In This Issue

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Fewer forks make faster progress

Just as a large number of forks can inhibit someone unaccustomed to formal dining, an excess of replication forks can slow the rate of DNA synthesis, Zhong et al. reveal.

Cdc7 is the catalytic subunit of the Dbf4-dependent kinase, which initiates DNA replication by activating the DNA helicase complex MCM at replication origins. In response to DNA damage, the checkpoint protein Rad53 limits origin firing by inhibiting Cdc7. Rad53 also slows the progression of existing replication forks, but whether Cdc7 influences fork dynamics is unclear.

Zhong et al. found that, although *cdc7* mutant yeast fired fewer origins than wild-type cells, their replication forks proceeded faster than normal from the origins that did initiate DNA synthesis, even in the presence of the DNA-damaging agent MMS. *orc1* mutant yeast, which fail to recruit MCM to replication origins, also showed faster fork progression at the handful of origins that managed to fire, indicating that reduced levels of origin firing increase replication fork speed. *mec1* mutants, on the other hand, initiated synthesis at more origins than wild-type cells and consequently showed decreased rates of replication fork progression.

Because there are fewer replication forks in yeast lacking Cdc7 or Orc1, MMS only weakly activated the DNA damage checkpoint and Rad53 in these cells, suggesting that reduced checkpoint activation is one reason these cells show rapid fork dynamics. But *mec1* mutants have slow replication forks even though they lack the DNA damage checkpoint. This suggests that the amount of origin firing influences fork progression in other ways, too, possibly because individual forks must compete for limited amounts of essential replication factors or nucleotides.

Zhong, Y., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201208060.

Bub3's function doesn't stop at the checkpoint

GFP-labeled cells lacking Bub3 and expressing the apoptosis inhibitor p35 quickly fill the abdomen of an adult fly.

Morais da Silva et al. show that the checkpoint protein Bub3 suppresses tumorigenesis independently of its role in chromosome segregation.

The spindle assembly checkpoint prevents cells from entering anaphase until all their chromosomes are correctly attached to the mitotic spindle, thereby avoiding chromosome missegregation and aneuploidy. Aneuploidy is widely thought to drive tumorigenesis, but whether the loss of checkpoint proteins contributes to tumor progression is unclear.

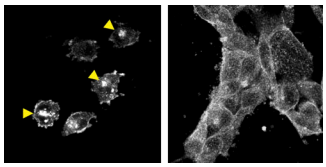
Morais da Silva et al. knocked down the checkpoint protein Bub3 in the developing wings of *Drosophila* larvae. Cells lacking Bub3 missegregated their chromosomes and died, but when the researchers overexpressed an anti-apoptotic protein to keep them alive, the cells hyperproliferated and formed tumors when transplanted into the abdomens of adult flies.

Though the Bub3 and apoptosis-deficient cells were aneuploid, missegregated chromosomes weren't the cause of these tumors. When Morais da Silva et al. induced aneuploidy by knocking down CENP-E—a kinesin motor that helps attach spindle microtubules to kinetochores—*Drosophila* wing cells didn't hyperproliferate even when apoptosis was inhibited. In addition, when the researchers blocked apoptosis and knocked down the kinetochore protein Nsl1, Bub3 was no longer recruited to kinetochores, and the spindle checkpoint was impaired. Yet these cells didn't form tumors unless the cytoplasmic pool of Bub3 was also depleted.

This suggests that, rather than suppressing tumorigenesis via the spindle checkpoint, Bub3 has an additional function that limits cell proliferation. The authors now want to determine what this extra function might be.

Morais da Silva, S., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201210018.

EphA2 cleavage helps tumor cells go it alone



Cells expressing an easily cleaved version of EphA2 (white) invade collagen as single, rounded cells (left), whereas cells expressing a cleavage-resistant mutant invade as a group (right).

A matrix metalloproteinase promotes the invasion of individual cancer cells by cleaving the receptor tyrosine kinase EphA2, Sugiyama et al. show.

Tumor cells can invade surrounding tissues as a group or as single cells that adopt either a rounded, amoeboid shape or an elongated, "mesenchymal" morphology. The EphA2 receptor promotes cancer cell invasion, though how it does this is unclear because tumors with high EphA2 levels often show reduced expression of the corresponding ligand, ephrinA1. EphA2 up-regulates MT1-MMP, a membrane-bound protease that promotes collective and mesenchymal invasion by degrading the extracellular matrix. Sugiyama et al. found that MT1-MMP then binds and cleaves EphA2 in certain breast cancer cell lines, boosting their ability to invade as single, rounded cells.

MT1-MMP cleaved EphA2 at the cell surface, triggering the receptor's internalization. Once inside the cell, EphA2 activated the small GTPase RhoA to enhance cellular contractility and repulsion, prompting cells to round up and detach from their neighbors. Cells expressing a cleavage-resistant form of EphA2 tended to invade as a group, whereas a cleavage-sensitive mutant promoted the dissemination of single cells from tumors formed in mice.

EphA2 and MT1-MMP were often coexpressed in human tumor samples, suggesting that this pathway may operate in vivo to determine how cancer cells invade their surroundings. Senior author Kaisa Lehti now wants to investigate why and how intracellular EphA2 activates RhoA and rounded, single-cell invasion, whereas uncleaved, cell-surface EphA2 stimulates the invasive growth of more coherent cell colonies.

Sugiyama, N., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201205176.