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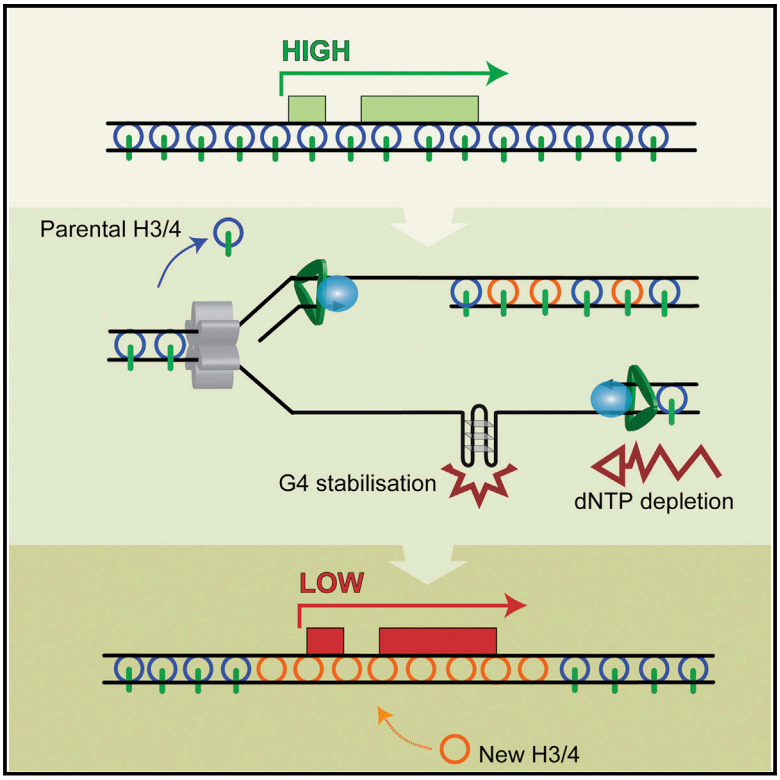
Cell Reports

Nucleotide Pool Depletion Induces G-Quadruplex-Dependent Perturbation of Gene Expression

Graphical Abstract



Authors

Charikleia Papadopoulou, Guillaume Guilbaud, Davide Schiavone, Julian E. Sale

Correspondence

guilbaud@mrc-lmb.cam.ac.uk (G.G.), jes@mrc-lmb.cam.ac.uk (J.E.S.)

In Brief

Slowing replication forks by depleting nucleotide pools enhances the ability of G quadruplexes to stochastically perturb gene expression during replication. Papadopoulou et al. find that a common global replication stressor interacts with local DNA secondary structures to cause epigenetic instability.

Highlights

- Hydroxyurea (HU) stochastically perturbs gene expression
- G quadruplex (G4) formation potentiates HU-induced epigenetic changes
- HU induces G4-dependent DNA damage and heterochromatin formation
- HU and G4 helicase mutations cause similar changes in expression

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Nucleotide Pool Depletion Induces G-Quadruplex-Dependent Perturbation of Gene Expression

Charikleia Papadopoulou,[1] Guillaume Guilbaud,[1,*] Davide Schiavone,[1] and Julian E. Sale[1,*]
[1]Medical Research Council Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK
*Correspondence: guilbaud@mrc-lmb.cam.ac.uk (G.G.), jes@mrc-lmb.cam.ac.uk (J.E.S.)
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SUMMARY

Nucleotide pool imbalance has been proposed to drive genetic instability in cancer. Here, we show that slowing replication forks by depleting nucleotide pools with hydroxyurea (HU) can also give rise to both transient and permanent epigenetic instability of a reporter locus, *BU-1*, in DT40 cells. HU induces stochastic formation of Bu-1^{low} variants in dividing cells, which have lost the H3K4me3 present in untreated cells. This instability is potentiated by an intragenic G quadruplex, which also promotes local H2Ax phosphorylation and transient heterochromatinization. Genome-wide, gene expression changes induced by HU significantly overlap with those resulting from loss of the G4-helicases FANCJ, WRN, and BLM. Thus, the effects of global replication stress induced by nucleotide pool depletion can be focused by local replication impediments caused by G quadruplex formation to induce epigenetic instability and changes in gene expression, a mechanism that may contribute to selectable transcriptional changes in cancer.

INTRODUCTION

The term replication stress describes the slowing or stalling of replication forks by endogenously or exogenously derived impediments to DNA polymerization (Zeman and Cimprich, 2014). Replication stressors can be local factors, such as DNA damage or secondary structures that affect forks randomly as they are encountered, or global ones, such as nucleotide pool depletion or imbalance that simultaneously slows all forks (Poli et al., 2012; Anglana et al., 2003). It is now recognized that replication stress induced by nucleotide pool imbalance is an important consequence of the activation of some oncogenes, which drive cells into S phase without upregulation of nucleotide supply (Bester et al., 2011). The resulting loss of polymerase processivity is thought to lead to localized uncoupling of the replicative helicase and polymerase and formation of tracts of single-stranded DNA (Byun et al., 2005; Pacek and Walter, 2004). While this normally induces checkpoint activation and senescence (Bartkova et al., 2006; Di Micco et al., 2006), in cells that can bypass the checkpoint, such replication stress provides a fertile source of genetic instability, particularly in the vicinity of fragile sites and sites capable of forming secondary structures (De and Michor, 2011; Tsantoulis et al., 2008).

In addition to the extensive genetic changes that have been well documented in many types of cancer, there are also extensive local and global alterations in histone and DNA modifications. The consequent changes in chromatin structure are accompanied by significant dysregulation of gene expression (Timp and Feinberg, 2013; Berdasco and Esteller, 2010), which, since it is not accompanied by changes in the DNA sequence, may be considered epigenetic (Berger et al., 2009). These epigenetic changes could act alongside genetic instability to produce clonal variation within a tumor, upon which selective pressure can act, and so may contribute to tumor evolution. Mutations in histone and DNA-modifying enzymes, and even histone proteins themselves, have been found in several cancers and are likely to explain at least some of the observed epigenetic instability (Timp and Feinberg, 2013). However, it is not clear that mutations in histone-modifying enzymes account for all the alterations observed in different cancer types.

We recently provided evidence that deficiencies in enzymes responsible for replicating G quadruplex (G4) structures, such as the specialized DNA polymerase REV1 and helicases FANCJ, WRN, and BLM, can lead to localized changes in histone modifications and gene expression (Sarkies et al., 2010, 2012; Schiavone et al., 2014). G4s can form within motifs comprising four short runs of dG bases, separated by linker sequences. The dG bases in the motif form planar Hoogsteen-bonded quartet structures that can stack on top of each other, resulting in an often highly thermodynamically stable secondary structure, the G4 (reviewed in Maizels and Gray, 2013). We proposed that persistent replication fork stalling at G4s in mutants such as *rev1* or *fancj* leads to pathologically long daughter strand gap formation, resulting in local uncoupling of DNA synthesis from parental histone recycling. This, in turn, leads to loss of the histone modifications present on the parental chromatin, which, if in the vicinity of a gene promoter, results in changes in transcription (Sarkies et al., 2010, 2012; Schiavone et al., 2014). A prediction of this model is that global replication stressors that lead to loss of processive DNA polymerization with uncoupling of the replicative helicase and polymerase also should promote epigenetic instability by dissociating DNA synthesis from histone recycling.