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Conflicts with transcription make early replication late

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By sequencing sites of mitotic DNA synthesis in cells lacking homologous recombination, Groelly et al. (2022) and Bhowmick et al. (2022) show how conflicts between transcription and replication in early S phase can cause under-replicated DNA to persist into mitosis.

Genomic instability is a potential driving force in cancer development, and DNA replication stress has been implicated in contributing to genomic instability in tumour cells. Replication stress can result from oncogene activation or loss of tumour suppressors. In this issue of Molecular Cell, two reports by Groelly, Bhowmick and colleagues shed new light on the genomic features and molecular mechanisms of replication stress in cells lacking the tumour suppressor pathway homologous recombination (HR) (Bhowmick et al., 2022; Groelly et al., 2022).

Replication stress occurs when replication forks slow down, stall or even speed up. This can lead to subsequent problems during mitosis, which exacerbate genomic instability. Common fragile sites (CFS) are difficult-to-replicate, late-replicating genomic regions that are sensitive to mild replication stress (Debatisse et al., 2012). The high frequency of genomic rearrangements at CFS in cancer samples provides some of the strongest evidence for the importance of replication stress in cancer (Dereli-Oz et al., 2011). Under conditions of mild replication inhibition, CFS enter mitosis before completing replication. This results in mitotic DNA synthesis (MiDAS), a specialised break-induced replication pathway using RAD52 that helps prevent genomic instability (Minocherhomji et al., 2015). The phenomenon of MiDAS further emphasises the connection between late-replicating genomic regions and the mitotic effects of replication stress. However, early-replicating genomic regions can also be especially susceptible to damage, for example under high levels of replication stress (Barlow et al., 2013).

The new studies by Groelly and Bhowmick use cutting-edge sequencing approaches to provide evidence that early replicating genomic regions can be hotspots of replication stress phenotypes manifesting in mitosis. They exploit MiDAS to understand how replication stress contributes to genomic instability in cells lacking critical HR proteins. HR, catalysed by the recombinase RAD51, can rescue and repair stalled replication forks (Wassing and Esashi, 2021). Several HR factors also prevent and resolve conflicts between transcription and replication (TRCs). The tumour suppressor BRCA2, involved in RAD51 loading onto DNA, can counteract the accumulation of replication-blocking RNA-DNA hybrids called R-loops (Bhatia et al., 2014). Groelly et al. use human H1299 non-small cell lung carcinoma cells shRNA-depleted for BRCA2, while Bhowmick et al. siRNA-deplete RAD51 in human U2OS osteosarcoma cells.

Both studies use previously established cell synchronisation and EdU-labelling protocols to measure DNA synthesis during mitosis in cells that have experienced replication stress in the preceding S phase, either due to low-dose Aphidicolin treatment or BRCA2 or RAD51 depletion (Figure 1). They then employ a newly developed method, MiDAS-seq, where the EdU-labelled genomic areas are isolated and sequenced for high resolution mapping (Macheret et al., 2020). Intelligent combination with existing datasets allows comparison of the genomic features of MiDAS sites in HR-deficient cells and canonical MiDAS sites, which are induced by Aphidicolin.

The most important finding is that MiDAS regions in HR-deficient cells, which the authors call “atypical” MiDAS sites (Bhowmick et al., 2022), are vastly different from the canonical MiDAS sites. They are in early replicating regions that are also transcribed in early S phase, and are characterised by open chromatin, high density of replication origins, the presence of short- to average-length genes and increased R-loop formation. These features, combined with known roles of HR factors, point towards TRCs as a potential cause of MiDAS. Indeed, both studies provide evidence for such conflicts. Groelly et al. use existing datasets from DNA-RNA immunoprecipitation sequencing (DRIP-seq) to support that atypical MiDAS events map to regions of R-loop formation, with the caveat that the datasets were obtained in different cell lines, and rescue MiDAS in BRCA2-deficient cells with RNase H1 overexpression. Bhowmick et al. quantify both nascent and steady-state transcription levels by combining the high throughput sequencing approaches SLAM-seq and QUANT-seq. This reveals that genes up-regulated in early S-phase are over-represented among MiDAS sites in HR-deficient cells. Both studies show that these MiDAS events require transcription in early S phase. Other lines of evidence include increased co-localisation of replication and transcription factors in early S phase combined with transcription-dependent RAD51 recruitment in S-phase. Therefore, the data suggest that both BRCA2 and RAD51 are required to prevent conflicts between transcription and replication at early replicating genomic regions.

