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TRF2 and Apollo Cooperate with Topoisomerase 2α to Protect Human Telomeres from Replicative Damage

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SUMMARY

Human telomeres are protected from DNA damage by a nucleoprotein complex that includes the repeat-binding factor TRF2. Here, we report that TRF2 regulates the 5′ exonuclease activity of its binding partner, Apollo, a member of the metallo-β-lactamase family that is required for telomere integrity during S phase. TRF2 and Apollo also suppress damage to engineered interstitial telomere repeat tracts that were inserted far away from chromosome ends. Genetic data indicate that DNA topoisomerase 2α acts in the same pathway of telomere protection as TRF2 and Apollo. Moreover, TRF2, which binds preferentially to positively supercoiled DNA substrates, together with Apollo, negatively regulates the amount of TOP1, TOP2α, and TOP2β at telomeres. Our data are consistent with a model in which TRF2 and Apollo relieve topological stress during telomere replication. Our work also suggests that cellular senescence may be caused by topological problems that occur during the replication of the inner portion of telomeres.

INTRODUCTION

Telomeres are essential for the maintenance of chromosome stability (Blackburn, 2001). The key to how telomeres perform this function resides in very special features that prevent their recognition as, and the accidental generation of, DNA double-strand breaks (Lundblad, 2000). Among the many mammalian telomere-associated proteins identified in the past decade, telomere repeats binding factor 2 (TRF2) plays a crucial role in protecting chromosome ends against instability (Bilaud et al., 1997; Broccoli et al., 1997; van Steensel et al., 1998). TRF2 binds specifically to double-stranded telomeric DNA and is copurified from nuclear extracts with five other telomeric proteins (TRF1, TIN2, TPP1, POT1, and Rap1), which form a multiprotein complex called shelterin (Palm and de Lange, 2008). Notably, TRF2 appears to associate with telomeres in distinct complexes, whose exact composition and biological significance remain to

be determined (Mattern et al., 2004). Upon TRF2 inhibition, telomeres associate with DNA damage response factors, forming telomere dysfunction-induced foci (TIFs) (Takai et al., 2003). The different aspects of telomere damage response are very similar to those elicited by the double-strand breaks induced by ionizing radiation (Denchi and de Lange, 2007).

The visualization of purified telomeric DNA from various eukaryotic organisms has revealed that at least some chromosomal termini adopt a looped configuration called t loop (Griffith et al., 1999). T loops have been proposed to protect chromosome ends from degradation and repair. Therefore, the contribution of TRF2 to telomere end protection may be explained, at least in part, by its role in t loop formation. Indeed, in vitro studies have shown that TRF2 can remodel a telomeric DNA substrate into a t loop (Stansel et al., 2001), a reaction probably facilitated by an unwinding activity (Amiard et al., 2007).

In addition to its DNA binding role, TRF2 recruits a number of factors and enzymes required for telomere protection, including Apollo, (Freibaum and Counter, 2006; Lenain et al., 2006; van Overbeek and de Lange, 2006), an Artemis paralog, which, interestingly, is also involved in DNA repair (Bae et al., 2008; Demuth et al., 2008; Demuth et al., 2004). Reduced expression of Apollo causes telomere defects in S phase cells and accelerates the onset of senescence in primary fibroblasts (van Overbeek and de Lange, 2006). The mechanism by which Apollo controls telomere replication and senescence is not known. The fact that Apollo exhibits a 5′ exonuclease activity in vitro (Lenain et al., 2006) suggests that an Apollo-mediated nucleolytic step could be required to protect telomeres. In this study, we present evidence that the nuclease domain of Apollo is required to protect the internal tracts of telomeric repeats from DNA damage and is regulated by TRF2. Moreover, we found that topoisomerase 2α (TOP2α) acts in synergy with TRF2 and Apollo for telomere protection. We propose a model in which TRF2 regulates the Apollo-mediated processing of topologically constrained structures that occur during telomere replication.

