Title: Telomere replication: Mre11 leads the way

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Abstract/Summary: In this issue of *Molecular Cell*, Faure et al, 2010 establish a critical role for the Mre11 complex in the recruitment of telomerase to leading but not lagging strand telomeres of budding yeast.

Body Text

Chromosomes of most eukaryotic organisms terminate in specialized nucleoprotein structures called telomeres, which contain double-stranded DNA (dsDNA) with 3' single-stranded DNA (ssDNA) overhangs. Most chromosomal regions can be replicated by replication forks moving in either direction but a telomere can only be replicated by a fork moving in a single direction, towards the chromosome end. The inherent directionality of telomere replication means that there is a clear distinction between those telomeric DNA strands that are replicated by leading or lagging strand replication machinery (Fig 1A). The G-rich, 3' strands, are replicated by lagging stand machinery, while the C-rich, 5' strands, are replicated by leading strand machinery. Lagging strand replication leaves 3' single-stranded DNA (ssDNA) overhangs at the telomere, as the replication machinery is unable to replicate the region of the template strand that is occupied by the RNA primer of the terminal Okazaki fragment and this constitutes the well recognized "end replication problem". Leading strand polymerases are presumed to be able to replicate to the end of the chromosome, producing blunt ends. Thus, while lagging strand synthesis has the inherent ability to produce telomeric DNA structures with 3' overhangs, the leading strand most likely requires further processing to convert blunt ends into 3' overhang structures. In this issue, Faure *et al.* provide important insights into the processing of leading and lagging strands after telomere replication.

Telomeric DNA in eukaryotes is bound by a number of specialized telomere capping proteins and more general DNA Damage Response (DDR) proteins, which cooperate to maintain telomere function (de Lange, 2009; Lydall, 2009). Faure *et al.* asked whether specific proteins bind to telomeres replicated by the leading strand machinery, the lagging strand machinery, or both. To do this the authors incorporated BrdU into DNA during a single S phase and performed ChIP after either an initial or a successive round of DNA replication to measure binding of specific proteins at the leading and lagging strand telomeres.

Budding yeast telomeres are capped in part by binding of the CST complex (Cdc13-Stn1-Ten1) to telomeric 3' ssDNA (Gao et al., 2007). Interestingly, Faure *et al.* found that the CST complex and telomerase bound both leading and lagging strand telomeres. This suggested that blunt ends generated by leading strand synthesis of telomeric DNA (Fig 1A) are modified to generate ssDNA, the substrate for CST binding. Consistent with this hypothesis, Faure *et al.* showed that Mre11, a component of the MRX complex (Mre11-Rad50-Xrs2/Nbs1) bound only to the leading strand telomeres and was critical for CST complex and telomerase binding to leading strand telomeres (Fig. 1B). Importantly, Mre11 was not required for binding of all proteins to leading strand telomeres because the Ku complex (Yku70-Yku80), a DDR component that binds nonspecifically to dsDNA ends, could bind telomeres in the absence of Mre11.

One outstanding question arising from the work of Faure *et al.* is how MRX functions at leading strand telomeres. MRX has multiple functions at DNA ends; one is to recruit the checkpoint kinase Tel1 (ATM) via the Xrs2 subunit, and this aids in telomerase recruitment to telomeres; another MRX activity is to help generate 3' ssDNA overhangs via the endonuclease Sae2 (CtIP) (Mimitou and Symington, 2009; Sabourin et al., 2007). Therefore at least two plausible mechanisms could explain the role of Mre11 at leading strand telomeres – one activating the Tel1 checkpoint kinase, the other regulating

nuclease activity. It will be interesting to see if Sae2 or Tel1 also preferentially bind leading strand telomeres.

Interestingly, it seems that blunt-ended telomeres induced by making DNA Double Strand Breaks (DSBs) near telomeric DNA in metaphase-arrested cells are converted to 3' ssDNA overhangs using similar mechanisms to the resection of internal DSBs (Bonetti et al., 2009; Mimitou and Symington, 2009). In both cases, Mre11 cooperates with Sae2 to create an initial overhang, which is then extended by Sgs1/Dna2 in cooperation with Exo1. It is therefore possible that during normal telomere replication Mre11/Sae2-generated overhangs on the leading strand and the overhangs on the lagging strand are processed by nuclease activities dependent upon Sgs1/Dna2 and Exo1 (Fig 1B). Alternatively, Mre11/Sae2, Sgs1 and Exo1 might be confined to the leading strand telomere with 3' ssDNA on the lagging strand being generated entirely by the removal of the RNA primer. The latter hypothesis would likely require a specific mechanism for exclusion of nuclease activities from the lagging strand and would be consistent with the role of Cdc13 in inhibiting nuclease activities at telomeres. Using the approaches developed by Faure et al it should now be possible to ask if Sae2, Sgs1, Exo1 or Dna2 show any preference for binding to leading or lagging strand telomeres.

Intriguingly, in fission yeast it has recently been demonstrated that leading strand replication of telomeres precedes lagging strand replication (Moser et al., 2009), suggesting perhaps that earlier replication of the leading strand provides more time for post-replicative processing of this strand. Furthermore, Mre11 is important for telomere function in *Drosophila*, an organism that does not use telomerase to maintain chromosome ends (Ciapponi et al., 2004), and therefore Mre11-dependent post-replicative processing of leading strands might be a fundamental property of replication to the end of all linear DNA molecules in eukaryotes. It is clear that leading and lagging strand telomeres of mammalian cells also behave differently. For example in one study of human cells inactivation of the telomere-binding protein TRF2 caused

telomere-telomere fusions specifically between leading strands telomeres (Bailey et al., 2001). In another study of mouse cells, inactivation of Trf2 combined with deletion of Mre11 biased fusions to leading rather than lagging strand telomeres (Deng et al., 2009). Finally very recent experiments have shown that other telomere binding proteins inhibit nuclease activities at yeast telomeres (Bonetti et al, 2010). Future studies should help clarify the important roles of telomere binding proteins, nucleases and other DNA damage response proteins at leading and lagging strand telomeres.

