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DNA2, a new player in telomere maintenance and tumor suppression

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Telomeres form special structures to shield chromosome ends from inappropriate DNA repair activities, therefore protecting genome stability. Telomeres are maintained by telomerase, telomere-specific binding proteins termed shelterins, as well as proteins involved in general DNA metabolism. The primary function of telomerase is to extend short telomeres, thereby revoking the proliferation barrier caused by telomere attrition. The shelterin complex, comprising of TRF1, TRF2, POT1, TPP1, TIN2, RAP1, binds to telomere DNA and suppresses ATM/ATR-mediated DNA damage response, thereby inhibiting DNA repair activities at telomeres. Aside from shelterin proteins, numerous proteins involved in general DNA repair/replication also associate with telomeres and play essential roles in maintaining telomere integrity. Although much has been learned about the functions of telomerase and shelterins in telomere maintenance, understanding of the roles of non-shelterin proteins has lagged.

We have recently identified mammalian DNA2 as a new player in protecting telomere integrity.[1] Complete DNA2 knockout in mice is embryonic lethal, suggesting that DNA2 is an essential gene. DNA2+/– mice are viable, but with high occurrence of lung adenocarcinoma, lymphoma, and hepatoma. Mouse embryonic fibroblast (MEF) cells derived from DNA2+/– mice display a variety of genome instabilities, including chromosome bridges and aneuploidy. Notably, telomeres in both normal and tumor tissues derived from DNA2+/– mice are significantly shorter than wild-type controls. DNA2 deficiency also induces DNA damage response at telomeres, suggesting that DNA2 deficiency induces telomere dysfunction. Further in-depth analyses reveal that DNA2 deficiency impairs efficient replication of telomeric DNA, leading to accumulation of fragile telomeres, sister telomere associations and telomere loss. Collectively, our results demonstrate that mammalian DNA2 reduces replication stress at telomeres and is an important player in preserving genome stability and suppressing cancer development.

Due to the unique features of telomeres, telomeric DNA is difficult to replicate. Conventional replication machinery needs additional assistance to completely replicate telomeres. One obstacle to fork progression is the formation of the G-quadruplex (G4) structure within TTAGGG repeats.[2] G4 is a highly stable structure formed by guanine residues through Hoogsteen hydrogen bonding. Stable G4 structure is expected to block fork progression and stall replication. Stalled replication forks may collapse, leading to sudden loss of telomeres and telomere instability. Given that telomeric G4 needs to be resolved to allow efficient fork progression, the overarching question that arises is, "How are G4 structures at telomeres resolved?"

Apparently, resolving G4 requires enzymatic activities from helicases and/or nucleases. To this end, helicases have been deeply studied. RecQ helicases such as BLM and WRN catalyze G4 unwinding in vitro. Consistently, deficiency in BLM or WRN results in telomere replication defects.[3,4] There has long been a presumption that G4 unwinding is the primary way of resolving G4. Now, using in vitro substrates that mimic G4 formed in telomeric sequences, we have found that purified mammalian DNA2 cleaves the intramolecular G4 structures formed in telomeric sequences.[1] The G4 cleavage activity is nuclease-dependent and helicase-independent. In agreement with the in vitro data, DNA2-deficient MEFs show a significant increase of fragile telomeres when treated with G4 stabilizing molecules. Our observation suggests that multiple pathways may be employed by mammalian cells to efficiently remove G4 arising at telomeres. While helicases like BLM and WRN may unwind G4, nucleases like DNA2 can be active participants in G4 removal. Availability of multiple pathways may be important for removing G4 under certain conditions, for instance, when the accessibility of helicases to G4 is limited. It is perceivable that other nucleases may help in removing telomeric G4 as well.

The roles of mammalian DNA2 in telomere maintenance are just beginning to be explored. Several questions pertinent to this study need to be addressed. First, nucleases/helicases must work together with shelterin proteins to keep telomeres stable, and their enzymatic activities should be tightly regulated to avoid unwanted processing of telomeric DNA. DNA2 is no exception. In fact, DNA2 interacts with TRF1/TRF2.[1] It remains to be delineated whether the activity of DNA2 at telomeres is regulated by shelterin, and whether DNA2 exerts its telomere-protective functions via the shelterin complex. Second, telomeres terminate with ss G-rich overhangs at the 3' ends (G-overhangs), and telomerase needs to bind to G-overhangs to extend telomeres. Given the considerablh long G-overhang lengths in human cells (> 100 nt),[5]

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formation of G4 at G-overhangs is highly plausible. Such G4 formation will block telomerase binding and inhibit telomere elongation. It will be of interest to determine whether DNA2-mediated G4 cleavage also plays a role in assisting telomerase extension of telomeres. Finally, post-replicative processing occurs at the ends of telomeres to generate G-overhangs. Given that telomere ends resemble DSB ends, it is possible that factors for processing DSB ends may also participate in resecting telomere ends. At least two pathways involving DNA2 and Exo1 modulate DSB end resection.[6,7] Interestingly, Exo1 resects telomere ends at the G_2 phase after Apollo makes initial resection of the telomere ends at the leading strand.[8] It will be of particular interest to learn whether DNA2 resects telomere ends, and, if so, whether the two pathways operate independently of each other, or whether they normally operate in concert to process telomere ends.

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