Mechanisms of Ageing and Development 133 (2012) 421–434

Contents lists available at SciVerse ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Post-translational modifications of TRF1 and TRF2 and their roles in telomere maintenance

John R. Walker, Xu-Dong Zhu *

Department of Biology, LSB438, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada

ARTICLE INFO

Article history:
Received 12 March 2012
Received in revised form 27 April 2012
Accepted 4 May 2012
Available online 23 May 2012

Keywords:
Post-translational modification
Human
Telomere binding proteins
TRF1
TRF2

ABSTRACT

Telomeres, heterochromatic structures, found at the ends of linear eukaryotic chromosomes, function to protect natural chromosome ends from nucleolytic attack. Human telomeric DNA is bound by a telomere-specific six-subunit protein complex, termed shelterin/telosome. The shelterin subunits TRF1 and TRF2 bind in a sequence-specific manner to double-stranded telomeric DNA, providing a vital platform for recruitment of additional shelterin proteins as well as non-shelterin factors crucial for the maintenance of telomere length and structure. Both TRF1 and TRF2 are engaged in multiple roles at telomeres including telomere protection, telomere replication, sister telomere resolution and telomere length maintenance. Regulation of TRF1 and TRF2 in these various processes is controlled by post-translational modifications, at times in a cell-cycle-dependent manner, affecting key functions such as DNA binding, dimerization, localization, degradation and interactions with other proteins. Here we review the post-translational modifications of TRF1 and TRF2 and discuss the mechanisms by which these modifications contribute to the function of these two proteins.

1. Telomeres and the shelterin complex

Telomeres are specialized heterochromatic structures found at the ends of linear eukaryotic chromosomes. Human telomeric DNA consists of double-stranded TTAGGG tandem repeats ranging from 2 to 30 kilobase pairs in length (Cheng et al., 1989; de Lange et al., 1990; Moyzis et al., 1988) as well as a G-rich 3′ single-strand protrusion (~150 nt) (Makarov et al., 1997; McElligott and Wellinger, 1997; Wright et al., 1997). Invasion of the 3′ G-rich single-strand protrusion into the duplex region of telomeric DNA gives rise to the formation of a higher order structure at telomeres, referred to as the t-loop (Griffith et al., 1999). The t-loop structure has been found not only in mammals but also in plants and protozoa, suggesting a conserved role of t-loops in masking chromosome ends from being recognized as double-strand breaks (Griffith et al., 1999; Munoz-Jordan et al., 2001; Murti and Prescott, 1999).

Mammalian telomeric DNA is coated with a telomere-specific protein complex, referred to as shelterin/telosome, which consists of TRF1, TRF2, TIN2, POT1, TPP1 and hRap1 [reviewed in de Lange 2005; Liu et al., 2004a; Palm and de Lange, 2008]. TRF1 interacts directly with TIN2 (Kim et al., 1999), which binds to TRF2 and TPP1 (Houghtaling et al., 2004; Kim et al., 2004; Liu et al., 2004b; Ye et al., 2004a; Ye et al., 2004b). While POT1 binds to TPP1 (Houghtaling et al., 2004; Liu et al., 2004b; Ye et al., 2004b), TRF2 interacts and forms a complex with hRap1 with a ~1:1 stoichiometry (Li et al., 2000; Zhu et al., 2000). Components of the shelterin complex do not exist in an equal abundance in cells (Takai et al., 2010) and, therefore, sub-complexes of shelterin lacking one or more subunits have been observed in cell extracts (Kim et al., 2008b; Liu et al., 2004a; Ye et al., 2004a).

The shelterin complex plays an essential role in maintaining the integrity of telomere length and structure. Disruption of shelterin proteins at telomeres not only promotes deregulation of telomere length homeostasis but also induces telomere de-protection, resulting in the formation of telomere abnormalities including telomere end-to-end fusions, telomere loss, telomere-containing double-minute chromosomes (TDM) and telomere doublets/fragile telomeres (more than one telomeric signal at a single chromatid end) [reviewed in de Lange, 2005; Palm and de Lange, 2008]. These dysfunctional telomeres are recognized as damaged DNA (McKerlie and Zhu, 2011; Mitchell et al., 2009; Sfeir et al., 2009; Takai et al., 2003; Wang et al., 2004) and can contribute to genomic instability, a hallmark associated with tumorigenesis and ageing.

