

# In This Issue

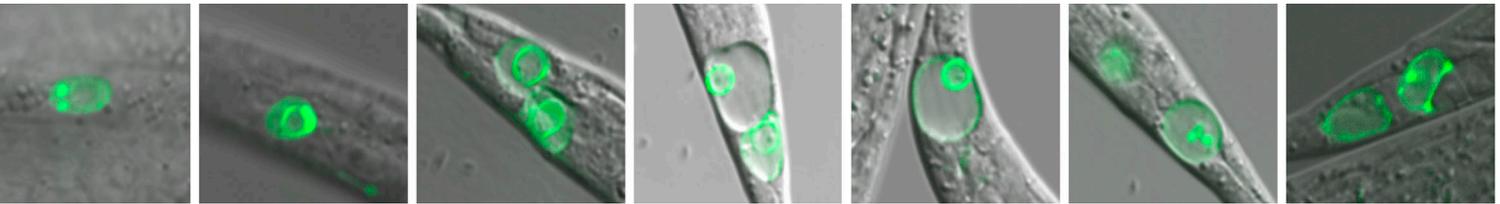
## Spill and kill

**L**ysosomes spill their guts and kill their host cells, as revealed on page 231. Artal-Sanz et al. show that cells are more likely to escape a necrotic death sentence if they contain less acidic lysosomes.

Necrosis—considered the unprogrammed counterpart to apoptotic death—is the deadly result of a severe and abrupt loss of energy, nutrients, or oxygen. These stimuli create sudden alterations in intracellular pH or ion levels that ultimately turn on destructive proteases within the cell.

Lysosomes also have to be acidified to be necrosis inducing. The authors found that alkalization of the organelles using weak bases suppressed necrosis. It also further reduced death in mutant neurons with unusually low amounts of cathepsins.

The authors suspect that lysosomes start leaking their contents at early stages of necrosis. Their idea is supported by past evidence that the protease calpain—which is activated by high  $\text{Ca}^{2+}$  levels during necrosis—damages lysosomal membranes. The damage might open the door for a slow escape of cathep-



As necrosis progresses (left to right), protease-containing lysosomes enlarge, aggregate at the nucleus, and then fuse to surround the swollen nucleus of the dying neuron.

Some of these proteases, such as cathepsins, work best at very low pH. Acidification of the cell occurs during necrosis. Further, a pump that acidifies lysosomes, where cathepsins are normally sequestered, is required for necrosis in worms.

The new study shows that lysosomes are themselves needed during necrosis. By interfering with lysosomal biogenesis, the authors hindered neuronal necrosis in worms. Mutations that created abnormally large lysosomes, by contrast, aggravated necrosis.

sin and  $\text{H}^+$ —thus both freeing proteases that dismantle the cell and creating the acidic environment in which they thrive. At later stages of death, the authors saw, lysosomes ruptured completely.

Necrosis dominates the neuronal death caused by ischemia during a stroke. Perhaps the damage can be minimized if therapies are devised to prevent lysosomal acidification locally. The drugs would probably only be beneficial, however, if administered very shortly after the stroke. **JCB**

## Flux without sliding

**O**n page 173, Cameron et al. find that kidney cells do not depend on sliding forces to generate flux in their mitotic spindles.

Flux is the poleward translocation of spindle microtubules relative to the poles that often helps chromosomes reach separate poles. Flux can be achieved by pushing microtubules outward from the center, by pulling them in from their ends, or by a combination of the two.

In fly S2 cells and in frog extracts, Eg5 is found at the central spindle, where antiparallel microtubules overlap. By cross-linking these microtubules and sliding them toward the poles, Eg5 has been proposed to create flux.

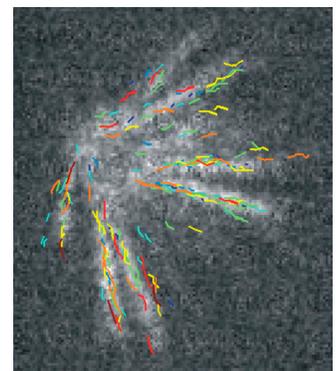
As Eg5 pushes from the middle, kinesin 13 lops off tubulin subunits at the poles. Kinesin 13 inhibition lengthens spindles but does not prevent the sliding component of flux, suggesting that central pushing forces are the major components of flux. But Cameron and colleagues find that, in marsupial kidney cells, flux continues without pushing forces.

The authors made careful measurements of flux rates in PtK1 cells and found only a 25% dampening in the absence of Eg5 activity. Even monopolar spindles, which lack

overlapping antiparallel microtubules, maintained almost normal flux rates.

Since pushing forces were not needed, the group tried to knock out pulling forces as well. Kinesin 13, however, was not easily removed from the equation. RNAi is not yet possible in PtK1 cells, and antibody-mediated inhibition was unsuccessful. The group even tried knocking the motor off poles, but it still clung to microtubule minus ends. Thus, although pulling forces seem to be dominant, in this cell type at least, it is unclear whether kinesin 13 is the motor responsible.

