Review

CellPress

No DDRama at chromosome ends: TRF2 takes centre stage

Sascha Feuerhahn[1*], Liuh-yow Chen[2], Brian Luke[3,†], and Antonio Porro[1*]

[1] Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne 1015, Switzerland
[2] Institute of Molecular Biology, Academia Sinica, 128 Academia Road, Section 2, Taipei 11529, Taiwan
[3] Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Deutsches Krebsforschungszentrum (DKFZ)–ZMBH Alliance, Heidelberg, Germany

Telomeres are nucleoprotein structures capping the natural termini of eukaryotic linear chromosomes. Telomeres possess an inherent ability to circumvent the activation of a full-blown DNA damage response (DDR), and hence fusion reactions, by limiting inappropriate double-strand break (DSB) repair and processing activities at eukaryotic chromosome ends. A telomere-specific protein complex, termed shelterin, has a crucial function in safeguarding and securing telomere integrity. Within this complex, TRF2 has emerged as the key player, dictating different states of telomere protection during the replicative lifespan of a cell. How TRF2 prevents activation of DSB repair activities at functional telomeres has now been extensively investigated. In this review we aim at exploring the complex and multifaceted mechanisms underlying the TRF2-mediated protection of eukaryotic chromosome ends.

Telomeres protect chromosomes against genome instability

Telomeres are highly conserved nucleoprotein structures that discriminate naturally occurring chromosome ends from DNA DSBs [1]. Understanding how telomeres ensure genome stability and control rates of replicative senescence remains an ongoing challenge. In humans, telomeric DNA is composed of several kb consisting of the short 5′-TTAGGG-3′/3′-CCCTAA-5′ hexanucleotide repeat sequence (see Glossary). This long double-stranded (ds) repetitive region ends in a 30–100 nt single-stranded (ss) TTAGGG, 3′ G-rich overhang [2–6]. Telomeric DNA is bound by the shelterin complex [7], which is anchored to the ds repeat region by two homeodomain-containing dsDNA-binding factors, TRF1 (telomere repeat-binding factor 1) and TRF2 (telomere repeat-binding factor 2). Apart from the N-terminal region, which is acidic in TRF1 and highly basic in TRF2, both proteins share significant sequence homology and have similar domain architectures. Despite their apparent similarities, TRF1 and TRF2 have distinct functions and, in turn, discernable knockout phenotypes [8]. TRF2 directly associates with hRAP1 (repressor activator protein 1), whereas both TRF1 and TRF2 interact with TIN2 (TRF1-interacting nuclear factor 2). TIN2 acts as a bridge between the ds telomeric DNA-binding proteins (TRF1 and TRF2) via TPP1 (TINF2-interacting protein 2) to the ss telomeric DNA-binding protein, POT1 (protection of telomeres 1) [7,9]. Although an obvious speculation would be that the differential hRAP1 binding accounts for the functional differences of TRF1 and TRF2, it is very unlikely considering that deletion of hRAP1 does not elicit a phenotype comparable to the loss of either TRF1 or TRF2 in cells with normal telomere length [10].

Functional telomeres prevent nucleolytic processing events and in turn prevent the activation of an inappropriate DDR which would otherwise lead to checkpoint-mediated cell cycle arrest and unscheduled repair events. Telomere deprotection does however occur in aging cells,

