Nature Reviews Molecular Cell Biology | Published online 23 Dec 2015; doi:10.1038/nrm.2015.24

DNA REPAIR

The cell cycle flavours of repair

> Both studies highlight the complexity of cell cycle-dependent control of DNA repair factors

Untimely DNA damage repair can be deleterious, for example, when homologous recombination (HR) is activated during the G1 phase of the cell cycle or if DNA breaks are repaired during mitosis. Recently published in *Nature*, Orthwein *et al.* describe a mechanism that supresses HR during G1, and Minocherhomji *et al.* reveal how mitotic DNA replication prevents instability at genomic regions known as common fragile sites (CFSs).

HR is tightly suppressed in G1 owing to the lack of sister chromatids that can serve as accurate repair templates. This is mediated by p53-binding protein 1 (53BP1), which prevents the recruitment of the BRCA1–PALB2 (partner and localizer of BRCA2)–BRCA2 complex and thus of other HR factors to double-strand breaks (DSBs) in G1. Orthwein *et al.* found that PALB2 and BRCA2 were not recruited to DSBs in G1 even in human cells not expressing 53BP1, owing to a lack of BRCA1–PALB2 association. A region in PALB2 that mediates its interaction with kelch-like ECH-associated protein 1 (KEAP1) was identified as being responsible for the BRCA1–PALB2 interaction. KEAP1 is a substrate adaptor (recruiter) for the E3 ubiquitin ligase CRL3 (cullin 3 (CUL3)–RING ubiquitin ligase), and PALB2 ubiquitylation by CRL3 at Lys20, Lys25 and Lys30 prevented its interaction with BRCA1. In cells lacking KEAP1 or KEAP1–CUL3 association, stable BRCA1–PALB2–BRCA2 complexes were detected at DSBs also in G1.

nobeastsofierce/iStock

As neither CRL3–KEAP1 activity nor its interaction with PALB2 is cell cycle regulated, the authors examined whether the KEAP1-, PALB2- and BRCA2-interacting deubiquitylase USP11, which is a HR factor of unknown function, is cell cycle regulated. USP11 was necessary for the formation of stable BRCA1–PALB2–BRCA2 complexes, but following the induction of DSBs in G1, it was ubiquitylated and degraded by the proteasome. Thus, the cell cycle-regulated expression of USP11 controls recruitment by BRCA1 of HR factors to DSBs.

CFSs are rearrangement-prone genomic regions that are intrinsically difficult to replicate. Following replicative stress, CFSs are prone to 'expression' as gaps or constrictions in metaphase chromosomes, and this is promoted by the MUS81–EME1 structure-specific endonuclease. Minocherhomji *et al.* showed that replicative stress can also induce DNA synthesis in early mitosis. Such mitotic DNA synthesis was also dependent on MUS81 and EME1, as well as on their scaffold protein SLX4, and approximately 80% of synthesis co-localized with expressed CFSs. Furthermore, SLX4 depletion resulted in reduced CFS expression and an increase in chromosome non-disjunction during anaphase. Chromosome non-disjunction was also observed following mitosis-specific replication arrest.

DNA polymerase δ-subunit 3 (POLD3) was also required for mitotic DNA synthesis at CFSs, CFS expression and suppression of chromosome non-disjunction; conversely, its depletion led to an increase in DNA breaks on metaphase chromosomes. Importantly, MUS81 depletion reduced POLD3 recruitment to chromatin in S phase as well as in prometaphase. Thus, the authors propose that replication forks stalled at CFSs during S phase are cleaved in early mitosis by SLX4–MUS81–EME1 to promote POLD3-dependent DNA synthesis. This process, which manifests as CFS expression when adjacent regions begin to condense, suppresses the formation of DNA breaks and chromosomal rearrangements.

Both studies highlight the complexity of cell cycle-dependent control of DNA repair factors. In G1, suppression of HR is revealed to be multi-layered and more tightly controlled than previously appreciated. In mitosis, DNA repair is a salvage mechanism to prevent chromosome missegregation.

Eytan Zlotorynski

ORIGINAL ARTICLES Minocherhomji, S. *et al.* Replication stress activates DNA repair synthesis in mitosis. *Nature* **528**, 286–290 (2015) | Orthwein, A. *et al.* A mechanism for the suppression of homologous recombination in G1 cells. *Nature* http://dx.doi.org/10.1038/nature16142 (2015)