In addition to the shelterin complex, mammalian telomeres are also associated with a number of accessory factors that are involved in DNA metabolism but not unique to telomeres (de Lange, 2005; Liu et al., 2004a; Palm and de Lange, 2008). These factors also play an important role in the maintenance of telomere integrity. Examples include XPF/ERCC1 (Zhu et al., 2003), the Mre11 complex (Zhu et al., 2000), Apollo (Lenain et al., 2006; van Overbeek and de Lange, 2006), Ku70/Ku80 (Hsu et al., 1999; Hsu et al., 2000) and ORC (Atanasiu et al., 2006; Deng et al., 2007).

* Corresponding author. Tel.: +1 905 525 9140x27737; fax: +1 905 522 6066.
E-mail address: zhuxu@mcmaster.ca (X.-D. Zhu).

0047-6374/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.mad.2012.05.002

2. Double-stranded telomere binding proteins TRF1 and TRF2

Within the shelterin complex, only TRF1, TRF2 and POT1 can interact with telomeric DNA directly (Palm and de Lange, 2008). POT1 contains multiple OB folds and binds to the G-strand telomeric DNA (Baumann and Cech, 2001; Kelleher et al., 2005; Lei et al., 2004; Loayza and De Lange, 2003; Loayza et al., 2004). On the other hand, TRF1 and TRF2 are double-stranded telomeric DNA binding proteins (Bilaud et al., 1997; Broccoli et al., 1997; Chong et al., 1995), and these two proteins will be the focus of this review.

TRF1 and TRF2 are related proteins that share a common architecture (Fig. 1), consisting of an N-terminal domain, a TRF homology domain (TRFH) which mediates dimerization, a flexible linker region and a C-terminal homeodomain which has been historically described as a SANT/Myb-like DNA binding domain (Bilaud et al., 1997; Broccoli et al., 1997; Court et al., 2005). While there is a high degree of sequence similarity for the respective TRFH and the Myb-like domains of TRF1 and TRF2, little sequence and structural similarity is found in their respective flexible linker region (Bianchi et al., 1999). Furthermore, the N-terminal domains of TRF1 and TRF2 differ significantly from one other, with TRF1 possessing a unique acidic domain and TRF2 carrying a basic domain rich in glycine and arginine residues, also referred to as a GAR domain.

Both TRF1 and TRF2 bind to duplex telomeric DNA as a dimer (Bianchi et al., 1997; Bilaud et al., 1997; Broccoli et al., 1997; Shen et al., 1997; van Steensel and de Lange, 1997), but they do not interact with each other (Broccoli et al., 1997; Zhu et al., 2000). Three-dimensional structural analysis showed that the homodimer formation of TRF1 or TRF2 is mediated by their respective TRFH domain; however, unique features at their respective dimerization interface prohibit them from forming a heterodimer (Fairall et al., 2001). The TRFH domains of TRF1 and TRF2 contain a similar peptide-docking site that is differentially used to interact with proteins containing the motif FxLxP for docking with TRF1, and YxLxP for docking with TRF2 (Chen et al., 2008; Kim et al., 2009). TRF1 binds to the FxLxP motif of TIN2 whereas TRF2 binds to the YxLxP motif of several shelterin accessory factors including Apollo, XPF, the Mre11 complex, MCPH1 and PNUTS (Chen et al., 2008; Kim et al., 2009). In addition, TRF1 and TRF2 can also interact with proteins through regions outside of their TRFH domains. The acidic domain in the N-terminus of TRF1 contains the RxxADG sequence that is bound by tankyrase 1 and tankyrase 2 (Guettler et al., 2011; Kaminker et al., 2001; Sbodio and Chi, 2002; Sbodio et al., 2002; Smith et al., 1998). Several proteins are found to interact with either the N-terminus or the C-terminus of TRF2 including WRN, ORC and FEN1 (Atanasiu et al., 2006; Muftuoglu et al., 2006; Opresko et al., 2002).

