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G4 DNA: at risk in the genome

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Regions bearing G-quadruplex (G4) DNA motifs can be sites of genomic instability and are frequently depleted in streamlined genomes, but are nevertheless maintained in many other genomes. Whether G4 secondary structures form *in vivo*, and how they are maintained or eliminated, remains little known. In this issue of *The EMBO Journal*, Lopes *et al* (2011) provide new evidence that G4 structures form and contribute to genomic instability in living cells, and identify specific mechanisms that minimize the risks posed by G4 motifs.

G4 DNA structures can form within regions bearing at least four runs of guanines, with at least three guanines per run ($G_{\geqslant 3}N_xG_{\geqslant 3}N_xG_{\geqslant 3}N_xG_{\geqslant 3}$). In the human genome, the rDNA, telomeres, and immunoglobulin switch regions are rich in G4 sequence motifs, as are some highly unstable minisatellites and single copy genes. Evidence that G4 structures can form within these regions has been provided by single molecule imaging, immunofluorescence microscopy, and genetic and biochemical characterization of factors that bind and unwind G4 DNA (reviewed by Maizels, 2006). Conservation of both position and structural potential, though not necessarily of sequence, suggests that G4 structures participate in key cellular processes. G4 structures at specific sites are intracellular targets of regulatory processes, including control of pilin antigen variation in *Neisseria gonorrhoeae* (Cahoon and Seifert, 2009), and p53 mRNA 3′-end formation in stressed cells (Decorsiere *et al*, 2011).

G4 structures have considerable thermal stability and could block progression of both DNA and RNA polymerases if not resolved. Cellular helicases that actively unwind G4 DNA may facilitate polymerase progression. RecQ family helicases (human BLM and WRN, *Escherichia coli* RecQ) share a conserved RQC domain that promotes G4 recognition, and unwind with 3′–5′ directionality. Werner syndrome, a human genetic disease characterized by premature ageing, is caused by WRN helicase deficiency, and cells lacking WRN exhibit sequence loss at telomeres at which the G-rich strand is the template for lagging strand replication (Crabbe *et al*, 2004). FANCJ-related helicases (human FANCJ, nematode dog-1) are DEAH/X superfamily II helicases, and unwind with 5′–3′ directionality. In nematodes deficient in dog-1, long DNA deletions with the 3′-end defined by G4 motifs occur (Kruisselbrink *et al*, 2008), and patient cells deficient in FANCJ echo this phenotype.

The new paper by Nicolas and collaborators (Lopes *et al*, 2011) combines genetic, chemical, and physical approaches to show that, in *Saccharomyces cerevisiae*, G4 DNA formed on the template for leading strand replication is normally unwound by Pif1, a 5′–3′ helicase related to *E. coli* RecD. These experiments take advantage of an elegant *S. cerevisiae*-based reporter assay, developed previously by the Nicolas laboratory, that quantifies the frequency of size variants of the human CEB1 minisatellite. The CEB1 repeat, which is rich in G4 motifs and highly polymorphic in human populations,

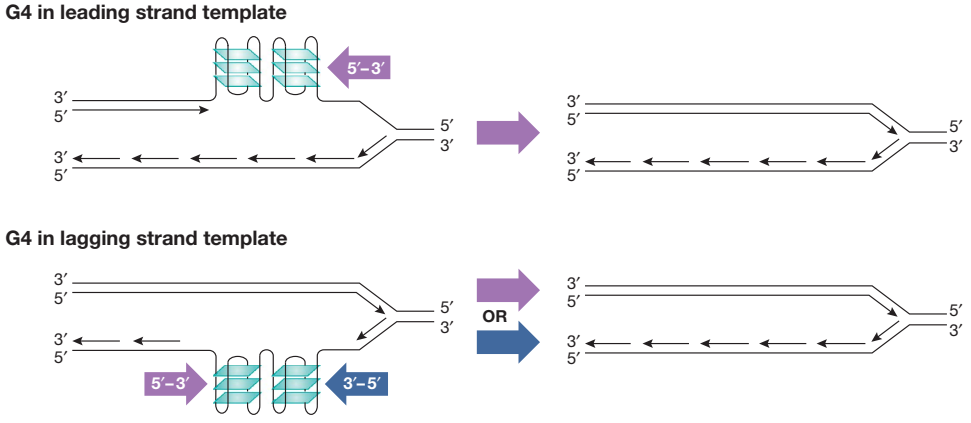

Figure 1 Maintenance of G4 motifs on leading or lagging strand templates. Replication can be blocked at G4 structures formed within regions denatured during replication of a leading or lagging strand template. A 5′–3′ helicase, such as Pif1, can load at the single-stranded region adjacent to a G4 structure on a leading strand template, whereas newly formed duplex DNA will prevent loading of a 3′–5′ helicase on that strand. In contrast, a helicase of either directionality could load and then successfully resolve a G4 structure on the lagging strand.