CFS are origin-poor and have been associated with a lack of backup replication origins as well as TRCs (Debatisse et al., 2012). The present studies agree with previous suggestions that TRCs are the main source of stress in early replicating genomic regions (Barlow et al., 2013).

New questions arise from this work. Why do HR-deficient cells display in no canonical MiDAS at CFS, even after Aphidicolin treatment (Bhowmick et al., 2022)? The authors suggest that in absence of RAD51, the late-replicating nature of CFS may allow for collapsed replication forks to be rescued by RAD52-dependent break-induced replication in G2 (Figure 1). Groelly et al. show that transcription and replication are predominantly co-directional at MiDAS sites in HR-deficient cells, and that the replication stress in these cells involves ATM signalling and 53BP1 foci formation, consistent with DNA double-strand break (DSB) formation at co-directional TRCs. Interestingly, DSB formation and RAD51-independent, RAD52-mediated fork restart have been proposed to occur at TRCs involving R-loops

(Chappidi et al., 2020). Similar mechanisms could occur, possibly at a higher rate, in BRCA2- and RAD51-deficient cells, with replication timing affecting whether this leads to MiDAS (Figure 1). In addition, Bhowmick et al. report that RAD51 recruitment in early S phase can be induced by overexpressing oncogenes MYC and Cyclin E. The authors speculate that "atypical" MiDAS sites in early replicating regions may be prone to amplification in cancer, and both studies provide evidence for such amplifications in the genomes of BRCA2-mutated breast cancers. These findings suggest new directions for investigating the mechanisms around TRCs at early replicating regions, and the role of HR in preventing and resolving these conflicts.

To conclude, in cells lacking tumour suppressors, unresolved TRCs in early replicating regions can persist through S phase and into mitosis, resulting in MiDAS. The findings draw exciting new connections between transcription, replication, DNA repair and the cell cycle, with potential implications for genomic instability in cancer.

Figure legend
A: Summary of the experimental approach. Cells are synchronised and EdU labelled, followed by next generation sequencing of EdU-containing DNA. Hydroxyurea (HU) and Aphidicolin treatment protocols differ between the studies.
B: In the canonical MiDAS model, insufficient time to resolve replication stress, such as transcription-replication conflicts (TRCs), in late-replicating regions leads to DNA synthesis in mitosis. In the new model for MiDAS in homologous recombination (HR)-deficient cells, unresolved TRCs in early S phase persist and lead to mitotic DNA synthesis, while stress in late-replicating regions might be resolved by RAD52-dependent repair (dotted lines).

Declaration of interests
The author declares no competing interests.

