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Review

Telomeres and the nucleus

Clara Lopes Novo, J. Arturo Londoño-Vallejo*

Telomeres & Cancer Laboratory, UMR3244, Institut Curie-CNRS-UPMC, 26 rue d'Ulm, 75248 Paris Cedex 05, France

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ABSTRACT

Telomeres are crucial for the maintenance of genome stability through "capping" of chromosome ends to prevent their recognition as double-strand breaks, thus avoiding end-to-end fusions or illegitimate recombination [1–3]. Similar to other genomic regions, telomeres participate to the nuclear architecture while being highly mobile. The interaction of telomeres with nuclear domains or compartments greatly differs not only between organisms but also between cells within the same organism. It is also expected that biological processes like replication, repair or telomere elongation impact the distribution of chromosome extremities within the nucleus, as they probably do with other regions of the genome. Pathological processes such as cancer induce profound changes in the nuclear architecture, which also affects telomere dynamics and spatial organization.

Here we will expose our present knowledge on the relationship between telomeres and nuclear architecture and on how this relationship is affected by normal or abnormal telomere metabolisms.

1. An overview of telomeres

1.1. Structure and function

1.1.1. Primary structure of chromosome ends

In most organisms, from yeast to humans, chromosomes end in a highly repetitive DNA sequence, which in vertebrates comprises tandem repeats of 5′-TTAGGG-3′ and in most other organisms is rich in guanines (G-rich). The double-stranded DNA portion of telomeres (which in humans can extend up to 10–20 kb) consistently ends in a single G-rich overhang of variable length depending of the organism [4,5]. This single stranded portion is essential for the telomere protective function and serves as a substrate for de novo repeat addition by telomerase, a dedicated reverse transcriptase. In humans, the most proximal region of the telomeric DNA is characterized by an interspersion of the consensus telomeric repeat (TTAGGG) with variant repeats (the most common being TTGGGG, TCAGGG and TGAGGG), whereas the distal region is formed by a homogeneous array of the consensus repeat [6,7].

1.1.2. T-loop

It has been proposed that the 3′-G rich overhang is essential to prevent the recognition of the chromosome end as a double strand break by the cell. It would do so through the formation of a t-loop structure in which the telomeric double strand portion folds back allowing the G-rich single strand to invade the double-stranded region, thus creating a displacement loop (D-loop) at the point of insertion. This model is supported by the detection of lasso-like structures at the end of chromosomes and isolated from live cells as well as in vitro experiments showing invasion and displacement of double strand sequences by a 3′ overhang [8,9]. This "cap" structure is expected to bear a dynamic behavior during the cell cycle, particularly during S-phase when telomeres undergo replication [10].

1.1.3. Shelterin

Chromosome capping and telomere integrity also requires the presence of telomere specific protein complexes. In mammals, this complex is called shelterin [11] and comprises DNA binding proteins as well as protein–protein bridging components. Telomeric Repeat binding Factors 1 and 2 (TRF1 and TRF2, respectively) bind to the double-stranded portion of telomeric DNA while Protection of Telomeres 1 (POT1) binds to the single-stranded overhang. These proteins recruit three additional proteins: TRF2 recruits the Repressor Activator Protein 1 (RAP1), while TRF1-Interacting Nuclear factor 2 (TIN2) associates with both TRF1 + 2 as well as with the telomeric adrenocortical dysplasia homolog (TPP1), which in turn is a POT1-binding partner, required for POT1 binding to the telomere overhang [12].

The shelterin complex promotes t-loop formation and prevents non-homologous end-joining (NHEJ) activity through the TRF2/RAP1 complex [13–15], while regulating telomere length through TRF1, which, in collaboration with POT1, negatively regulates telomere length most probably by physically preventing telomerase access to telomere ends [16]. The binding of TRF1 is modulated by TIN2 [17] through the control of tankyrase 1, an

* Corresponding author. Tel.: +33 156 246611; fax: +33 156 246674.
E-mail address: arturo.londono@curie.fr (J.A. Londoño-Vallejo).

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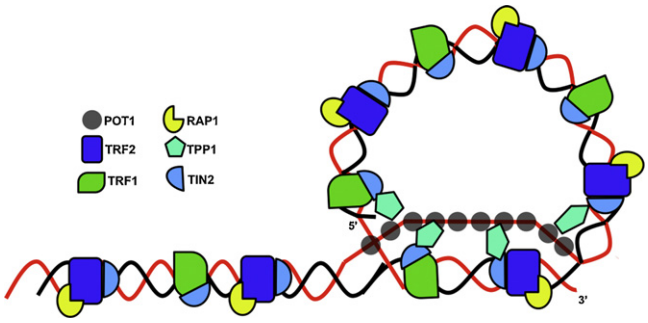

Fig. 1. Telomere protecting cap. The 3′ G-rich overhang at the end of a linear chromosome can loop back and invade a proximal double-stranded region to form a displacement structure, the D-loop. The resultant cap structure, known as t-loop, protects the telomere from being recognized as a double-strand break. The shelterin complex, formed by the direct binding of TRF1, TRF2 and POT1 to the telomeric repeats and consequent recruitment of RAP1 (TRF2), TPP1 (POT1) and TIN2 (TRF1, TRF2 and TPP1), promotes the formation of and stabilizes the t-loop.

