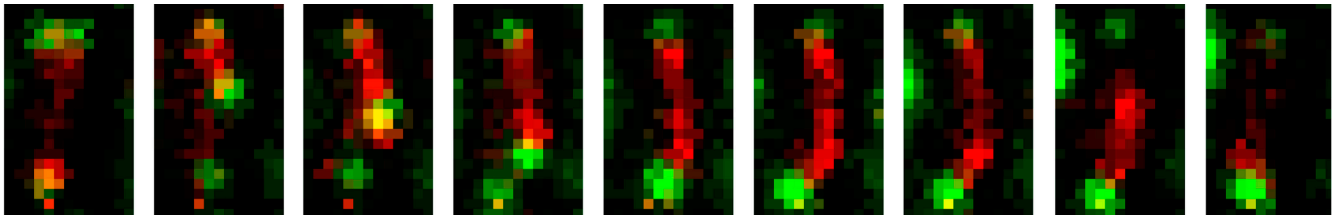
In This Issue



Plasmids (green) are pushed (left to right) around a bacterium by polymerizing filaments of ParM (red).

ParM pushes plasmids apart

A bacterial protein that looks like actin but acts like microtubules makes a Sisyphean effort to push plasmids apart, as revealed in videos by Campbell and Mullins.

The prokaryotic actin look-alike is ParM, which forms a filament that, like microtubules, is dynamically unstable. ParM is encoded by plasmid operons that also contain centromeric sequences and the gene for a DNA-binding protein that hooks plasmids to ParM. To watch ParM in action, the authors imaged bacteria containing a low-copy plasmid that is segregated to daughter cells. The videos unveiled a sloppy, dynamic segregation machinery.

When ParM protein was present, plasmids were pushed around much faster than by diffusion. In cells that had two plasmid copies, these erratic movements occasionally brought plasmids close enough together for a bundle of ParM filaments to link the two. Filaments then elongated, thereby pushing the plasmids to opposite ends of the bacterium.

Once plasmids reached the poles, the filaments rapidly collapsed, perhaps triggered by the force of the plasmids' contact on the cell membrane. The cycle then repeated: plasmids were again nudged along by new ParM filaments, found each other, and were pushed apart. This cyclic behavior continued until the cell divided, usually landing one plasmid in each daughter.

The group studied plasmid separation because it's easy to spy on in vivo, but plasmids might have pilfered the system from bacterial chromosomes. Mullins says that this DNA separation system is good enough for bacteria, whose large numbers can withstand occasional errors. It's 100-fold more efficient than no system and probably requires less energy than do high fidelity eukaryotic segregation systems. And the need for only two proteins, as opposed to the dozens eukaryotes use, helps keep the genome compact. JCB

Reference: Campbell, C.S., and R.D. Mullins. 2007. *J. Cell Biol.* 179:1059–1066.

Apoptotic inaccessibility with maturity

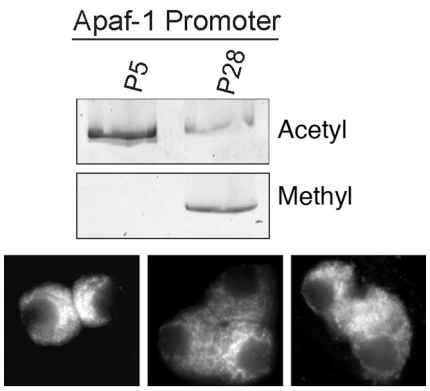
Mature neurons wrap up chromatin around death-sensitizing genes to prevent unwanted apoptosis, say Wright et al.

Neurons die off by the handful during development, when their proliferating brethren can easily replace them. But once neurons mature, they shut off proliferation pathways, and survival becomes precious. One way older neurons become less sensitive to apoptosis is by preventing a protein called Bax from reaching mitochondria, where it pokes holes that let out cytochrome *c* (cyt *c*). The new findings identify an additional protective step downstream of cyt *c* release.

Older sympathetic neurons survived injections of cyt *c* because they had less Apaf-1, a protein that recruits cyt *c* to the death-inducing apoptosome complex. Apoptosis, however, still killed mature neurons in response to DNA damage, the authors found. This death resulted from the return of Apaf-1.

In young neurons, *Apaf-1* expression is induced by a cell cycle–regulated transcription factor called E2F1. When neurons stop proliferating, E2F1 is shut down along with other cell cycle proteins. DNA damage restored E2F production. But the team found that giving cells E2F1 alone was not enough to force older, undamaged neurons to express *Apaf-1*. The cells also had to be prodded to open up the chromatin around the *Apaf-1* promoter.

In mature neurons, the histones at the *Apaf-1* promoter were decorated with methyl groups that signify inaccessible, silent chromatin. The same promoter in young neurons was instead tagged with acetyl groups, which indicate accessibility. When mature neurons were given both E2F1 and drugs that open chromatin by inhibiting histone deacetylases, they again made Apaf-1 and underwent apoptosis in response to cyt *c*. Since similar drugs are used in chemotherapy, oncologists might want to keep an eye out for neuronal side effects.



***Apaf-1* is acetylated and open in young neurons (P5) but methylated and silent in mature neurons (P28). Its derepression allows older neurons to apoptose (bottom, left to right) upon release of cyt *c* (white).**

It is currently unclear how DNA-damaged neurons initiate pathways that unwrap chromatin around *Apaf-1* (and possibly other apoptotic genes). And whether the same thing happens under pathological conditions such as a stroke or neurodegenerative disorders is unknown. JCB

Reference: Wright, K.M., et al. 2007. *J. Cell Biol.* 179:825–832.