Glossary

DNA damage response (DDR): an elaborate signaling network that detects and repairs DNA cytotoxic lesions that can arise in the cell spontaneously or in response to exogenous agents.
Heterochromatin: a tightly packed form of chromatin, which consists of DNA, protein, and RNA, and that results in the suppression of gene expression.
Histone methyltransferases: histone-modifying enzymes that catalyze the transfer of methyl groups to a histone lysine residue.
Lysine methyltransferases: enzymes that can add methyl groups to histone and non-histone lysine residues.
Nonhomologous end-joining (NHEJ): a DNA repair pathway occurring mainly during G1 phase whereby two ends of a double-stranded (ds) break are directly ligated together.
Shelterin: a multisubunit protein complex (six in humans: TRF1, TRF2, POT1, TPP1, TIN2, Rap1) that specifically associates with telomeric DNA and is essential for telomere capping.
Stochastic optical reconstitution microscopy (STORM): a single-molecule super-resolution technique that is capable of providing optical resolution below the diffraction limit.
Telomere dysfunction-induced foci (TIFs): consist of DDR markers, such as phosphorylated H2AX (γH2AX) or 53BP1, that accumulate at chromosome ends upon telomere deprotection.
t-loops: telomeric structures in which the single-stranded (ss) telomeric 3′ G-overhang loops back and invades the ds telomere repeat, thereby forming a loop.
Telomeric DNA: consists of the short TTAGGG/CCCTAA hexanucleotide sequence that is repeated in contiguous tracts at chromosome ends.
Telomere repeat-containing RNA (TERRA): a long non-coding RNA that is transcribed from subtelomeric regions and represents an integral component of telomeric chromatin.

Corresponding authors: Luke, B. (B.Luke@imb-mainz.de); Porro, A. (porro@imcr.uzh.ch).
Keywords: TRF2; telomeres; DNA damage response; chromatin; TERRA; t-loops.
[*] *Current address:* Institute of Molecular Cancer Research, University of Zürich, Switzerland, Winterthurerstrasse 190, Zürich 8057, Switzerland.
[†] *Current address:* Institute of Molecular Biology (IMB), 55128 Mainz, Germany.

0968-0004/
 http://dx.doi.org/10.1016/j.tibs.2015.03.003

and coincides with the natural erosion of chromosome ends. When telomeres reach a critically short length, or become damaged, the DDR is no longer kept in check and cells enter a state of irreversible arrest despite continued metabolic activity; this is referred to as replicative senescence [11]. DNA damage-induced senescence is frequently associated with a persistent DDR originating from telomeres, which leads to the activation of DNA damage signaling kinases and p53-dependent growth arrest. This latter finding also supports the notion that damaged or eroded telomeres may be considered a source of irreparable DNA damage [12,13].

TRF2 is a master regulator of telomere integrity

The shelterin component TRF2 is a key factor in regulating telomere integrity. Disruption of TRF2 results in telomere deprotection and unleashes the activity of the ataxia telangiectasia mutated (ATM) kinase [14–16] (Box 1). The subsequent localization of DDR components such as phosphorylated H2AX (γH2AX) and 53BP1 to chromosome ends, results in the formation of so-called 'telomere dysfunction-induced foci' (TIFs) [17]. In addition, TRF2 loss leads to the induction of chromosome end-to-end fusions, mediated by the Ku70/80- [18] and DNA ligase IV-dependent [19] classical non-homologous end-joining pathway (c-NHEJ). Fused chromosomes ultimately result in strong proliferative defects and an increased risk of genome instability.

The ability of TRF2 to maintain a telomere state that prevents the activation of the ATM pathway requires a cooperative interaction with the other shelterin subunits. In particular, TRF2 requires TIN2 for its optimal binding to telomeres [20] and, in accordance, a TRF2 mutant that lacks the TIN2-binding domain is partially defective in preventing ATM activation [20]. Strikingly, the forced telomere localization of a TIN2 mutant that is deficient in TRF1 binding rescues telomere deprotection phenotype following TIN2 loss [21], suggesting that the physical interaction between TIN2 and TRF1 is dispensable for the telomere protective functions of TRF2 [21]. Further work, however, will help to determine the biochemical features of the TRF2–TIN2 interaction to fully understand which molecular properties of TIN2 are required to affect the way TRF2 protects chromosome ends from DNA repair activities.