Figure Legend

Telomere replication.

A. Telomeres are nucleoprotein caps at the end of eukaryotic chromosomes. Replication of telomeric DNA occurs from sub-telomeric origins. This dictates that the G strand is replicated by the lagging strand machinery, resulting in a 3' ssDNA overhang, while the C strand is replicated by the leading strand machinery, resulting in a blunt end.

B. In budding yeast, telomeres are bound by the Ku complex (Yku70-Yku80) and the CST complex (Cdc13-Stn1-Ten1), which cooperate to recruit telomerase to the telomere. The CST complex only binds 3' ssDNA overhangs, while the Ku complex can bind to both blunt-ended telomeres and those with a 3' ssDNA overhang. The work of Faure *et al.* (presented in this issue) and others in the field, supports a model where the MRX/MRN (Mre11-Rad50-Xrs2/Nbs1) complex converts blunt-ended products of leading strand synthesis into 3' overhangs to which the CST complex can bind, in a process essential for telomerase recruitment.

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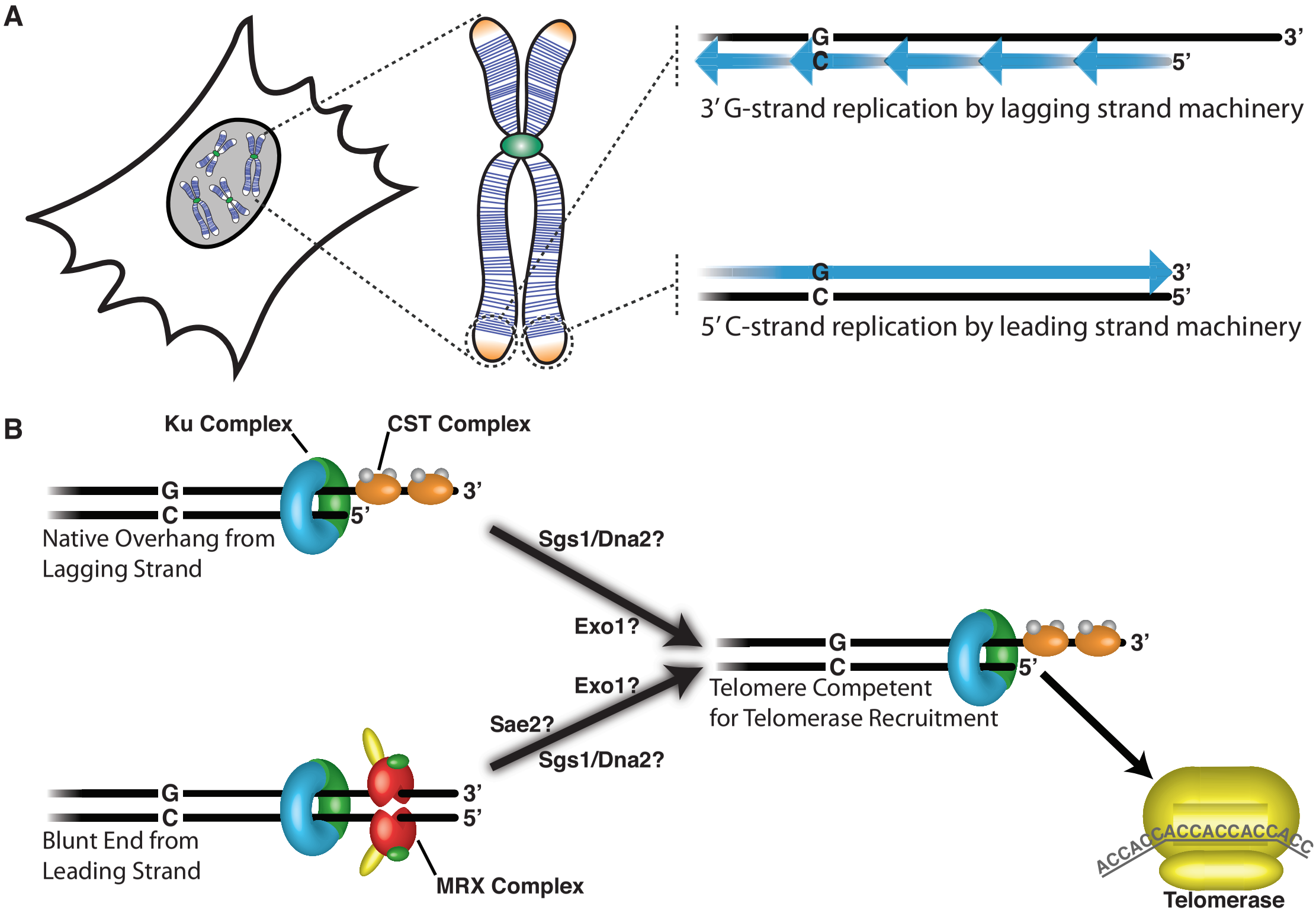
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Native Overhang from
Lagging Strand
Sgs1/Dna2?
Exo1?
Exo1?
Sae2?
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