ADP-ribosyl-transferase that can modify TRF1 thus diminishing its affinity for telomeric DNA [18]. TRF2 plays a key role by stabilizing the 3′ overhang and POT1 can regulate the nucleolytic processing responsible for the 3′ overhang formation [19]. The shelterin assembly seems to depend on a concerted interaction of TIN2 with TPP1 and POT1 that guarantees the transport of these proteins to the nucleus [20]. Finally, the shelterin complex interacts with (and recruits to telomeres) many DNA repair/recombination factors, which at telomeres paradoxically appear to limit rather than to promote DNA repair, thus ensuring the integrity of telomeres [21] (Fig. 1).

1.1.4. Replication mechanism

It is accepted that telomeres are replicated passively and unidirectionally by replication forks progressing from the last subtelomeric origin of replication. The semi-conservative nature of this replication poses a problem for the complete replication of distal DNA sequences due to the "end-replication problem" [22,23]. At every replication cycle, removal of the RNA-primer of the very last Okazaki fragment leaves a gap in the lagging strand. This gap naturally recreates the G-rich overhang on the lagging-replicated sister telomere. Because of a receding C-strand in the parental chromosome, the telomere of the sister chromatid arising from leading strand replication will be naturally shorter. Most importantly, this telomere will be blunt ended after termination of replication, thus requiring active degradation mechanisms to form a functional G-rich overhang. In the absence of telomerase, this process is responsible for the shortening of the telomere on the leading-replicated sister chromatid at every cell division, thus leading to overall shortening of telomeres with accumulated cell divisions. In addition to the end-replication problem, the repeated nature of telomeres constitutes another challenge for the DNA replication machinery, a situation that can lead to genomic instability. Indeed, telomeres have been shown to cause replication forks to stall [24]. Obstacles to the progression of the replication fork may appear on the G-rich strand, which has the potential to form secondary structures such as guanine-quadruplexes (G-quadruplexes or G4) [25,26], or on both strands as the t-loop structure itself may constitute both a physical as well as a topological barrier potentially increasing the positive supercoiling of DNA ahead of the fork [27]. Finally, unidirectionality prevents any stalled fork from being rescued by converging replication forks.

Although telomeres seem to be prone to replication-derived errors that could lead to genomic instability, a vast and complex network of proteins involved in DNA replication and repair are recruited by the shelterin complex to ensure an accurate telomere replication. Amongst those proteins, Bloom (BLM) and Werner (WRN) RecQ helicases seem to be essential to stabilize and/or resolve stalled replication forks at telomeres, since both associate with telomeres during S-phase [28,29] and WRN has been shown to be essential for efficient lagging-strand replication on the telomeric G-strand [30,31]. This is perhaps related to the ability of RecQ helicases to resolve secondary structures (like G4) formed during the replication fork progression. Another model proposes that TRF2 and POT1 recruit WRN and BLM to release the invading 3′ overhang strand (forming the D-loop) and allow the replication fork to proceed [32].

1.1.5. Mitotic senescence

In 1961, Hayflick and Moorhead noticed that normal human diploid fibroblasts could not grow indefinitely in culture. After 60–80 populations, somatic cells stop dividing and enter a state phenotypically characterized by large and flat cells, with vacuolated morphology and inability to synthesize DNA. Later it was observed that the progressive telomere shortening in these cells eventually results in structural telomere changes that can induce a P53- and P16/retinoblastoma protein (P16/pRB)-dependent replicative senescence [33,34]. At this stage, cells stop dividing and enter a replicative-senescent state or mortality stage 1 – M1 [35]. The introduction of TERT (the catalytic subunit of telomerase) in cells before they reached senescence resulted in both telomere lengthening and the acquisition of indefinite replication capacity (immortalization) thus directly linking mitotic senescence and telomere shortening [36].

1.2. Telomere chromatin: histone modifications, TERRA vs. ARRET

Telomeres have long been classified as constitutive heterochromatic domains due to the presence of epigenetic modifications characteristic of transcriptional repression, like Histone H3 trimethylation at lysine 9 (H3K9m3), Histone H4 trimethylation at lysine 20 (H4K20m3), Histone hypoacetylation and the presence of various isoforms of Heterochromatin Protein 1 (HP1) [37–39]. Furthermore, despite the absence of CpG dinucleotides susceptible to undergo DNMT-mediated methylation at telomeric repeats, the subtelomeric regions contain highly methylated cytosines at CpG dinucleotides in human somatic cells [40–43].

Therefore, it came as a surprise the discovery of transcripts containing telomeric repeats, suggesting that a dynamic "open-and-close" chromatin remodelling process at telomeres. TERRA (Telomeric Repeat containing RNA) is present at mammalian telomeres [44,45] and has also been described in yeast [46] as well as in Arabidopsis thaliana [47] suggesting that these RNAs have a conserved function. Indeed, experimental evidence indicates that telomeric abnormalities arise from either depletion or accumulation of these transcripts [44,48].

TERRA molecules range from 100 bases to around 9 kb and are transcribed from subtelomeric promoters probably found at all chromosome ends. The transcripts contain both tracts of UUAGGG repeats (a few hundreds of base pairs) and subtelomeric sequences [44,49]. TERRA molecules are transcribed by the PolII polymerase, have a 5′-cap and are mostly (around 90%) not polyadenylated [49]. In humans, TERRA molecules are displaced or degraded from telomeres by factors participating in the nonsense-mediated RNA decay (NMD) pathway [44,50] and promoter CpG methylation by DNMT3b and DNMT1 can suppress their expression [51]. TERRA is restricted to the nucleus, with poly(A)+ fractions accumulating in the nucleoplasm whilst poly(A)− molecules are chromatin-associated [49]. TERRA levels are under cell-cycle control: there is an accumulation peak in G1 followed by a continued decrease that reaches the lowest levels between late S and G2 phases [49]. TERRA has been also reported to mediate an association of