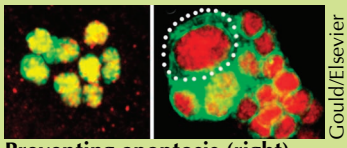
Research Roundup

Hox signals death for neuronal precursors

The number of neurons in an adult fly is determined by a death-inducing blast of a homeodomain protein, based on results from Bruno Bello, Frank Hirth, and Alex Gould (National Institute for Medical Research, UK).



Gould/Elsevier

Preventing apoptosis (right) enables an NSC (outlined) to make more neurons than usual.

When the fly larva hatches, neurons are nearly evenly distributed along the major body axis. But in the adult, they are more numerous in the segments of the thorax than the abdomen, in part because neural stem cells (NSCs) in the thorax divide for a longer period of time. The new article describes "how division is stopped in its tracks and why this happens earlier in the abdomen than in the thorax," says Gould.

His group has found that a pulse of an abdomen-specific Hox transcription factor, called AbdA, in late larval stage NSCs determined the final number of neurons. Rather than signaling exit from the cell cycle, AbdA limited proliferation by inducing cell death through the *grim/hid/reaper* pathway. Artificial premature expression of AbdA further decreased the number of abdominal neurons by inducing early cell death.

Ectopic expression of thorax-specific Hox genes *Antp* and *Ubx* also induced NSC apoptosis. However, these genes were not normally expressed in thoracic NSCs, thus allowing the cells to propagate longer. Gould is now looking for more players in the Hox-induced death pathway, including factors that induce AbdA expression. He is also interested in features of NSCs that make them sensitive to Hox-induced apoptosis, even while many other Hox-expressing cell types are spared. ■

Reference: Bello, B., et al. 2003. *Neuron.* 37:209–219.

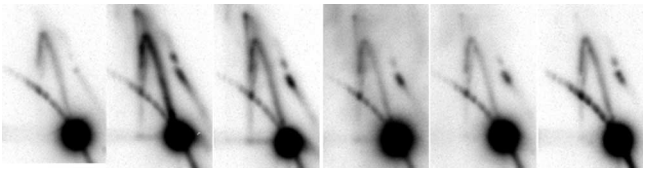
Rec'd and repaired

DNA replication stalls when the polymerase encounters lesions in the DNA, but recovers soon after lesion repair. In a recent work, Justin Courcelle and colleagues (Mississippi State University, Mississippi State, MS) examine what happens to the replication fork during this downtime. The results show that maintaining the correct fork structure depends on recombination proteins that may help to prevent illegitimate strand exchanges.

Courcelle's group used two-dimensional gel electrophoresis to examine the shapes of a replicating bacterial plasmid. Advancing replication forks yielded the expected Y-shaped structure. But UV-induced lesions stalled the replication fork and produced X-shaped structures. These structures represent the nascent DNA backing up from the apex of a Y-shaped fork. The stalled structures were processed by RecQ and RecJ and maintained by RecA and RecF, which are the same proteins that promote homologous DNA pairing during recombinational processes.

A mid-replication stall is "like catching a cell with its pants down," according to Courcelle. "It can't live as one and a half cells for eternity," he says. Unchecked free DNA ends are recombinogenic. Fork stabilization by these Rec proteins may be essential for preventing unwanted mitotic recombination and its potentially cancerous consequences. In addition, fork regression and Rec binding probably delays replication long enough for repair enzymes or SOS polymerases either to repair the lesion or to replicate past it. Whether RecA and RecF recruit repair enzymes or simply maintain an open fork remains to be determined. ■

Reference: Courcelle, J., et al. 2003. *Science.* 10.1126/science.1081328.



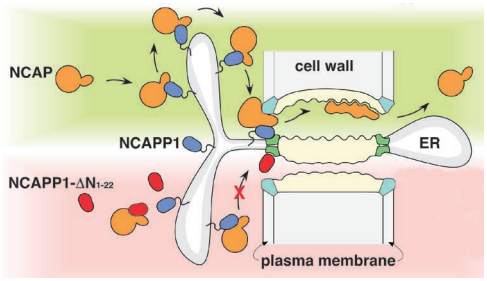
Courcelle/AAAS

The structures of replicating DNAs change (left to right) to allow repair work.

Promoting passage in plasmodesmata

In plants, cell-to-cell communication is achieved through plasmodesmata, unique intercellular organelles that establish cytoplasmic and ER continuity between neighboring cells. Jung-Youn Lee, William Lucas (University of California, Davis, CA), and colleagues now identify a selective gatekeeper for this system, which they call NCAPP1.

Plasmodesmata are the conduits for many non–cell-autonomous proteins (NCAPs). Lee et al. figured that some NCAPs might bind to plasmodesmal proteins, so they used an affinity column based on an NCAP called CmPP16 and a cell wall fraction highly enriched for plasmodesmal proteins to identify NCAPP1.



Lucas/AAAS

When NCAPP1 (blue) is blocked (bottom), its cargo (orange) does not pass through plasmodesmata.

