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Lisby, Michael; Géli, Vincent

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DNA damage response to eroded telomeres

Michael Lisby¹ and Vincent Géli²

¹Department of Biology; University of Copenhagen; Copenhagen N, Denmark; ²CNRS; Unité Propre de Recherche 3081; Genome Instability and Carcinogenesis
Convened by l'Université d'Aix-Marseille 2; Marseille, France

Telomeres are nucleo-protein structures at the ends of linear chromosomes that play important roles in both ageing and cancer biology. Telomeres contribute to maintain genome integrity by protecting linear DNA molecules of chromosomes against degradation and end-to-end fusion. Telomeres also aid in chromosome end replication, which is hampered by the inability of the DNA polymerase to replicate the extreme end of the lagging strand template. In both yeast and human, telomere length can be maintained by reverse transcription catalyzed by the telomerase enzyme. However, in the absence of telomerase, telomeres progressively shorten with each cell cycle until cells enter replicative senescence with critically short telomeres. Current models suggest that telomeres protect chromosome ends by preventing their recognition as DNA double-strand breaks (DSBs) by the DNA repair machinery, but the underlying molecular mechanism is far from being understood (reviewed in ref. 2).

In a recent work, we showed that a single short telomere in yeast Saccharomyces cerevisiae is sufficient to trigger a DNA damage response and to advance replicative senescence. Loss of telomerase is accompanied by an ordered recruitment of the Mre11 protein, the telomerase sequence-specific single-strand binding protein Cdc13, the DNA single-strand binding replication protein A (RPA), the ATRIP-like Ddc2 checkpoint protein, and the Rad52 recombination protein. Furthermore, we found that eroded telomeres while remaining at the nuclear periphery relocalize to the nuclear pore complex.

Single-cell analysis of a single inducible short telomere was accomplished by fluorescently marking a telomere using an array of Lac repressor binding sites placed adjacent to a Flp recombinase-excisable telomere sequence in cells expressing the Lac repressor fused to yellow fluorescent protein (YFP). This analysis showed that a Cdc13 focus consisting of 13–18 molecules forms at the shortest telomere in the cell. Interestingly, a single Cdc13 focus is also observed during the collective shortening of all telomeres in telomerase-negative cells, which is hampered by the inability of the DNA polymerase to replicate the extreme end of the lagging strand template. In both yeast and human, telomere length can be maintained by reverse transcription catalyzed by the telomerase enzyme. However, in the absence of telomerase, telomeres progressively shorten with each cell cycle until cells enter replicative senescence with critically short telomeres. Current models suggest that telomeres protect chromosome ends by preventing their recognition as DNA double-strand breaks (DSBs) by the DNA repair machinery, but the underlying molecular mechanism is far from being understood (reviewed in ref. 2).

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Importantly, the DNA damage response to eroded telomeres occurs many generations before the onset of replicative senescence, which coincides with a block to further telomere shortening. Mre11 and Tel1 (the orthologue of human ATM)
associate with eroded telomeres throughout the cell cycle in pre-senescent cells, while Rad52, Ddc2 and Cdc13 foci reform in every S phase. Remarkably, cell proliferation is largely unaffected by the DNA damage response in pre-senescent cells, indicating that the extent of recruitment of Tel1 and/or Ddc2-Mec1 is not sufficient to mediate cell cycle arrest. Perhaps, replicative senescence takes place only after a threshold of checkpoint signaling from multiple eroded telomeres is reached. Alternatively, checkpoint signaling from telomeres is reduced because the binding to TG1-3 repeats of RPA, which recruits the Ddc2-Mec1 checkpoint kinase to single-stranded DNA, is outcompeted by Cdc13.

It was recently reported that persistent DSBs localize to nuclear pores at the nuclear periphery to enhance repair by gene conversion. We found the same to be true for telomeres that are recognized by the DNA damage response in senescing cells. Although the biological function of the telomere relocalization is unknown, it is tempting to speculate that recombinational lengthening of telomeres is stimulated at the nuclear pore complex.

Figure 1. Recombination at telomeres. (A) Recombinational restart of collapsed replication fork. A collapsed replication fork within the telomeric TG1-3 repeats may lead to fork reversal or a Rad52-dependent template switch to restart replication. (B) Telomere recombination initiates at single-stranded subtelomeric sequences. Single-stranded TG1-3 repeats formed by a burst of resection in S phase are bound preferentially by Cdc13. Upon telomere shortening in telomerase-negative (tlc1Δ) cells, resection has a greater chance of extending into subtelomeric X and Y' sequences, which are bound preferentially by RPA. Subsequently, RPA recruits Rad52 to initiate recombination.

References