication perturbations can advance the replication of telomeres, altering the telomere length equilibrium [11].

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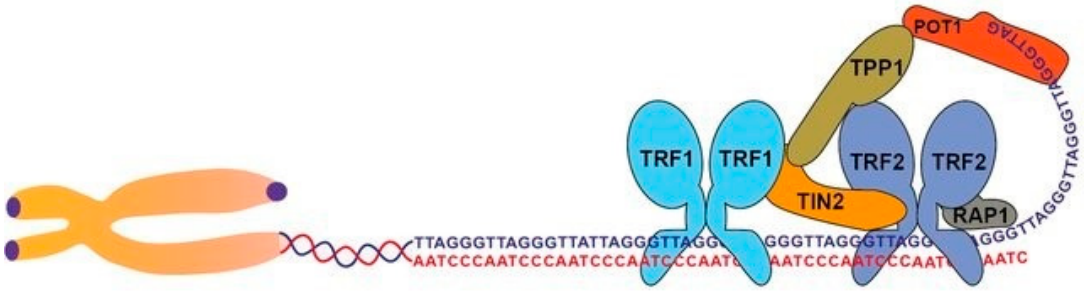

Figure 1. The role of shelterin in protecting and regulating telomeres. This is a diagram of how the shelterin complex attaches to the telomeric DNA. The shelterin components TRF1 and TRF2 form dimers that bind to specific regions of the telomeric DNA. Image source: Doksani Y. (2019). The Response to DNA Damage at Telomeric Repeats and Its Consequences for Telomere Function. Genes, 10(4), 318. https://doi.org/10.3390/genes10040318

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 G-quadruplex (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 [2]. This can lead to chromosome instability and telomere loss [12]. To prevent this, cells have several strategies to overcome the telomere replication problem, such as helicases, nucleases, and fork protection complex (FPC). The FPC is part of the replisome and ensures proper fork pausing and passage [13]. The shelterin complex also helps to promote efficient telomere replication and prevent fork stalling and collapse [2,14]. Thus, 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 [2,15]. WRN may be involved in G4 resolution at telomeres by interacting with replication factor A complex (RPA), PCNA, Pol δ, and TRF2 [16]. RPA can also bind and unfold G4 structures by itself, or recruit other helicases through physical interactions [17]. 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 [18,19]. The proliferating cell nuclear antigen (PCNA) may coordinate this network by recruiting different factors to the replisome [19].

BLM may collaborate with TRF1, which has the FxLxP motif for BLM binding [20]. TRF1 may also recruit BLM to remove G4 and avoid telomere fragility [20]. Another helicase that can resolve G4 with a 5′–3′ polarity is RTEL1, which is essential for DNA replication and recombination [21]. RTEL1 may be associated with the replisome by its PIP box domain that binds PCNA [21]. BLM and RTEL1 have different roles, as their deficiency causes additive telomere fragility [2,21]. 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 [22]. Rrm3 travels with the replication fork and helps replicate telomeric repeats [23]. In humans and mice, PIF1 also unwinds G4 and interacts with TERT [24]. In fission yeast, Pfh1 is essential for replicating difficult regions and resolving G4 at telomeres [2,25]. 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 [26].

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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. RTEL1 is a helicase that participates in this process by interacting with the shelterin protein TRF2, that binds to the T-loop base [27]. RTEL1 also associates with the replisome through PCNA to promote replication [28]. How RTEL1 coordinates its interactions with PCNA and TRF2 throughout the cell cycle is unclear, as well as how it distinguishes between different replication barriers such as G4, T-loops, or others. Helicases, such as WRN, BLM, and RECQL4, may also be involved in T-loop resolution [2,29]. If RTEL1 fails, the SLX1–SLX4 nucleases resolve the T-loop inappropriately, causing telomere instability [30]. TRF2 also recruits Apollo, a 5′-exonuclease that prevents topological stress at the T-loop base [31]. 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 [32]. TERRA can form RNA:DNA hybrids with the telomeric DNA, displacing the G-rich strand and creating R-loops [33]. This R-loop can interfere with the replication of telomeric repeats and cause telomere fragility and genomic instability [34]. To prevent this, TERRA levels are regulated during the cell cycle, peaking at G1–S and declining from S to G2 [2,35]. Moreover, several factors are involved in resolving TERRA R-loops, such as RNase H, which degrades the RNA strand [36], ATRX, which is a chromatin remodeler that may recognize or modify G4 structures [36], and UPF1, which is a helicase that participates in telomere replication [37]. These mechanisms ensure that TERRA does not impair the completion of leading-strand telomere replication and maintain telomere integrity.

TERRA also has many positive roles in telomere biology, such as regulating telomere length, replication, protection, chromatin structure, and mobility [38]. Therefore, TERRA levels and R-loop formation must be tightly controlled to avoid replication–transcription conflicts [2]. 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 [39]. 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 [40]. 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. Telomeres are attached to the NE on one side of the nucleus and centromeres on the other in yeast cells [41]. 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 [2]. Fft3, a chromatin remodeler, also contributes to telomere anchoring [42]. Human telomeres, however, are distributed throughout the nucleus and interact with the NM via shelterin and lamins [43]. Only some telomeres are found at the NE [10]. 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. These include secondary structures (G4 and T-loops), transcription, and topological constraints due to compaction and anchoring of telomeric chromatin. These factors 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 [9], while TRF2 resolves supercoiling generated by fork progression [31]. 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 [44] may play a key role in this

process. Telomere replication also influences telomere length maintenance by telomerase, an enzyme that adds DNA repeats to telomeres. Two models have been proposed to explain how telomerase elongates short telomeres preferentially [45]. Both models involve the association of telomerase with the replication fork and the dissociation of telomerase due to natural barriers at telomeres. Thus telomere replication and elongation are tightly linked processes.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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