Beginning at the end: DNA replication within the telomere

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Using single molecule analysis of replicated DNA (SMARD), Drosopoulos et al. (2015; *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201410061) report that DNA replication initiates at measurable frequency within the telomere of mouse chromosome arm 14q. They demonstrate that resolution of G4 structures on the G-rich template strand of the telomere requires some overlapping functions of BLM and WRN helicase for leading strand synthesis.

Double-strand breaks in DNA can wreak havoc in cells if not repaired. Therefore, it was proposed that the ends of chromosomes may be specialized cap structures that are not recognized as double-strand breaks, thus preventing cell cycle arrest, degradation, and recombinational fusion (Muller, 1938; McClintock, 1939). We now know that telomeres comprise the ends of chromosomes and are essential for genome stability. Telomeres are composed of tandem head-to-tail repeats of a short G-rich sequence; for example, human telomeres are 2–20 kb of $(TTAGGG)_n$ repeats. The chromosome ends are not blunt, and the 3′ end (G-rich strand) overhangs in a single strand that can invade the interior of the telomere to displace the internal G-rich sequence and form a T-loop structure (Griffith et al., 1999; Cesare et al., 2003; Doksani et al., 2013), thus protecting the chromosome ends from being recognized by the cell as double-strand breaks, in addition to protection by proteins that bind the telomere.

Eukaryotic chromosomes are duplicated via semiconservative replication with a leading (continuous synthesis for net growth at the 3′ end of the nascent leading strand) and lagging (discontinuous Okazaki fragment synthesis for net growth at the 5′ end of the nascent lagging strand) elongating strand as shown in Fig. 1. In chromosomal semiconservative replication, the short 5′ RNA primer is removed from the nascent strand and the gap is filled in by DNA that is ligated to the adjacent nascent DNA. However, at the end of the chromosome, the gap after removal of the 5′ terminal RNA primer on the lagging strand cannot be filled in, and the chromosome may become shorter with each ensuing round of replication. This has been termed the end-replication problem (Watson, 1972; Olovnikov, 1973), and telomerase helps to solve this problem (Greider and Blackburn, 1987; Soudet et al., 2014).

Semiconservative replication occurs before the action of telomerase. Previously it was thought that DNA replication began at an origin in chromosomal DNA adjacent to the telomere repeats, with the replication forks moving bidirectionally away from the subtelomeric origin (Fig. 1 A), thus replicating the telomere. However, the question remained whether DNA replication might initiate with some frequency within the telomere itself (Fig. 1 B). This question has now been answered in the affirmative in this issue by Drosopoulos et al., who used single molecule analysis of replicated DNA (SMARD; Norio and Schildkraut, 2001). In this approach, replicating cells are sequentially labeled by two different nucleotide analogues that are subsequently identified by immunofluorescence. For example, in bidirectional replication, red signals from the first pulse will be flanked at each end by green signals from the second pulse. Earlier reports using SMARD had concluded that most replication initiates at subtelomeric regions in the mouse and human genome and rarely in the telomeres themselves (Sfeir et al., 2009; Drosopoulos et al., 2012). In the recent study by Drosopoulos et al. (2015), fluorescence in situ hybridization (FISH) using probes from the telomere region allowed the replication pattern to be analyzed for a 320 kb genomic segment from the end of mouse chromosome arm 14q. Due to the long time (4 h) for the first (red) pulse, usually only red tracts of signal within the telomere were seen, but since many such molecules did not have the red signal extend into the subtelomeric region, it can be comfortably concluded that replication must have initiated within the telomere (Fig. 1 B). Moreover, some molecules did have red signal in the telomere flanked by green signal, supporting this conclusion. Although in these cases there was chromosome-proximal green signal, chromosome-distal green signal was rarely seen. Thus, although there was limited evidence for bidirectional replication originating in the telomere, it is very clear that a replication origin can exist within the telomere proper with a replication fork that extends over time into the subtelomere. It remains to be investigated whether replication initiates at a relatively high frequency in the telomeres of chromosomes other than 14q.

These findings raise the question of whether the origin for DNA replication coincides with the simple sequence repeat found in telomeres or instead if it coincides with some other sequence that might be interspersed within the telomere. The former is suggested by a study with *Xenopus* cell-free extracts that could assemble the pre-replication complex and undergo some DNA replication on exogenous DNA containing exclusively telomeric repeats (Kurth and Gautier, 2010). Similar conclusions that

