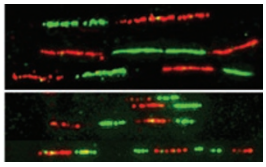
Replication speed bumps



The ring-shaped cohesin complex links sister chromatids and functions as a structural barrier for the transcriptional machinery at insulators and boundary elements. As a result, cohesin frequently accumulates in certain regions of the genome, and all replication forks must pass through one or more cohesin-associated regions during S phase. Sister chromatid cohesion requires the replication factor C (RFC)–CTF18 clamp loader (RFC^{CTF18}) and the acetylation of cohesin subunit SMC3 by acetyltransferases ESCO1 or ESCO2. Using single-molecule DNA synthesis assays, Jallepalli and colleagues now show that RFC^{CTF18} regulates the speed, spacing and restart activity of replication forks in human cells. They also demonstrate that RFC^{CTF18} deficiency is accompanied by reduced SMC3 acetylation. Moreover, cells from individuals with Roberts syndrome (RBS), who carry mutations in the *ESCO2* gene, or cells expressing an SMC3 mutant that cannot be acetylated, showed replication fork slowing and DNA damage accumulation. This suggests that cohesin acetylation is required for processive DNA replication, but how? Non-acetylatable SMC3 copurifies with increased amounts of the regulatory cofactors WAPL and PDS5A compared to the wild type, and the interaction of these cofactors with cohesin is negatively correlated with SMC3 acetylation levels. Furthermore, RNA interference–mediated depletion of WAPL or PDS5A rescues fork progression in cells lacking ESCO1, ESCO2 or RFC^{CTF18}. Altogether, these findings suggest that non-acetylated cohesin obstructs the replication fork, whereas acetylation of cohesin causes dissociation of WAPL and PDS5A, enabling the fork to advance. Whether the widespread slowing of the replication fork in cells from individuals with RBS contributes to the disease phenotype remains a question for the future. (*Nature* **462**, 231–234, 2009) *AH*

FActories in repair

The human cancer predisposition syndrome Fanconi anemia (FA) results from mutations in a pathway involved in repair of DNA interstrand cross-links (ICLs). Thirteen FA complementation groups (corresponding to 13 *FANC* genes) have been identified. Eight FANC proteins form a core complex that has ubiquitin E3 ligase activity. Two other proteins, FANCD2 and FANCI, form a complex that is monoubiquitinated by the core complex. The mechanistic relationship between these proteins and ICL repair has not been fully defined. To provide such insight, Walter and colleagues have used the *Xenopus laevis* cell-free extract system. When a plasmid containing a cisplatin cross-link is incubated with the extract, the lesion is repaired in a replication-dependent manner. The authors first confirmed that recombinant frog FANCD2 and FANCI interact in the extract, are monoubiquitinated, and bind chromatin in a replication- and DNA-damage-dependent manner. Mutation of the monoubiquitination site in FANCD2 prevented chromatin binding. When FANCD2 was depleted from the extract, repair of the lesion was significantly reduced, although the damage-signaling kinase ATR was still functional in checkpoint activation. Further experiments defined the molecular activity in ICL repair that was defective in the FANCD2-depleted extracts. Although the replication forks remained able to approach the lesion normally, a reduction in repair extension products was observed. Depletion of polymerase ζ, which promotes synthesis after insertion of the nucleotide across from the damaged template, likewise affects the production of extension products. However, with FANCD2 depletion, the nucleolytic incision events that precede insertion do not occur. From these data, the conclusion is that monoubiquitinated FANCD2–FANCI promotes the incision and/or base insertion steps in ICL repair. (*Science* published online, doi:10.1126/science.1182372, 12 November 2009) *AKE*

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Switched on

Cells respond to viral nucleic acid by producing type I interferon as part of the innate immune response. Cytosolic viral RNAs are recognized by RIG-I–like receptors, which activate the mitochondrial antiviral signaling protein (MAVS). This protein, in turn, activates downstream transcription factors such as IRF3. Ubiquitination is known to regulate this pathway, but its exact role was not known. Zeng *et al.* used a cell-free system to examine how mitochondria taken from virus-infected cells activate IRF3. By fractionating the cytosolic extract, they identified Ubc5 as the ubiquitin-conjugating enzyme required for IRF3 activation, and by using RNA interference, they demonstrated that the catalytic activity of Ubc5c is essential to this process. As previous studies had indicated that cells lacking NEMO do not activate the IRF3 response, the authors used their cell-free system to show that both of NEMO's ubiquitin-binding domains are required. Although ubiquitination was thought to be involved in the pathway, no genetic evidence for a role of Lys63 polyubiquitination was available until now. Zeng *et al.* replaced endogenous ubiquitin with the K63R mutant and found that this abrogated IRF3 activation. Thus, Lys63 polyubiquitination involving Ubc5 is essential for IRF3 activation. (*Mol. Cell* **36**, 315–325, 2009) *MH*

Histones gone wild!

Sepsis is an acute systemic inflammatory response triggered by infection; severe cases can result in multiple organ dysfunction and failure, septic shock and death. The use of recombinant activated protein C (APC) to treat severe sepsis has been approved by the FDA. Protein C is a plasma serine protease with anticoagulant effects, but its anti-inflammatory effects are not fully understood, with different substrates implicated so far in protection against sepsis. Now Esmon and colleagues identify a novel target for APC during sepsis: extracellular histones. The authors found that the cytotoxicity of conditioned medium from activated macrophages was reduced by APC treatment, and by comparing the treated and untreated media, they observed fragments of histones H2A, H3 and H4, formed by APC. The authors then directly showed that purified histones are cytotoxic to human endothelial cells, and that APC can indeed cleave H3 and H4 *in vitro*. The toxicity of histones was further demonstrated in mice: the animals died 1 hour after being injected with histones; co-injection with APC prevented lethality. Infusion of anti-H4 antibody also protected mice from the septic response induced by a high dose of lipopolysaccharide (LPS). Finally, the authors challenged baboons (a primate model for sepsis) with a lethal dose of bacteria, and observed up to 15 μg ml^{-1} histone H3 in the plasma after 8 hours; treatment of the animals with APC protected them from sepsis and led to the presence of cleaved H3 in their plasma. Analyses of plasma samples from human sepsis patients yielded similar observations. How extracellular histones exert their cytotoxic effects remains to be determined, but this work clearly points to new ways of treating sepsis and other inflammatory conditions. (*Nat. Med.* **15**, 1318–1321, 2009) *IC*