Nature Reviews Molecular Cell Biology | AOP, published online 15 February 2012; doi:10.1038/nrm3294

TELOMERES

Damage response cut short

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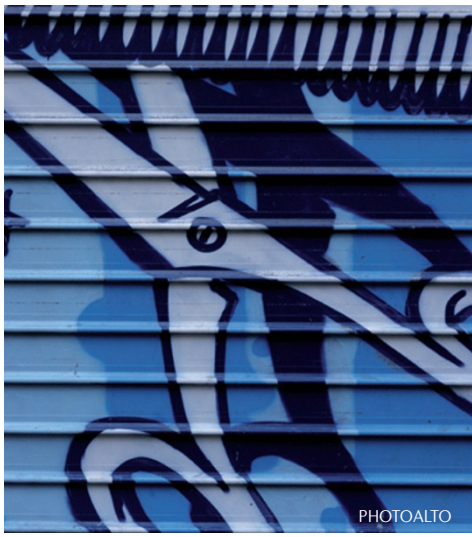
Telomere capping affords protection against checkpoint mechanisms that would normally sense and respond to the presence of DNA double-stranded breaks (DSBs). Ribeyre and Shore now find that the telomere-interacting factors RAP1-interacting factor 1 (Rif1) and Rif2 each use distinct mechanisms to prevent checkpoint activation at short telomeres and that they can exert this effect *in trans* to block a response at neighbouring telomeres.

Telomere capping complexes protect telomeres from resection throughout the cell cycle. Rif1 and Rif2 also inhibit resection of telomeres and alter checkpoint protein binding. However, as they also influence telomere elongation, their direct role in protecting telomeres has been unclear. Ribeyre and Shore therefore set out to address how Rif1 and Rif2 act at *de novo* telomeres, using an established single cell assay in which telomeric sequences of defined length are placed on either side of an induced DSB.

Uncapped telomeres, or telomeres that have become critically short, normally trigger a DNA damage response and cell cycle arrest. By contrast, the authors showed that cells can tolerate the presence of short, elongating telomeres in wild-type cells. Introduction of either long or short tracts of telomeric repeats did not trigger a pronounced cell cycle arrest. However, when either Rif1 or Rif2 was deleted, cells underwent a transient cell cycle arrest specifically in response to short telomere tracts. This suggests that these factors normally prevent cells from initiating a DNA damage response at short, actively elongating telomeres.

Next, they asked how Rif1 and Rif2 might provide protection. DSBs are resected to produce single-stranded DNA (ssDNA) ends that are recognized by damage response factors such as replication protein A (Rpa). Consistent with previous links between Rif2 and DNA resection, the authors showed that Rif2 mutation increased the presence of ssDNA at DSBs. Chromatin immunoprecipitation (ChIP) analysis showed that Rif1, by contrast, seemed to prevent checkpoint activation by directly preventing the association of damage response factors, including Rpa, rather than through strong inhibition of DNA resection. Rif1 and Rif2 mutants also showed increased recruitment of Rad24 to DSBs, which is required for activation of the checkpoint kinase Mec 1 (mitosis entry checkpoint protein 1). Thus, the authors concluded that Rif1 and Rif2 prevent checkpoint activation at short telomeric ends through independent and additive mechanisms.

It has previously been proposed that telomeric tracts might elicit an 'anti-checkpoint' function in response to adjacent uncapped telomeres. To test this, the authors modified their construct to place a short tract on one side of a DSB together with a long tract on the other side; in this case, no pronounced cell cycle delay was observed when Rif1 and/or Rif2 were lost. This delay did not seem to be affected either by the number of short tracts present or the position of the short tract, ruling out dosage effects or checkpoint suppression through DNA degradation. Thus, they concluded that telomeres can indeed induce an anti-checkpoint effect on a nearby uncapped or short telomere.



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How might the checkpoint be switched off? Using ChIP analysis, the authors looked at which factors associate with the uncapped DNA end at the point when the checkpoint has been suppressed. They found that suppression correlated with decreased binding of ssDNA-binding proteins that are important for the damage response. Thus, telomere tracts seem to suppress recognition of nearby uncapped DNA ends.

The authors predict that this anti-checkpoint role of Rif1 and Rif2 is coupled to their effects on telomere elongation, and that feedback between these two processes allows robust regulation of telomere stability. It will be interesting to see how these are coordinated to ensure checkpoint activation only when necessary in a physiological context.

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ORIGINAL RESEARCH PAPER Ribeyre, C. & Shore, D. Anticheckpoint pathways at telomeres in yeast. *Nature Struct. Mol. Biol.* 12 Feb 2012 (doi:10.1038/nsmb.2225)