Antics at the telomere: uncoupled polymerases solve the end replication problem

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Replicating the end of a linear chromosome poses a problem that can be solved by the combined action of the general DNA replication machinery, DNA repair factors, telomere proteins and telomerase. In this issue of *The EMBO Journal,* a new study by Moser *et al* examines the timing of replication, repair and telomere factor association with fission yeast telomeres. The study demonstrates the dynamic nature of protein binding and provides a framework for understanding how leading and lagging strand polymerases, DNA damage signalling and telomere factors cooperate during telomere replication.

Telomere replication is an essential process because incomplete replication leads to telomere shortening and replicative senescence, whereas failure to duplicate the terminal DNA structure leads to loss of the G-strand overhang, chromosome fusions and cell death (Smogorzewska and de Lange, 2004). The basic steps in telomere replication involve duplication of the telomere duplex by the standard replication machinery, generation of a G-overhang by C-strand resection of the telomere replicated by the leading strand polymerase, addition of G-strand repeats by telomerase and fill-in of the complementary C-strand by Pol α/primase (Figure 1A) (Gilson and Geli, 2007). Currently, it is unclear how these steps are integrated, but given the complexity of a replication fork, it is most likely that a large number of factors are needed to link the general replication machinery to the telomere-specific replication machinery. The ATM and ATR DNA damage signalling pathways may be used to monitor and regulate this process (Verdun and Karlseder, 2007; Sabourin and Zakian, 2008).

Past studies with yeast and mammalian cells have established that a wide variety of proteins bind the telomere, but they do not all bind simultaneously. Instead, their association and dissociation seem to be part of a tightly choreographed series of events that are needed to replicate and then protect the chromosome terminus (Verdun and Karlseder, 2006; Chan *et al*, 2008). The current study by Moser *et al* (2009) uses a series of timed chromatin immunoprecipitation (ChIP) analyses to provide our first high-resolution view of these events. The authors performed quantitative ChIP with synchronized cultures of *Schizosaccharomyces pombe* harvested at 20-min intervals during progression through S phase. This provided a detailed picture of the association/disassociation kinetics of replication, repair and telomere factors. To determine whether binding of the various factors depended on DNA replication, hydroxyurea (HU) was used to inhibit the late S-phase replication of telomeres. By way of comparison, the authors also examined the timing and level of association of the various factors at an early firing replication origin (ars2004).

The ChIP analysis indicated that initial replication events are similar at telomeres and ars2004. The general timing of MCMs and Pol ε recruitment was the same and DNA replication, as monitored by BrdU incorporation, initiated at the time of Pol ε recruitment at both loci (Figure 1B). However, recruitment of other replication factors, repair factors and the response to HU treatment were startlingly different. As expected, the leading strand polymerase Pol ε, and the lagging strand polymerases Pol α and δ associated with ars2004 simultaneously and relatively little RPA or Rad 26 (ATRIP) was present during an unperturbed S phase. However, at the telomere, binding of Pol α and δ was delayed by ~20 min relative to Pol ε. Moreover, the amount of telomere-bound RPA and Rad26 increased in conjunction with Pol ε association. Subsequent binding of Pol α and δ coincided with telomerase association and a
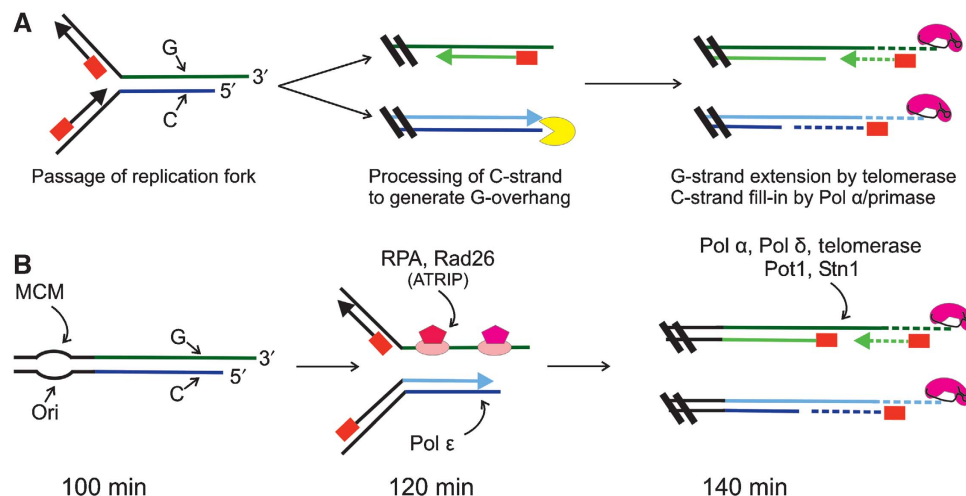
Figure 1 Telomere replication. (**A**) Stages in replication. (**B**) Dynamics of protein association.

decrease in RPA and Rad26. The conclusion that can be drawn from these data is that leading and lagging strand replication of the telomeric tract are temporally separated with leading strand replication occurring first. The remaining template for lagging strand replication is coated by RPA and thus recruits sensors linked to the ATR-mediated DNA damage checkpoint. Subsequent lagging strand replication is then temporally linked to telomerase recruitment.

Analysis of HU-treated cells yielded yet more interesting information. Although the replication block inhibited telomere association of Pol α, δ, ε, Pot1, RPA and Rad26, binding of telomerase was only partially blocked and binding of Nbs1 (a component of the MRN complex) and Stn1 was largely unaffected. Thus, it appears that telomerase may be able to act independently of DNA replication. Perhaps progression into S phase is sufficient to allow binding of the MRN complex and subsequent C-strand processing and/or disruption of the telomeric chromatin allows telomerase access to the DNA terminus. Once G-strand DNA becomes available it appears that Stn1 can outcompete RPA and Rad26 for binding.

As Pot1 binding depends on replication but Stn1 binding does not, one wonders whether the role of Stn1 is to replace RPA on telomeric DNA in a replication-independent manner.

Taken together, these results begin to reveal how eukaryotic cells have harnessed both the general replication and DNA damage response machinery to take care of the end replication problem on a linear chromosome. Inevitably, the replication fork has to disappear at the chromosome terminus but it appears that the cell has devised ways to exploit the resulting separation of leading and lagging strand synthesis to ensure that the telomeric G-strand can be extended by telomerase and the C-strand filled in by the lagging strand replication machinery.

Acknowledgements

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