3. Roles Of TRF1 and TRF2 at telomeres

TRF1, the first shelterin protein discovered to bind telomeric DNA (Chong et al., 1995), is implicated in telomere replication, telomere protection, telomere length maintenance and the resolution of sister telomeres. The gene encoding TRF1 is essential since knockout of TRF1 leads to embryonic lethality (Karlseder et al., 2003). Deletion of TRF1 promotes the formation of fragile telomeres, a type of telomere abnormality that can be caused by replication-dependent defects (Martinez et al., 2009; Sfeir et al., 2009). It has been suggested that TRF1 is required to support efficient replication of telomeric DNA (Sfeir et al., 2009). Cells null or depleted for TRF1 accumulate telomere fusions (Iwano et al., 2004; Martinez et al., 2009; McKerlie and Zhu, 2011; Sfeir et al., 2009), indicative of its role in telomere protection. Overexpression of TRF1 promotes telomere shortening whereas loss of TRF1 from telomeres has been shown to induce telomerase-dependent telomere lengthening, implying that TRF1 negatively regulates telomerase-dependent telomere extension, perhaps by restricting the access of telomerase to the ends of telomeres (Ancelin et al., 2002; Smogorzewska et al., 2000; van Steensel and de Lange,

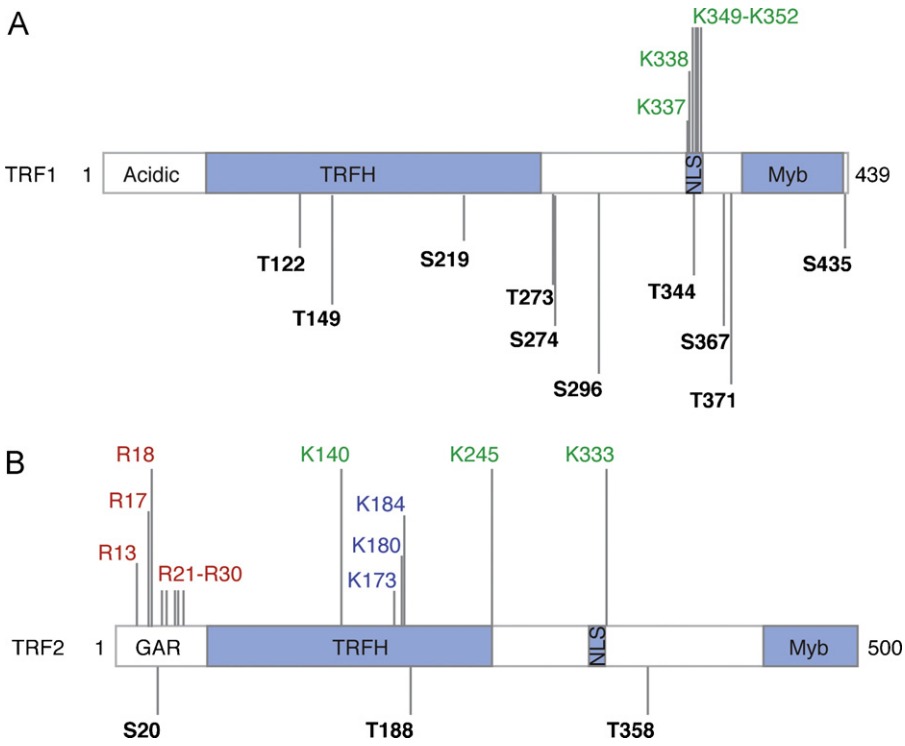

Fig. 1. Schematic diagrams of TRF1 (A) and TRF2 (B) along with their respective post-translational modification. The major domains of TRF1 and TRF2 are shown. NLS: nuclear localization signal. Phosphorylation sites are indicated in black, ubiquitylation sites in blue, SUMOylation sites in green, and arginine methylation sites in red (for interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).