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DNA REPLICATION

Quad-jumping

DNA G-quadruplexes (G4) are structures formed at guanine-rich sequences, with the capacity to impede DNA replication. Factors such as the helicase Fanconi anaemia group J (FANCJ) and the polymerase REV1 facilitate their replication, thus maintaining genomic stability. Schiavone *et al.* now report that the primase–polymerase PrimPol is another factor responsible for facilitating replication through G4 structures.

As PrimPol is required for replicative bypassing of ultraviolet-induced, DNA-distorting lesions, the authors examined its capacity to bypass G4 structures in chicken DT40 cells. The *BU-1A* locus contains a G4, which was previously shown to stall replication in cells lacking FANCJ or REV1. The stalling led to local uncoupling of DNA synthesis from histone recycling and erasure of epigenetic marks, thereby resulting in stochastic loss of Bu-1a protein expression. When investigating Bu-1a expression in PrimPol-knockout cells, the authors found three populations with discrete expression levels: Bu-1a^{high} (wild-type levels), Bu-1a^{medium} and Bu-1a^{low} (essentially nil). Bu-1a^{medium} and Bu-1a^{low} cells arise spontaneously, sequentially and irreversibly from Bu-1a^{high} cells, through stochastic loss of active epigenetic marks followed by heterochromatinization. Importantly, deletion and reintroduction of the G4 on both alleles confirmed that it was essential and sufficient for Bu-1a expression instability, and that only G4 structures present in the template of the leading strand cause gene silencing.

> “the primase–polymerase PrimPol is another factor responsible for facilitating replication through G4 structures”

Additional experiments *in vitro* established that PrimPol is unable to replicate through G4 structures, so the authors examined its capacity to reprime (restart) replication distal to the structures. They found that PrimPol synthesized ~6-nt-long primers almost immediately downstream of the G4. *In vivo*, both the PrimPol catalytic domain and its carboxy-terminal domains, which bind to single-stranded DNA (ssDNA), as well as the ssDNA-binding protein RPA (replication protein A), were needed for efficient G4 replication.

Thus, blocking leading-strand replication by a G4 results in ssDNA formation downstream of the structure. The authors propose that PrimPol is recruited to these sites of replication stalling by binding to the G4, RPA and the ssDNA itself, to reprime DNA synthesis close to the structure. This ‘close-coupled’ repriming limits the size of the ssDNA gap generated. How the G4 left within this gap is subsequently replicated remains to be elucidated.

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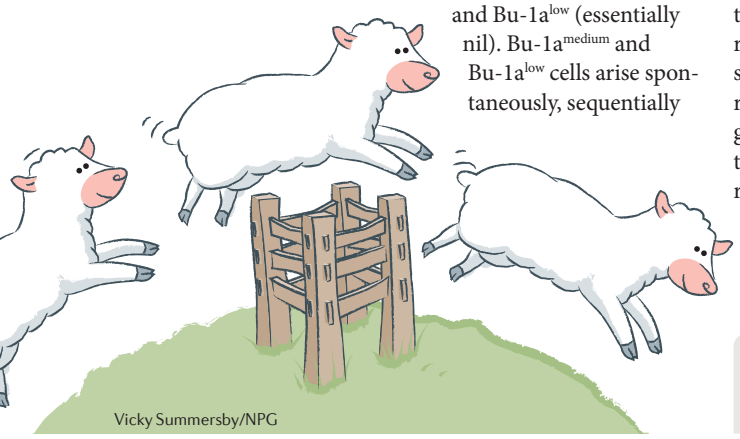

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ORIGINAL ARTICLE Schiavone, D. *et al.* PrimPol is required for replicative tolerance of G quadruplexes in vertebrate cells. *Mol. Cell* http://dx.doi.org/10.1016/j.molcel.2015.10.038 (2015)