RESULTS

TRF2 Regulates the Exonuclease Activity of Apollo

We first asked whether the 5′ exonuclease activity of Apollo can be modulated by TRF2. When Apollo was incubated with purified TRF2, its 5′ exonuclease activity on a single-stranded telomeric substrate was stimulated (Figures 1A and 1B). TRF2 did not stimulate the 5′ exonuclease activity of RecJf or Artemis (Figures 1A–1C) and enhanced Apollo 5′ exonuclease activity on a blunt substrate that ended with a nontelomeric sequence (Figure 1D), while purified TRF2 alone did not modify the substrate (Figures S1A and S1B available online). TRF1 did not stimulate the nuclease activity of Apollo (Figures 1B and 1C).

TRF2 inhibited Apollo activity on substrates that mimics telomeric DNA ends, i.e., 5′ recessed strands of double-stranded substrates ending with 3′ G tails (Figure 1E). This could stem from the preferential binding of TRF2 to the junction between the single- and the double-stranded part of the telomeric DNA (Stansel et al., 2001), physically preventing the access of Apollo to its substrate.

The stimulating effect of TRF2 on exonuclease activity did not lead to an observable activation of an endonuclease activity. The same result was obtained in the presence of purified DNA-PK under conditions in which this kinase phosphorylates and activates Artemis (Figure 1 and data not shown). Therefore, the existence of any endonuclease activity for Apollo remains hypothetical.

The Nuclease Domain of Apollo Is Required for Telomere Protection and Prevention of Senescence

The regulation of Apollo exonuclease activity by TRF2 leads credence to the view that this catalytic activity is involved in telomere protection. To test this hypothesis, we constructed three alleles of Apollo bearing mutations in conserved residues within the metallo-β-lactamase domains (Apm1, 2, and 3) of Apollo, Artemis, and hSNM1 (Figure S1C) (Pannicke et al., 2004; Poinsignon et al., 2004). We substituted one histidine (H33) or aspartate (D14, D35) residue either separately in Apm2 (D35N) and Apm3 (D14N) or as a double substitution in Apm1 (H33A, D35N). In contrast to wild-type Apollo, the purified mutants did not display any cleavage activity when tested with a single-stranded substrate in vitro (Figure S1B). These results are in agreement with a previous mutation comparable to D35N that abolished the exonuclease activity of hSNM1 (Hejna et al., 2007). It is interesting that mutations of D14, H33, and D35 in Artemis impaired its endonucleolytic activity but left its exonucleolytic activity intact (Pannicke et al., 2004), although recent results seem to question the reality of this latter exonucleolytic activity (Pawelczak and Turchi, 2010). These discrepancies among the in vitro properties of Apollo, Artemis, and hSNM1—together with the fact that unlike hSNM1 (Hejna et al., 2007), Apollo does not seem to use RNA substrates (data not shown)—suggest that these three paralogs display distinct catalytic properties, likely to translate into different functions.

The wild-type and nuclease-inactive alleles of Apollo were expressed in human cells as green fluorescent protein (GFP) C-terminal fusions using retroviral vectors. These GFP-tagged proteins were named Apwt-G, Apm1-G, Apm2-G, and Apm3-G, respectively. The presence of a tag at the C terminus of Apollo did not modify the nuclease activity of Apollo in vitro (data not shown) (Lenain et al., 2006). In addition, these Apollo constructs were mutated to remove the target site for the small interfering RNA (siRNA) against Apollo (CL2 siRNA, Figure S1D), previously selected for its ability to diminish Apollo expression and cause telomere deprotection (Lenain et al., 2006). Both Apwt-G and mutant proteins were expressed in vivo at similar levels and were present at telomeres (Figures S2A–S2C). The fact that the nuclease activity is not required for targeting Apollo to telomeres confirms previous data showing that the TRF2-interacting region of Apollo lies outside of its nuclease domain (Chen et al., 2008; Lenain et al., 2006)

The expression of Apwt-G in Apollo-proficient cells did not lead to the loss of telomere protection and did not alter cell-cycle progression (Figures S2D and S2E). This, together with the fact that cells exhibiting the highest and lowest levels of Apwt-G display a similar number of foci containing both the checkpoint protein 53BP1 and the telomere marker protein TRF1, two features of TIFs (Figure S2F) (Takai et al., 2003), indicates that