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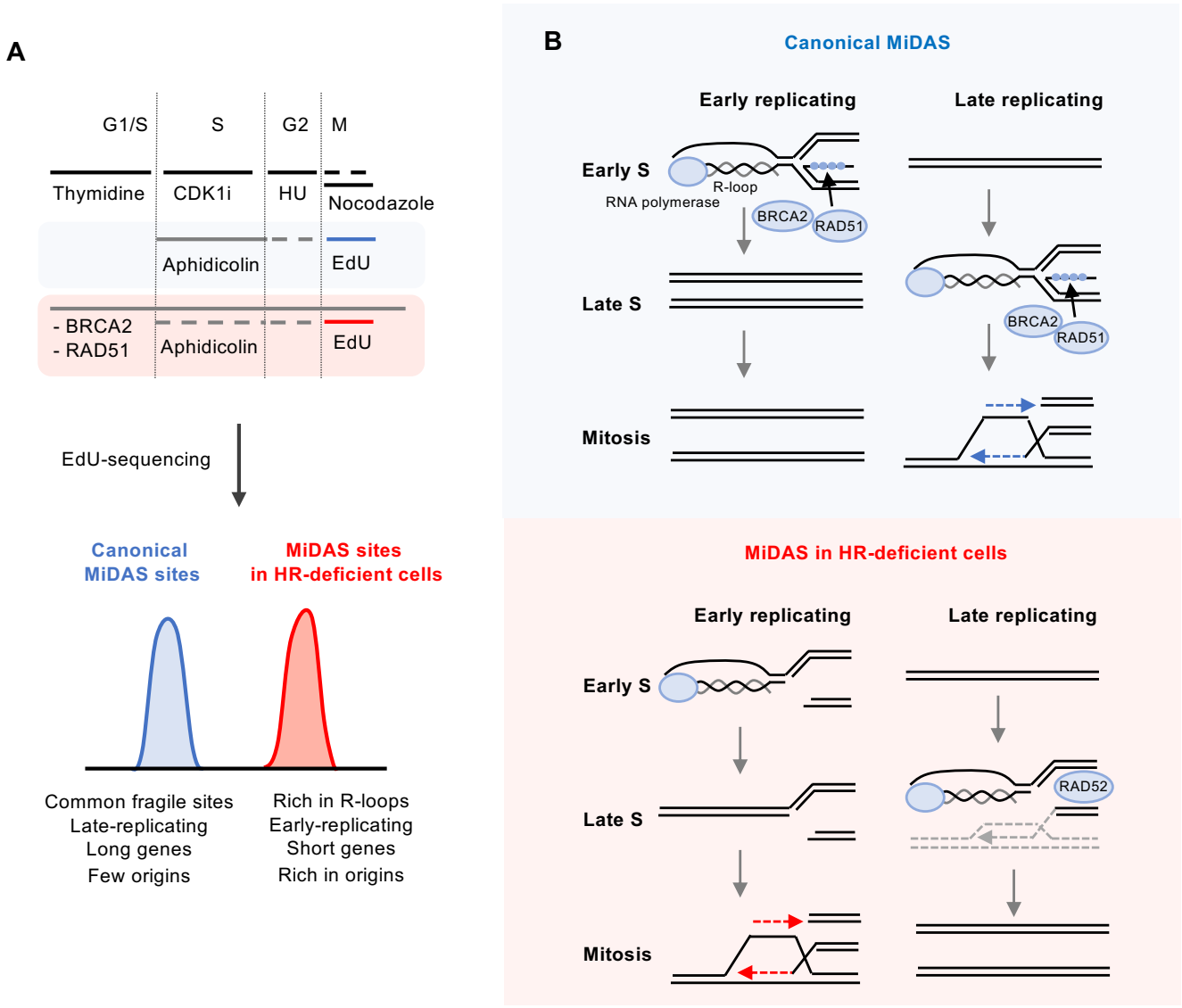
A
G1/S
S
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Thymidine
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HU
Nocodazole
Aphidicolin
EdU
- BRCA2
- RAD51
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EdU
EdU-sequencing
Canonical MiDAS sites
MiDAS sites in HR-deficient cells
Common fragile sites
Late-replicating
Long genes
Few origins
Rich in R-loops
Early-replicating
Short genes
Rich in origins
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Canonical MiDAS
Early replicating
Late replicating
Early S
R-loop
RNA polymerase
BRCA2
RAD51
Late S
BRCA2
RAD51
Mitosis
MiDAS in HR-deficient cells
Early replicating
Late replicating
Early S
Late S
RAD52
Mitosis

Figure 1