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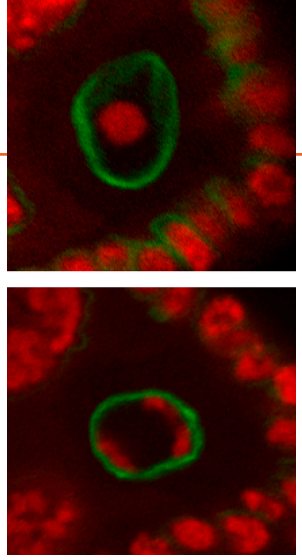
Chromosomes leave envelope for karyosome



Oocyte DNA (red) forms a compact karyosome before meiosis (top). In flies that have a mutant NHK-1, the DNA remains near the nuclear envelope (bottom).

In the large volume of an oocyte, chromosomes huddle together before the meiotic spindle forms. Fashioning this huddled mass—called a karyosome—requires that chromosomes first be released from the nuclear envelope, according to results from Lancaster et al.

Because oocytes lack centrosomes, they assemble spindle microtubules from chromatin. By clustering chromosomes together, karyosomes help make sure that only one spindle is generated. Karyosomes don't form in fly oocytes that lack a kinase called NHK-1. In the new work, the authors found that chromosomes in *nhk-1* mutant cells made widespread contacts with the nuclear envelope, whereas in normal cells, the karyosome formed away from the envelope.

The group found that an NHK-1 substrate from fly cells was an envelope-associated protein called BAF. In their model, BAF hooks chromatin to the nuclear envelope during DNA recombination. But phosphorylated BAF unfastened the connection and freed chromosomes to crowd together. Expression of a BAF mutant that could not be phosphorylated maintained a link between the DNA and an inner nuclear envelope protein called Otefin.

The group is now determining whether NHK1, which is itself phosphorylated late in meiosis, might be activated by cell cycle–regulated kinases. The mammalian versions of BAF and NHK-1 are needed for structural changes in the nuclear envelope during mitosis, but no one has yet looked at their role in meiosis. JCB

Reference: Lancaster, O.M., et al. 2007. *J. Cell Biol.* 179:817–824.

Slow-moving Alzheimer's

Traffic delays might do more than make you late to work. Findings from Kim et al. now suggest that slowed trafficking of the γ-secretase protease might cause Alzheimer's disease.

γ-Secretase is a multisubunit complex that includes presenilin. Mutations in presenilin are associated with inherited forms of Alzheimer's. Scientists do not yet understand how these varied mutations—which can fall at multiple points along the protein—all lead to the misprocessing of the amyloid precursor protein to create an aggregation-prone form of Aβ.

Presenilin is assembled with the rest of the γ-secretase complex within the secretory pathway. In the new report, the team found that trafficking through at least part of this pathway—out of the ER and into COPII vesicles—was slowed by presenilin mutations that cause Alzheimer's. Packaging of the mutants into COPII vesicles was reduced in in vitro ER budding assays.

Exit from the ER is often delayed by protein misfolding, which is a problem that affects many mutant versions of presenilin. Helping the sluggish mutants fold by treatment with a chemical chaperone restored their packaging into vesicles.

Retention of the mutants within the ER probably does not itself create the faulty Aβ, as the authors found only inactive γ-secretase in both the ER and COPII vesicles. The protease must therefore be processed and activated in a downstream compartment such as the Golgi. If folding-challenged presenilin mutants are also delayed when they exit this later compartment, prolonged exposure to processing enzymes might create their distorted cleavage properties. JCB

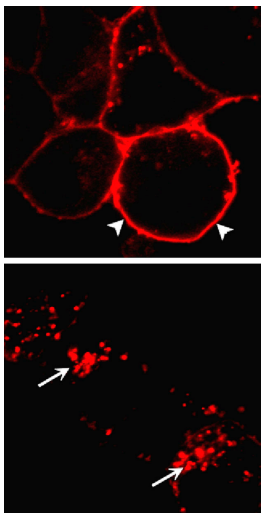
Reference: Kim, J., et al. 2007. *J. Cell Biol.* 179:951–963.

Peptidase frees receptors in endosomes

Internalized pain receptors are freed up by a peptidase for another round of agony, Padilla et al. reveal.

Peptidases on the cell surface cleave and thereby activate or inactivate small, extracellular peptides such as angiotensin. The enzymes also reside in internal compartments called endosomes, where their action is less apparent.

The new work shows that a peptidase called ECE-1 needs the low pH of the endosome to cleave several of its targets. One such peptide target was CGRP, which is released by cells during inflammation. Binding of CGRP to its receptor, CLR, induces pain signaling pathways in neurons. The peptide–receptor complex is then internalized into endosomes, which switches off the pain pathway.



The recycling (top, arrowheads) of CLR (red) back to the plasma membrane is blocked in cells lacking ECE-1 (bottom).

Padilla and colleagues found that the internalized peptide/receptor was accompanied by ECE-1 into endosomes. There, the low pH encouraged the peptide to fall off its receptor. Cleavage by ECE-1 helped to ensure that the peptide did not rebind.

The dissolution of the pair released an associated scaffolding protein called β-arrestin, whose liberation allowed the receptor to return to the cell surface. This ECE-1–induced recycling was necessary for cells to respond to a second round of CGRP. Inhibitors of ECE-1, which were developed to block activation of a peptide that raises blood pressure, might thus have analgesic and antiinflammatory effects. JCB

Reference: Padilla, B.E., et al. 2007. *J. Cell Biol.* 179:981–997.

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