Future studies should also address what factors determine the flux method in different cell types. Perhaps spindle–cortex interactions, which are lacking in frog egg extracts, are necessary for pulling forces. Or maybe the use of centrosome- rather than chromatin-driven spindle formation mechanisms has different effects on the motors that propel flux. **JCB**



Flux occurs even in monopolar spindles, which lack sliding forces.

## Repair road map

**A** report on page 195 by Bekker-Jensen et al. maps out the damage-induced rearrangement of proteins involved in repairing double-stranded DNA breaks (DSBs). The map, the authors hope, will help scientists assign functions to future putative damage-responsive proteins.

Treatments such as ionizing radiation (and chemotherapy) that induce DSBs lead to the accumulation of nuclear foci containing repair proteins. Yet other proteins associated with the cellular response to DSBs do not join such foci.

The authors of the new report used lasers to induce DSBs on a scale that mimics environmental damage by radiation. They then classified known proteins involved in DSB repair according to their arrangement after the laser-induced damage. A protein's location, they discovered, was well-suited to its function.

Three kinds of foci were seen. One

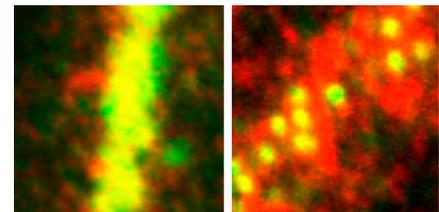
set was found on the single-stranded DNA that forms right at the break site. Proteins in these foci included those involved in the nitty gritty of damage repair via homologous recombination. Also included was ATR, which is an early-acting kinase that turns on the DNA damage checkpoint.

Another set of foci occupied a broader range, binding to chromatin as much as 1 Mb away from the lesion. Proteins in this set included adaptors that promote the interactions of checkpoint kinases (such as ATM) with their substrates or that simply concentrate repair proteins near the break site to increase repair efficiency.

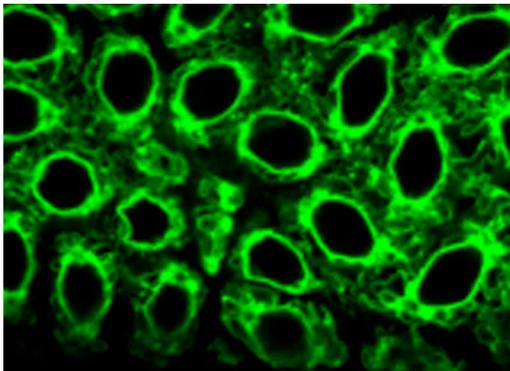
A few proteins, including regulators that turn on and off the damage-sensing kinases, bridged these other two foci. Many proteins did not form foci at all. The authors believe that some of these are true messengers, which check in at the DNA, get activated at lesions, and then dis-

seminate throughout the nucleus. These proteins include Chk1 and Chk2 kinases, which coordinate cell cycle progression, transcriptional changes, and replication fork stability in response to damage.

Checkpoint effectors such as Cdc25 and p53 never even approached the lesion but rather appeared merely to wait for messengers to reach them. They are thus already in position to stall DNA replication or alter transcription, as necessary. **JCB**



Some repair proteins, such as 53BP1 (yellow, left), are broadly distributed along chromatin (red) surrounding lesions. Others, including RPA (yellow, right), accumulate specifically at the lesions.



ER (green) is partitioned around each syncytial nucleus in the fly embryo.

## To each nucleus its own ER

**T**housands of nuclei share a common cytoplasm in the early fly embryo. But they do not share a common ER, as revealed by Frescas et al. on page 219.

The embryonic fly genome divides 13 times before each of the resulting 6,000 nuclei acquires its own plasma membrane. Partitioning the various organelles in this cytoplasmic soup at cellularization seems like a daunting task. The new results show that microtubules divvy up the ER several nuclear divisions before this point.

As in other cell types, the embryonic fly ER started out as a continuous interconnected membrane, in which fluorescent proteins diffused freely. But during the last few common divisions, after the nuclei have migrated from the embryo interior to the cortex, this diffusion was greatly reduced. At this point, the ER was compartmentalized around individual nuclei. Proteins did not exchange between compartments.

This ER isolation was weakened by microtubule depolymerization. Microtubule reorganization, resulting in ER isolation, is probably coordinated by centrosomes, which arrive at the cortex along with the nuclei.

During these final divisions, each nucleus also had its own devoted Golgi stacks. The segregation of this membrane system seems to allow the cell to target secreted proteins to restricted areas of the plasma membrane near an individual nucleus. Indeed, a block in the secretion of membrane proteins in one spot of the embryo was not rescued by the ER/Golgi units of neighboring nuclei. Perhaps this targeting ability helps to maintain protein gradients essential for fly development.

Many other organelles, including endosomes and mitochondria, are linked to the microtubule network. If they are all similarly sectioned off, each nucleus might be essentially the equivalent of a cell, even without a plasma membrane barrier. Whether microtubules form a physical barrier, and whether at least some cytoplasmic proteins are also compartmentalized, is under investigation. **JCB**