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# DNA damage response to eroded telomeres

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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.<sup>1</sup> 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.<sup>3,4</sup> Loss of telomerase is accompanied by an ordered recruitment of the Mre11 protein, the telomere 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 relocate 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 without affecting the organization of telomeres into 4–6 clusters at the nuclear periphery. This finding suggests that during senescence in the absence of telomerase, the shortest telomere or a cluster of telomeres experience a burst of resection allowing increased binding of Cdc13. Indeed, resection could be self-enhancing, because Rap1, which binds double-stranded TG<sub>1-3</sub> repeats and inhibits resection, is displaced from telomeres during resection.<sup>5</sup> Consistent with the DNA damage response elicited by a single telomere, the length of the shortest telomere is found to be the major determinant of the onset of senescence<sup>3,6</sup> and in wild-type cells telomerase acts preferentially on the shortest telomere.<sup>7</sup> Surprisingly, Cdc13 foci were observed even in telomerase-positive cells, albeit at a low frequency. In fact, 20% of spontaneous Rad52 foci colocalize with a Cdc13 focus, suggesting that telomeres are frequently recognized by the DNA damage machinery even in telomerase-positive cells. These foci could represent sites of collapsed telomere DNA replication, resulting in abrupt shortening of a telomere<sup>8</sup> (Fig. 1A). Consistently, Cdc13 foci were observed preferentially during S phase of the cell cycle.

Quantitative analysis of Cdc13, RPA and Rad52 foci in cells with different chromosome end structure demonstrated that resection of telomeres per se is not

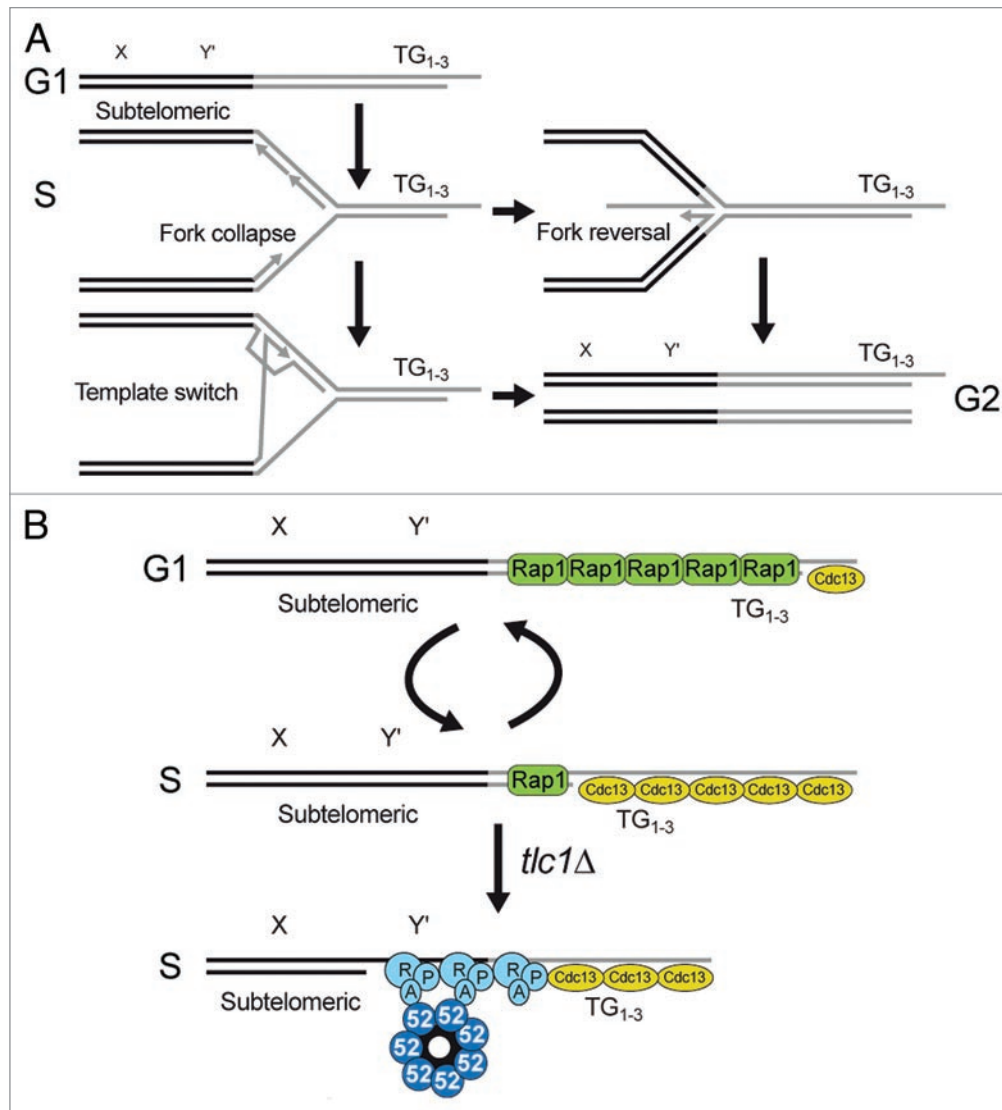
sufficient to trigger recruitment of the recombination machinery. Thus, long telomeres with single-stranded overhangs result in the recruitment of Cdc13 but neither RPA nor Rad52 to foci, while short telomeres with single-stranded overhangs were bound by all three proteins. This scenario contrasts to non-telomeric DSBs, where resection causes the recruitment of RPA and Rad52 but not Cdc13 to initiate homologous recombination. Furthermore, chromatin immunoprecipitation analysis of RPA and Cdc13 demonstrated a preferential binding of Cdc13 at TG<sub>1-3</sub> overhangs, while RPA bound more avidly to subtelomeric regions. These observations led us to propose that the predominant binding of Cdc13 rather than RPA to single-stranded TG<sub>1-3</sub> overhangs prevents the formation of a recombination-proficient RPA filament and subsequent recruitment of the Rad52 recombination protein (Fig. 1B). Only when resection extends into subtelomeric regions are RPA and Rad52 recruited to initiate homologous recombination. This notion is further supported by the observation that formation of Cdc13 foci in S phase precedes formation of Rad52 foci likely because TG<sub>1-3</sub> repeats become single-stranded before subtelomeric sequences during resection. Consequently, the recombinational rescue of telomeres by the ALT-like (alternative lengthening of telomeres (reviewed in ref. 9)) pathway might be limited to short telomeres, where most or all of the TG<sub>1-3</sub> sequences have been lost.

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)

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**Figure 1.** Recombination at telomeres. (A) Recombinational restart of collapsed replication fork. A collapsed replication fork within the telomeric  $TG_{1-3}$  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  $TG_{1-3}$  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.

associate with eroded telomeres throughout the cell cycle in pre-senescent cells,<sup>3,4,10</sup> 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  $TG_{1-3}$  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.<sup>11,12</sup> 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.

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