In This Issue

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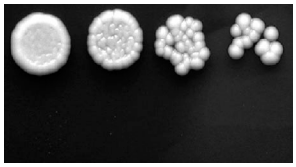
Double-checking DNA synthesis



Yeast cells lacking the replication and spindle assembly checkpoints are able to form colonies (top row) unless S phase is delayed (bottom row).

The replication and spindle assembly checkpoints work together to prevent premature mitosis, Magiera et al. report.

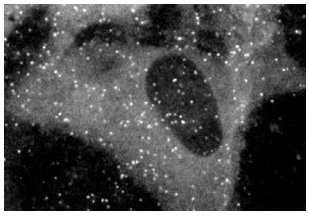
Cells need to copy their DNA before they can separate their chromosomes. The DNA replication checkpoint monitors the progress of the first step, and the spindle assembly checkpoint (SAC) regulates the second. Still uncertain is how cells dovetail the end of replication with the beginning of mitosis. If researchers disrupt DNA synthesis, the replication checkpoint kicks in and halts the cell cycle. Whether the checkpoint forestalls mitosis under normal circumstances remains unclear, however.

To find out, Magiera et al. turned yeast cells into procrastinators, deleting two cyclins to delay the beginning of S phase by 30 minutes. These cells are still copying their DNA when they should be entering mitosis, and the researchers found that this alteration postponed anaphase by 17 minutes.

Mec1, the yeast equivalent of ATR kinase, is a key protein for the replication checkpoint, and the team discovered that removing it from cells lacking the cyclins shortened the anaphase delay. Removing Mad2, a crucial component of the SAC, also allowed the cyclin-deficient yeast to begin mitosis slightly sooner. But deleting Mec1 and Mad2 caused cells to accumulate DNA damage, lose chromosomes, and die. The team's findings indicate that the replication checkpoint and the SAC are active at low levels during S phase, protecting the cell's genome by delaying mitosis until replication is complete.

Magiera, M.M., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201306023.

NEMO surfaces in NF-κB–activating clusters



Shortly after stimulation with IL-1, a cell sparkles with NEMO-containing molecular clusters (dots).

Tarantino et al. identify molecular clusters that help cells switch on the transcription factor NF-κB.

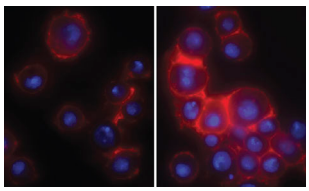
NF-κB controls processes as diverse as immune responses, cell growth, and apoptosis. Cells normally keep NF-κB under wraps in the cytoplasm, but the IKK complex removes this inhibition, enabling NF-κB to enter the nucleus. The on–off switch for the IKK complex is the NEMO subunit. NEMO activation depends on its binding to two types of ubiquitin chains: K63 chains, in which ubiquitin molecules link through their lysine at position 63; and linear chains, in which the ubiquitins link through their N-terminal methionine. Recent results suggest that the activation of NEMO and NF-κB by different inflammation-promoting cytokines requires different patterns of ubiquitin chain formation.

Tarantino et al. tested this possibility by tracking NEMO's movements in cells stimulated by IL-1 or TNF. Both cytokines spurred NEMO subunits to join large clumps of molecules at the cell membrane. These structures were rich in active IKK complexes, suggesting that their formation helps switch on IKK.

The researchers uncovered several differences between the IKK-containing structures triggered by the two cytokines. The clusters induced by TNF cozy up to the TNF receptor, but the structures spurred by IL-1 don't associate with the IL-1 receptor. Formation of IL-1–induced structures required K63 and linear ubiquitination, whereas TNF-induced clusters appeared in the absence of either type of ubiquitination. The results suggest that directing NEMO into large—but distinct—clusters enables cells to activate NF-κB in response to TNF and IL-1. Disassembly of the structures, possibly by removal of ubiquitins, might enable cells to quickly shut off NF-κB's activity.

Tarantino, N., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201307172.

The sources of cell stickiness



***Drosophila* S2 cells are normally nonadherent (left), but they become sticky when engineered to express DE-cadherin (right).**

By knocking down 14,000 *Drosophila* genes, Toret et al. uncover 17 control circuits that orchestrate cell–cell adhesion.

Cadherin proteins are the central component of the adherens junctions that connect epithelial cells to one another. Because the junctions are crucial for multicellular organisms, enumerating all of the proteins that organize them has been difficult.

To get around this problem, Toret et al. used *Drosophila* S2 cells, which are descended from macrophage-like cells and don't normally form adherens junctions or produce DE-cadherin, the fruit fly version of E-cadherin. The researchers engineered these cells to manufacture DE-cadherin, spurring the cells to adhere to one another. To find out which proteins were essential for these linkages, the team knocked down each *Drosophila* gene one at a time and gauged the cells' stickiness.

The researchers pinpointed 378 proteins whose loss dramatically reduced cells' ability to attach to their neighbors. By comparing these proteins to their human equivalents and tracing their interactions, Toret et al. teased out 17 networks that help control the formation and function of adherens junctions. One of the networks includes proteins that partner with cadherin, such as β-catenin, and another contains proteins that control actin dynamics. To their surprise, the researchers also fingered proteins that promote cell migration, such as elmo and cyfip. One of the largest protein hubs comprised nuclear proteins, some of which alter gene expression.

The researchers confirmed the function of many of these proteins in *Drosophila* oocytes and in mammalian epithelial cells, indicating that these different cellular pathways have conserved roles in regulating intercellular adhesion.

Toret, C.P., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201306082.