was inserted in both orientations near a well-defined replication origin (ARS305) in the *S. cerevisiae* genome. Strikingly, Pif1 was required for the stability of the repeats in only one orientation, with G4 motifs as the template for leading strand replication. Figure 1 depicts a simple model for Pif1 function in this context. A G4 structure on a leading strand template could halt replication but not duplex unwinding in advance of the replication apparatus. According to this model, Pif1 may normally bind 5′ of the G4 structure and unwind 5′–3′ to remove the G4 structure, allowing leading strand synthesis to resume. In the absence of Pif1, secondary structures would persist. Using two-dimensional gel electrophoresis, Nicolas and collaborators found Rad51/Rad52-dependent repair to generate characteristic recombination intermediates at such forks, resulting in deletions at CEB1 minisatellites.

The question of whether G4 structures form *in vivo* has been a polarizing one. The paper by Nicolas and collaborators brings two kinds of evidence to bear on this point. The role of Pif1 in CEB1 stability does not uniquely implicate a G4 structure, as Pif1 also unwinds a variety of other structures. While a mutant derivative of CEB1, lacking potential to form G4 structures, was shown to be immune to destabilization, a skeptic could still argue that Pif1 recognition of a key motif in duplex DNA had been eliminated by this sequence change. However, the second line of evidence is more difficult to rationalize on the basis of alternative mechanisms not involving G4 structure formation: treatment of wild-type cells with Phen-DC3, a selective and high-affinity G4 ligand (Monchaud *et al*, 2008), also promoted genomic instability at wild-type, but not at mutant, CEB1 sequences. Phen-DC3 would be predicted to stabilize G4 structures on either strand, making them more resistant to resolution. Consistent with this, Phen-DC3 treatment of wild-type cells phenocopied (albeit less dramatically) the leading strand-specific instability seen in *pif1*Δ cells.

A recent genome-wide analysis of Pif1 function and distribution in *S. cerevisiae* (Paeschke *et al*, 2011) did not identify strand specificity of instability caused by the lack of Pif1, in contrast to the report by Nicolas and collaborators. In that study, ChIP-Seq analysis showed Pif1 (the Pif1-K264A ATPase mutant derivative) to be enriched at endogenous G4 motifs. However, there was no exclusivity in that relationship. Most G4 motifs (75%) were not associated with Pif1-K264A, and most Pif1-K264A (89%) associations were in regions lacking G4 motifs. The ability of Pif1 to act on a variety of structures could explain its broad genomic distribution.

The results on leading strand specificity reported in this issue of *The EMBO Journal* are especially intriguing because typically the lagging strand template is more problematic than the leading strand. This is thought to reflect the fact that, during replication, the lagging strand is disproportionately exposed as gapped, single-stranded DNA, which confers a propensity to form structures that can impede accurate replication. We speculate that, paradoxically, this same propensity may protect the lagging strand from instability due to G4 structure formation. An adjacent single-stranded region is usually required to load helicases that unwind structured regions of DNA, and this may limit opportunities for resolving structures on the leading strand. As shown in Figure 1, after passage of the replication fork, a lagging strand template bearing a G4 structure contains both 5′ and 3′ single-stranded regions, which would in principle enable a helicase of either directionality to load and resolve the adjacent structure. In contrast, only a 5′–3′ helicase may be able to load adjacent to a G4 structure on a leading strand template.

Taken together, the results reported by Nicolas and collaborators provide compelling evidence for G4 structure formation in living cells and a model for how failure to resolve G4 structures can lead to recombinational repair in *S. cerevisiae*. Do these results have broader implications for stability of G4 motifs in more complex eukaryotes? The details of helicase participation in the maintenance of G4 motifs may vary, reflecting the many different helicases with overlapping and/or redundant functions that are active in different cells. Nonetheless, the mechanistic model for repair at G4 structures that emerges from this research provides an invaluable focus for future analyses of how cells minimize the risks posed by genomic G4 motifs.

Conflict of interest

The authors declare that they have no conflict of interest.

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