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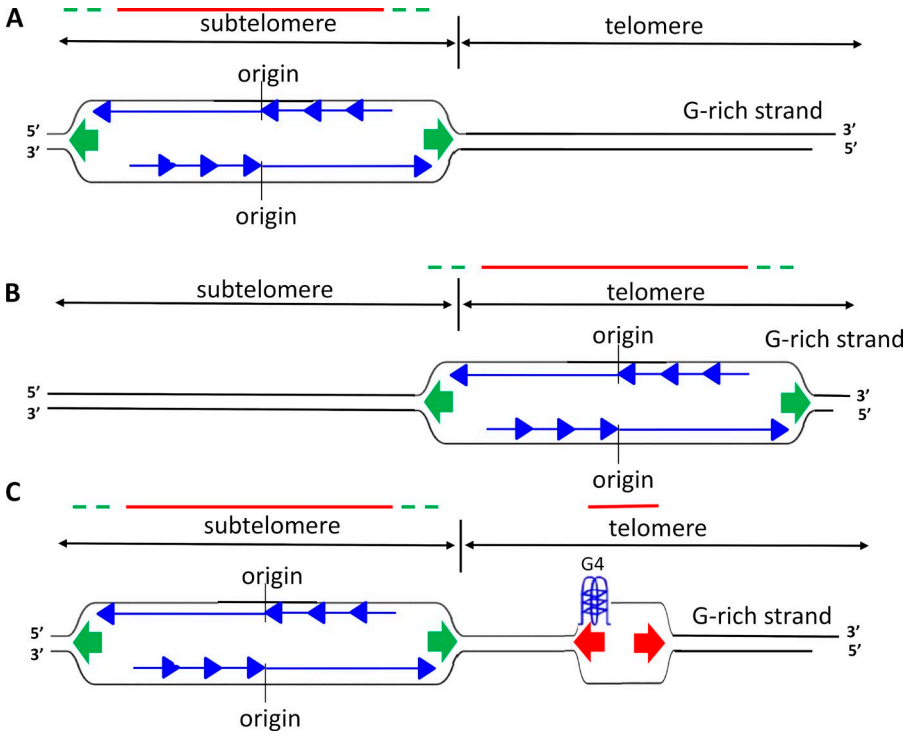

Figure 1. **DNA replication at the end of chromosomes.** (A) DNA replication can initiate within the subtelomeric region with replication forks (green arrows) progressing bidirectionally away from the origin. Telomere DNA is replicated by a replication fork that passes through this region. In each panel, leading nascent strand synthesis is indicated by a blue line with a single arrowhead; lagging nascent strand synthesis is indicated by a blue line with multiple arrowheads. At the top of each panel, the red line indicates the signal seen by microscopy of replication that initiated and continued during administration of the first pulse (IdU, red), and the dotted green line indicates the signal seen for replication extension during the second pulse (CldU, green). (B) On some DNA molecules from mouse chromosome 14q, DNA replication initiates within the telomere itself. In practice, the second (green) pulse was often not observed in the telomere. (C) Partially overlapping functions of BLM and WRN helicases are used to resolve G-quadruplex (G4) DNA (blue structure) that can form on the G-rich parental strand of the telomeres. In cells deficient of BLM and/or WRN helicase, progression of the nascent leading strand in the telomere is impaired; the slowed replication forks are indicated by red arrows. The resulting replication stress is accompanied by activation of dormant replication origins in the subtelomere. The cartoon is not drawn to scale, and the infrequently used subtelomeric replication origin in C is closer to the telomere than the subtelomeric origin in A.

DNA replication can initiate in the simple DNA repeats found in centromeres where replication bubbles have been observed in *Drosophila virilis* by electron microscopy have been reached (Zakian, 1976), and a recent study suggests that DNA replication initiates within human alpha-satellite DNA (Erliandri et al., 2014).

Replications forks move slowly through telomeric DNA (Ivessa et al., 2002; Makovets et al., 2004; Miller et al., 2006; Sfeir et al., 2009) due to the high thermal stability of GC-rich telomeric DNA as well as its propensity to form stable secondary structures, such as G-quadruplex (G4) DNA, which can pose problems for DNA replication (Lopes et al., 2011; Paeschke et al., 2011). Various helicases help solve this problem; for example, Pif1 helicase helps to unwind G4 (Paeschke et al., 2013). Bloom syndrome helicase (BLM) and the Werner syndrome helicase (WRN) have also been implicated in assisting telomere replication: BLM suppresses replication-dependent fragile telomeres (Sfeir et al., 2009), and WRN suppresses defects in telomere lagging strand synthesis (Crabbe et al., 2004). Drosopoulos et al. (2015) now report that leading strand synthesis that initiates within the telomere has a slower rate of progression into the subtelomere in BLM-deficient cells as visualized by SMARD. Moreover, there was a higher frequency of replication initiation in the 14q subtelomere of the BLM-deficient cells, originating closer to the telomere than in BLM-proficient cells. These observations suggest that dormant replication origins in the 14q subtelomere can be activated when fork progression is impeded in BLM-deficient cells (Fig. 1 C). Drosopoulos et al. (2015) also found an increase in subtelomeric replication initiation when replication fork progression from the telomere was hindered by aphidicolin, as an alternate means to activate dormant origins by replication stress. When cells were treated with the G4 stabilizer PhenDC3, 14q subtelomeric origin firing increased further in BLM-deficient cells. Collectively, the data suggest a slowdown of progression of leading strand synthesis from an origin in the 14q telomere (using the G-rich parental strand as the template) when G4 structures cannot be resolved in BLM-deficient cells. As further support for a role of BLM helicase to remove G4 structures, there was increased staining in BLM-deficient cells by the BG4 antibody (Biffi et al., 2013) against G4 in the whole genome and especially in telomeres.

WRN helicase can unwind G4 in vitro (Fry and Loeb, 1999; Mohaghegh et al., 2001). When Drosopoulos et al. (2015) used SMARD to analyze replication in cells doubly deficient of both BLM and WRN, they found a marked decrease of red replication signal in 14q telomeres, suggesting some functional overlap between BLM and WRN with regard to leading strand synthesis off the G-rich strand of telomeres. Supporting this conclusion, there was more G4 staining by the BG4 antibody in cells doubly deficient of both BLM and WRN than in cells deficient of just BLM or just WRN. This is the first direct demonstration in vivo of a contribution of BLM and WRN helicases in the resolution of G4 structures, which is especially needed for progression of leading strand synthesis that initiates in telomeres and is copied from the G-rich strand.

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