In This Issue

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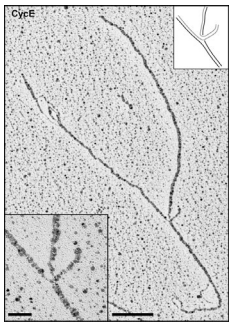
Oncogenes induce a reversal of replication's fortunes



An electron micrograph shows a reversed replication fork induced by Cyclin E overexpression.

Neelsen et al. describe how certain oncogenes can perturb DNA replication and induce DNA damage.

Several oncogenes, including Cyclin E and Cdc25A, push cells precipitously into S phase, resulting in numerous DNA double-strand breaks (DSBs). But how S phase deregulation disrupts replication and induces DNA damage is unclear.

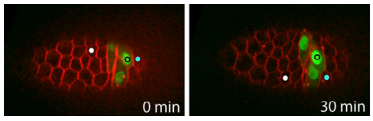
To find out, Neelsen et al. overexpressed either Cyclin E or Cdc25A in human cells. Within hours, both oncogenes slowed the progression of replication forks, inducing the formation of unusual replication intermediates called "reversed forks," in which newly synthesized DNA strands anneal to each other instead of their parental strands. Reversed forks are presumably caused by topological stress, which might arise because the oncogenes deregulate replication initiation and thus increase interference with transcription.

Though Cyclin E and Cdc25A caused similar replication defects, the two oncogenes induced DNA damage at different rates. Cyclin E–expressing cells took several cell cycles to accumulate significant numbers of DSBs because they could transiently delay mitotic entry and resolve most unusual replication intermediates. Cdc25A-expressing cells, on the other hand, are defective in the DNA damage checkpoint and therefore enter mitosis prematurely. These cells rapidly accumulated DSBs due, in part, to the activation of a mitotic nuclease called MUS81, which appears to target unresolved reversed forks, perhaps so that sister chromatids can be separated.

DSBs therefore do not arise directly from oncogene-induced replication defects but instead result from nuclease-mediated processing during mitosis. The DNA damage checkpoint limits this processing by allowing cells to fix their replication problems before mitotic entry. Because MUS81 depletion only partially suppresses DSB formation, Neelsen et al. now want to investigate other factors that may contribute to oncogene-induced replication stress and genotoxicity.

Neelsen, K.J., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201212058.

Misshapen orders an egg roll



Over 30 minutes, white and blue dots track the downward movement of wild-type follicle cells past a group of immobile cells (green) that lack Msn.

A protein kinase shapes *Drosophila* egg chambers by promoting cell migration, Lewellyn et al. reveal.

Fly egg chambers contain an inner core of germ cells surrounded by a layer of epithelial follicle cells and an overlying basement membrane. During development, the follicular epithelium polarizes and migrates perpendicular to the anterior–posterior axis of the egg chamber so that the entire chamber rotates within the basement membrane. This movement appears to organize the basement membrane into fibrils that encircle the egg chamber, forming a corset that may help the spherical egg chamber elongate.

The follicular epithelium fails to polarize in the absence of a protein kinase called Misshapen (Msn), resulting in abnormally rounded eggs. Lewellyn et al. found that individual follicle cells lacking Msn were immotile. Neighboring wild-type cells continued to migrate, and, if enough cells maintained Msn expression, the epithelium as a whole could move and rotate the egg chamber.

Depleting Msn increased the amount of integrin adhesion receptors at the basal surface of follicle cells, keeping them stuck to the basement membrane and unable to organize it into fibrils. Partially reducing integrin levels rescued follicle cell motility and basement membrane organization. Lowering integrin levels also restored the overall planar polarity of the follicular epithelium, suggesting that the tissue's polarization arises from the motility of the individual follicle cells. Senior author Sally Horne-Badovinac now wants to investigate how the follicle cells collectively choose their migration direction and to determine how Msn regulates integrin levels.

Lewellyn, L., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201209129.

CUL4B keeps replication firing on all cylinders

Zou et al. describe how a protein linked to mental retardation promotes DNA replication.

Mutations in CUL4B, a member of the cullin family of ubiquitin ligase scaffold proteins, are associated with mental retardation and other developmental defects. Human cells lacking CUL4B progress slowly through S phase, though how DNA replication is impaired in these cells is unknown.

Zou et al. found that depleting CUL4B decreased the ability of replication origins to "fire" and initiate DNA synthesis. Cells lacking CUL4B had reduced amounts of nuclear CDC6, a protein that licenses DNA replication by promoting the assembly of prereplication complexes at origin sites. Overexpressing CDC6 restored origin firing to normal rates.

CDC6 levels were lowered in CUL4B-deficient cells by the ubiquitin ligase APC^{CDH1}. The cyclin-dependent kinase CDK2 can phosphorylate and protect CDC6 from APC^{CDH1}-mediated degradation, but CDK2 expression was reduced in the absence of CUL4B, increasing CDC6's turnover. Zou et al. found that CUL4B up-regulates CDK2 by inhibiting the production of two microRNAs, miR-372 and miR-373, that target the kinase. In CUL4B's absence, the two microRNAs were up-regulated, leading to a decrease in CDC6 levels and replication origin firing.

How CUL4B represses miR-372 and miR-373 remains unclear. Because CUL4B-containing ubiquitin ligases interact with the histone-modifying complex PRC2, the authors speculate that the microRNAs may be regulated by histone methylation.

Zou, Y., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201206065.