Box 1. TRF2 and the DNA damage signaling kinases

TRF2 suppresses DDR signaling and c-NHEJ pathway at functional telomeres through its direct control of a number of kinases involved in the DNA damage signaling cascade. Through its homodimerization domain TRF2 associates with the ATM kinase, which is then held in its inactive state. TRF2 binds to a region of ATM near its S1981 residue, whose phosphorylation occurs concomitantly with the dissociation of ATM oligomers. This dissociation step is likely to be relevant for the formation of the active ATM monomers [98], which are then released from DNA and are able to propagate the checkpoint response by phosphorylating other 'soluble' substrates. By preventing the phosphorylation of ATM on S1981, TRF2 might block the interaction between the ATM kinase and the MRN complex, which acts as DNA damage sensor, thus ultimately abrogating ATM signaling at an early step [98]. Moreover, TRF2 physically interacts with and hinders the phosphorylation and activation of Chk2 [99], an ATM-dependent mediator of checkpoint responses and senescence, thus hindering telomeres from both a full-blown ATM-dependent DDR [17,37] and engaging in c-NHEJ [15,16]. Furthermore, through its interaction with the shelterin component hRAP1, TRF2 also impairs the activation of the DNA-dependent protein kinase (DNA-PK), a DSB repair complex, composed of the DNA-binding Ku70/80 heterodimer and the serine/threonine kinase catalytic subunit (DNA-PKcs), which is involved in the induction of c-NHEJ [100]. Specifically, it has been suggested that the TRF2/hRAP1 complex may cause a steric hindrance that impairs the proper inward sliding of Ku at telomeric termini. Moreover, TRF2 itself impedes the ability of Ku to synapse telomere ends [60]. Under such conditions, DNA-PK is maintained in its inactive form because its activation by auto-phosphorylation very likely requires Ku translocation and the subsequent melting of DNA termini [100]. The interaction between TRF2 and Rap1 appears dispensable to protect telomeres against c-NHEJ in cells with long telomeres [101], but it might well play a role when telomeres become critically short or when TRF2 becomes limiting.

TRF2 dictates the different states of telomere protection

The TRF2 protein is composed of four functional domains. The TRFH domain is required for the formation of homodimers and higher-order oligomers (Figure 1). TRF2 also harbors an N-terminal basic domain that is rich in glycine and arginine residues (GAR domain), a flexible hinge domain involved in protein–protein interactions, and a C-terminal Myb/homeodomain-like DNA-binding domain [22], which has exquisite specificity for telomeric TTAGGG repeats (Figure 1) [23–26]. Importantly, TRF2 does not exclusively bind to telomeric DNA repeats but also associates with various DNA conformational structures in a sequence independent manner, for example, the junction between duplex DNA and ss 3′ overhangs [27], Holliday junctions [28,29], and positively supercoiled DNA [30,31].

Although the telomeric signal that ultimately triggers replicative senescence remains unknown, growing evidence suggests that a deficiency in TRF2 might be a key contributor. In support of this notion, it has been shown that either the overexpression [32] or stabilization of TRF2 [33] can delay the onset of replicative senescence. As telomeres shorten, TRF2 pools at chromosome ends will diminish in a gradual manner, and hence different TRF2 levels at telomeres may correspond to distinct stages of senescence. Spontaneous telomere deprotection occurring in immortalized cancer cells and during replicative aging determines three different telomere states (closed, intermediate, and uncapped) [34–36], which are based on the gradual decline of TRF2 [37] (Figure 2). In non-senescent cells with long telomeres, and hence ample TRF2 occupancy, a 'closed state' is promoted, which prevents both DDR activation as well as chromosome fusions, likely through the sequestration of chromosome ends in higher-order secondary structures [32] (discussed in the next section).

The 'intermediate state' occurs following a limited amount of telomere shortening that results in reduced, but not fully depleted, TRF2 pools. The intermediate state has recently been mimicked experimentally by employing a partial depletion of TRF2 [37]. Cells harboring 'intermediate state' telomeres elicit a telomeric DDR including γH2AX-positive chromosome ends; however, the number of TRF2 molecules that remains bound is still sufficient to suppress chromosome fusions via the c-NHEJ pathway (Figure 2) [37]. Similarly to internal chromosome DNA