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Give me a break: How telomeres suppress the DNA damage response

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ABSTRACT

Linear organization of the genome requires mechanisms to protect and replicate chromosome ends. To this end eukaryotic cells evolved telomeres, specialized nucleoproteic complexes, and telomerase, the enzyme that maintains the telomeric DNA. Telomeres allow cells to distinguish chromosome ends from sites of DNA damage. In mammalian cells this is accomplished by a protein complex, termed shelterin, that binds to telomeric DNA and is able to shield chromosome ends from the DNA damage machinery. In recent years, we have seen major advances in our understanding of how this protein complex works due to the generation of mouse models carrying mutations of individual shelterin components. This review will focus on our current understanding of how the shelterin complex is able to suppress the DNA damage response pathways, and on the cellular and organismal outcomes of telomere dysfunction.

1. Telomeric DNA

Telomeric DNA across eukaryotes has a similar structural composition despite variability in sequence composition and length. Telomeres are constituted of double-stranded repetitive sequences that are maintained by telomerase. The DNA strand that contains the chromosomal 3′ end is rich in guanidines and devoid of cytidines. Based on this uneven distribution of nucleotides (nt), the two telomeric DNA strands are termed G-strand and C-strand (Fig. 1A). Rather than being blunt-ended, the tip of the telomeres in all eukaryotes terminates with a 3′ overhang due to the protrusion of the G-strand over its complementary region with the C-strand. In mammals, as in most eukaryotes, telomeres consist of long stretches of TTAGGG repeats. The length of the telomeric tracts varies between species, with human telomeres being on average 10–15 kbp in length and laboratory mice having telomeres as long as 50 kbp. How telomere length is set to a determined value in a given organism is still not understood.

Two opposing forces contribute to telomere length. On the one hand telomeres shorten at every round of cellular division due to incomplete replication of the lagging strand, the so called "end-replication problem". Additional erosion of telomeric DNA occurs due to post-replicative processing of chromosome ends that give rise to 3′ overhangs. The loss of terminal sequences is balanced by the action of telomerase, a riboenzyme that synthesizes new TTAGGG repeats at chromosome ends using its RNA component as a template. The G-rich 3′ overhangs are also variable in length, with mammals showing lengths that range between 50 and 500 nt. The mechanism that generates 3′ overhangs is currently unknown but that they are the product of telomerase activity has been excluded [1,2]. It has been postulated that G-overhangs are generated by the resection of the C-strand by a not yet identified nuclease [3].

The presence of single-stranded overhangs at chromosome ends is thought be crucial for the formation of secondary telomeric DNA structures. Indeed electron microscopy studies revealed that mammalian telomeres adopt a large secondary structure, termed t-loop [4] (Fig. 1B). In the t-loop conformation, the 3′ overhang is thought to invade the duplex telomeric DNA and anneals to the complementary C-strand, displacing the G-strand. t-loops have been observed in human cells, mouse cells, trypanosomes, ciliates, plants, *C. elegans*, and in some settings, in yeast [5–10]. As discussed in detail below it is speculated that t-loops have a critical role in the protection of chromosome ends since this structure sequesters the chromosome end that is thereby hidden from the DNA damage machinery [4]. Currently very little is known about the dynamics of t-loop formation, whether these structures are present at every single telomere, whether they are present throughout the cell cycle, and how they are displaced to allow proper DNA replication.

2. Proteins stably associated with mammalian telomeres: the shelterin complex

Telomeric DNA repeats are the anchor site for a set of proteins that are essential for proper telomere function. In mammals, the core protein complex that binds stably and exclusively to the TTAGGG repeats, has been termed shelterin. Localization of this complex at chromosome ends is what enables cells to

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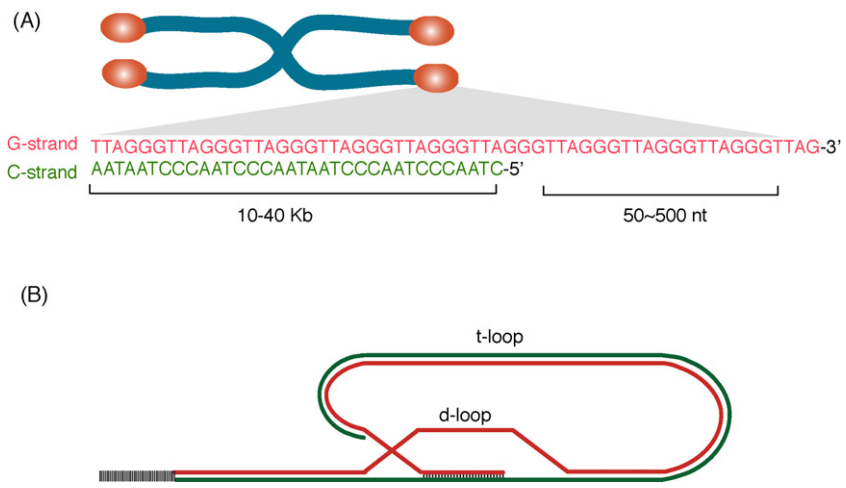

