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CHROMATIN

A fork load

In a recent study, Peter Adams and colleagues showed that repression of histone synthesis causes S-phase arrest. Adams' group now reports, in *Molecular Cell*, that the assembly of nucleosomes onto newly synthesized DNA near the replication fork — rather than histone synthesis *per se* — is tightly coupled to DNA synthesis in human cells.

Adams and co-workers showed that a dominant-negative mutant of the p150CAF-I subunit of the heterotrimeric chromatin-assembly factor CAF-I inhibits CAF-I-dependent chromatin assembly in an *in vitro* assay. The mutant protein (called HA-p150C) disrupts the endogenous CAF-I complex *in vivo* by preventing the binding of wild-type p150CAF-I to the p60CAF-I subunit. As a result, p60CAF-I can no longer associate with chromatin at replication foci.

To investigate what happens at the site of DNA synthesis, the replication fork, Adams and co-workers transfected cells with a plasmid encoding HA-p150C, and found that DNA synthesis and progression through S phase were blocked. Co-expression of the wild-type p150CAF-I subunit abolished cell-cycle arrest, confirming that perturbation of CAF-I inhibits DNA synthesis. As expected, the cells that were arrested in S phase had an abnormal chromatin structure that was hypersensitive to micrococcal nuclease digestion (that is, the DNA is more accessible to the nuclease,

because the chromatin structure is perturbed).

S-phase arrest can be caused by DNA damage. So was this the case for defective chromatin assembly-induced arrest? As it turned out, yes — Adams and colleagues found that HA-p150C-expressing cells contained fragmented DNA, and that histone H2AX was phosphorylated, presumably, in response to double-strand breaks.

What causes the inhibition of DNA synthesis and S-phase arrest? The S-phase checkpoint protects the cell's genome integrity during S phase, and is activated in response to DNA damage. So, Adams and colleagues reckoned that inhibition of chromatin assembly might activate the S-phase checkpoint, which is indeed what happens.

H2AX is a substrate for ATR and/or ATM, both S-phase checkpoint kinases, and the authors showed that ATR nuclear foci, the sites of active ATR, are present preferentially in HA-p150C-expressing cells arrested in S phase. Other substrates of ATR and ATM — p53 and BRCA1 — were also activated. Phosphorylation of p53 was induced, and dispersed BRCA1 foci, which are typical of activated BRCA1, were enriched in HA-p150C-expressing cells that contained ATR foci.

The authors propose that "...defects in S-phase chromatin assembly cause double-strand breaks due to stalling and inappropriate processing of replication forks". This indicates that defects in chromatin assembly, combined with inactivation of the S-phase checkpoint, might promote genome instability, and Adams and colleagues

are now testing whether genes encoding chromatin-assembly factors might be mutated in human cancers.

Arianne Heinrichs

References and links

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