Nature Reviews Molecular Cell Biology | AOP, published online 15 june 2011; doi:10.1038/nrm3142

DNA REPLICATION

Pif1 overcomes a quadruplex hurdle

G-quadruplex (G4) structures, formed by non-canonical intramolecular G•G base pairs, are predicted to be prevalent in the genome. Their stable nature suggests that they might pose problems for DNA *in vivo* and, indeed, Zakian and colleagues have found that, in *Saccharomyces cerevisiae*, G4 structures slow down replication forks and that this is counteracted by the Pif1 helicase.

Pif1 stands out among the helicases that can resolve G4 structures *in vitro* for its potent unwinding activity. *In vivo*, Pif1 is known to have several roles, including telomere maintenance and DNA replication. So, Zakian and colleagues set out to address whether the ability of Pif1 to unwind G4 structures might be relevant to these functions *in vivo*. Using chromatin immunoprecipitation, they showed that a substantial proportion of Pif1-binding sites overlap with G4 motifs — sequences that have the potential to form G4 structures *in vivo*. In synchronized cells, the association of Pif1 and DNA polymerase ε (also known as Pol II) with G4 motifs was found to peak shortly after replication.

This led the authors to ask whether Pif1 may be important for surveying the genome prior to mitosis for potential obstacles, such as G4 structures. They saw that, in Pif1-deficient cells, replication forks progressed more slowly in the regions where G4 motifs were present; this was demonstrated by analysing both DNA polymerase ε occupancy and replication intermediates on two-dimensional gels. Moreover, they showed that the presence of G4 motifs between DNA repeats resulted in increased recombination in Pif1-deficient cells, and this correlated with the formation of DNA double-stranded breaks. So, one possibility is that the pausing of replication forks that occurs when G4 structures accumulate in the absence of Pif1 promotes DNA breakage and subsequent recombination events.

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If G4 motifs do disrupt the normal progression of replication forks and increase DNA breaks, their accumulation might be expected to reduce cell growth when cells experience replication stress. The authors tested this by increasing the copy number of G4 motifs and then examining what happens when cells experience replication stress in hydroxyurea. They found that this stress led to reduced cell growth in Pif1-mutant cells and also correlated with spontaneous mutation at the G4 motifs and subsequent rescue of replication progression and DNA breakage.

The authors propose that a key role of Pif1 is to ensure that G4 structures are resolved so that DNA replication can progress normally. However, given that Pif1 associates with only a subset of G4 motifs, it is likely that it shares this responsibility with other helicases. It will therefore be important to see how these may act in concert to overcome such structural obstacles in the genome.

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ORIGINAL RESEARCH PAPER Paeschke, K., Capra, J. A. & Zakian, V. A. DNA replication through G-quadruplex motifs is promoted by the *Saccharomyces cerevisiae* Pif1 DNA helicase. *Cell* **145**, 678–691 (2011)