Fig. 1. Structure of mammalian telomeres. (A) Chromosomes in mammalian cells end with long arrays of [TTAGGG] repeats. The strand containing the 3′ terminus, termed the G-strand, is rich in guanidines and spans many nucleotides (nt) over its complementary (C-) strand. This G overhang varies in length between different organisms. (B) Schematic representation of a telomere in a t-loop configuration, the G-strand is labeled in red, the C-strand in green.

distinguish chromosome ends from sites of DNA damage, to regulate telomerase-mediated telomere elongation and to protect chromosome ends from nucleolytic attack. In addition, shelterin mediates protein interactions with accessory factors that bind transiently to telomeres and aid telomere function and homeostasis. Shelterin is composed of six proteins: TRF1, TRF2 (Telomeric Repeat binding Factor 1 and 2), TIN2 (TRF1- and TRF2-Interacting Nuclear Factor 2), POT1 (Protection Of Telomeres 1), TPP1 (formerly known as TINT1, PTOP or PIP1) and RAP1 (Repressor/Activator Protein 1) (Fig. 2A and B). Three members of this complex, TRF1, TRF2 and POT1, bind directly to telomeric DNA repeats and anchor the rest of the complex along the length of the telomeres. TRF1 and TRF2 bind the double-stranded portion of telomeres while POT1 binds to 3′ single-stranded G overhangs.

TRF1 and TRF2 bind DNA through a nearly identical SANT/Myb domain that shows high specificity for the double-stranded sequence 5′-YTAGGGTTR-3′ [11–13]. Both proteins bind DNA as homodimers or higher order monotypic oligomers [14–16] and this oligomerization has been shown to greatly increase their affinity for DNA [11]. TRF1 and TRF2 do not interact directly with each other and therefore bind to chromosome ends in an independent manner.

The link between these two proteins and the rest of the shelterin complex is provided by TIN2. This key protein can simultaneously bind to TRF1 and TRF2 utilizing two independent binding sites [17]. The interaction with TIN2 also contributes in stabilizing TRF1 and TRF2 at telomeres. In fact, in cells with reduced levels of TIN2, TRF1 is readily ADP-ribosylated by tankyrase 1, a modification that destabilizes TRF1 from DNA [18,19]. In addition, the ability of TIN2 to bridge TRF2 and TRF1 molecules at telomeres is important for the stabilization of TRF2 on DNA [20,21]. Finally TIN2 binds and recruits TPP1–POT1 complexes to the telomeres [20–24]. Thus TIN2 crucially links the double-stranded and single-stranded binding functions of the shelterin complex.

TPP1 is the protein that links TIN2 to POT1 [23–25]. In the absence of TPP1, POT1 is not recruited to chromosome ends and loss of TPP1 results in telomere deprotection and telomere length phenotypes that are consistent with complete loss of POT1 from telomeres. The inability of POT1 to bind directly to DNA in the absence of TPP1 is surprising given that POT1 contains two oligonucleotide/oligosaccharide-binding (OB) folds that are highly specific for telomeric single-stranded 5′-(T)TAGGGTTAG-3′ sequence [26–29]. However, TPP1 also appears to be crucial for the proper localization of POT1, as POT1 mutants lacking the TPP1 binding site are excluded from the nucleus [30].

Interestingly, mouse cells have two POT1 genes, POT1a and POT1b [31,32]. Both proteins associate with telomeres and share

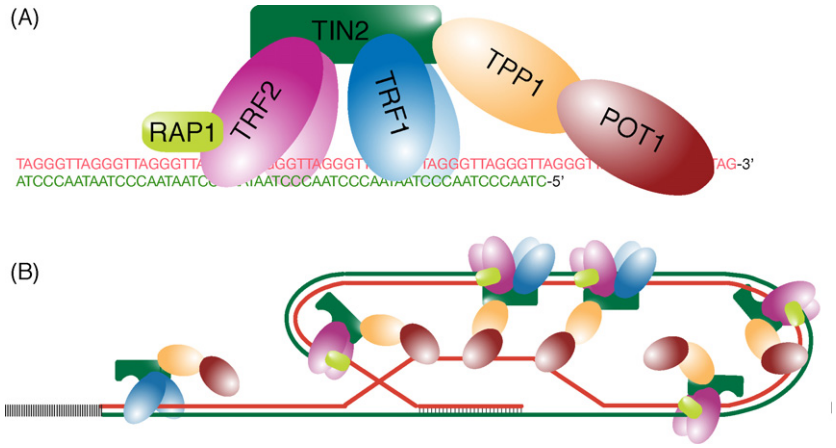

Fig. 2. The shelterin complex. (A) The six proteins that constitute the shelterin complex (see text for details). (B) Schematic model of shelterin complex bound to a telomere in a t-loop configuration. TRF1 and TRF2 bind both double-stranded TTAGGG repeats and TIN2 independently, thus different subcomplexes can exist at telomeres and are depicted in this scheme.