As expected for a plasmodesmal function, colocalization of NCAPP1 and CmPP16 was observed at the ER near the orifice to plasmodesmata. A dominant–negative form of NCAPP1 altered trafficking of the NCAPs CmPP16, LEAFY, and the movement protein of *Tobacco mosaic virus*, but left trafficking of other NCAPs unaffected. Thus, NCAP movement is a regulated process, similar to nuclear pore trafficking. Lucas' group is now purifying other potential plasmodesmal proteins that traffic NCAPP1-independent cargos. ■

Reference: Lee, J.-Y., et al. 2003. *Science.* 299:392–396.

Text By Nicole LeBrasseur
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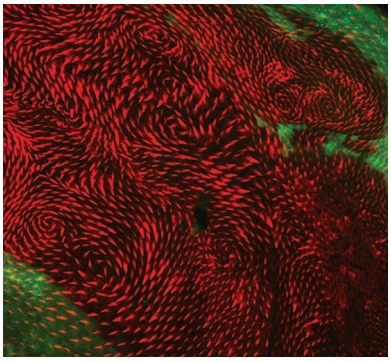
Keeping wing cells in line

Cell polarity in fly wing cells is set with extraordinary precision. Out of the distal end of each cell grows an actin-rich protrusion known as a wing hair. Although the wing contains over 30,000 epidermal cells, it produces this distal-specific hair pattern without error. Dali Ma, Jeffrey Axelrod (Stanford University, Stanford, CA), and colleagues report that this precision is achieved through cooperation between two pathways. They find that wide-ranging gradients and locally acting signaling molecules work together to ensure high fidelity throughout the wing.



Axelrod/Macmillan

Wing hairs are locally aligned but lose distal orientation when the global Ft signal is disturbed.

The local signaling is based on an intercellular feedback loop that has been shown to put Frizzled (Fz) on one side of the cell and keep it from the adjacent side of the neighboring cell. Fz localization is thus propagated from one wing cell to the next. Axelrod's group now shows that the initial polarity of this local pathway is set by global regulators that have thus far been characterized in the eye.

As in the eye, opposing gradients of the cadherin Dachsous (Ds) and a transmembrane protein Four-jointed (Fj) were found in the wing. In both cases, the Ds and Fj gradients activate another cadherin, called Fat (Ft), on the distal sides of the cells where there is more Fj and less Ds. They show that Ds/Ft polarity is necessary for Fz localization, and thus sets the local polarity system. Disruption of the global signal, by mutation of Ds and Ft, produced clones of cells with hairs pointing in a locally organized direction (due to the action of Fz), but the direction was uncoupled from the overall wing axis.

The authors propose that the global pathway, which sets overall direction, is a subtle signal that is prone to cell-to-cell variation. They suggest that deviations are removed through the local Fz pathway, which propagates among neighboring cells to correct errors. Vertebrate systems also exhibit similar precision in polarity—in hair cells in the human ear, for example. Mutations causing deafness syndromes have been mapped to cadherins, suggesting that similar high fidelity mechanisms may be at work. ■

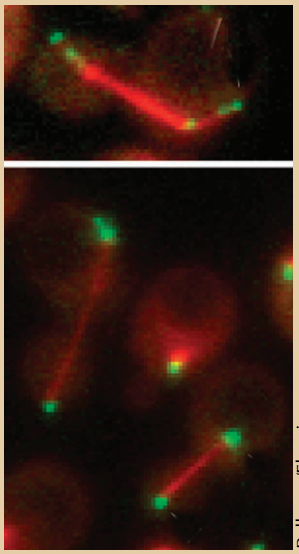
Reference: Ma, D., et al. 2003. *Nature.* 421:543–547.

A minus-end motor sees the plus side

New results from Brina Sheeman, David Pellman (Harvard Medical School, Boston, MA), and colleagues indicate that dynein, a minus end–directed microtubule motor, hops a ride to the membrane on plus ends. The results are inconsistent with current models of dynein activation and localization.

As a minus end–directed motor, dynein walks along astral microtubules toward the spindle pole, and thus could reel in the spindle during anaphase. The favored model for this movement predicts that dynein first binds to the bud cortex, where it captures passing microtubules. From there it could pull the spindle toward the bud. However, at least in budding yeast, significant amounts of dynein have not been found at the cortex. Nonetheless, according to Pellman, "the model is so intuitively appealing that the supposition is that [dynein] must be there even though we cannot see it." But his results now suggest that this is not the case.

The group looked at endogenous levels of dynein in budding yeast. Even a triple-GFP tagged version of dynein was not found at the cortex, but rather on microtubule plus ends. This localization depended on the yeast homologues of CLIP-170 and LIS1, proteins implicated in dynein function in mammalian systems. Dynein was also seen on the spindle poles, its expected end-point after its motility is activated. Disturbing dynein activity, by mutating either its ATPase domain or by inactivating the dynactin complex, caused the motor to accumulate at plus ends rather than moving to the spindle poles.



Pellman/Elsevier

Dynein (green) is found at the plus ends of microtubules (top) and at the spindle pole body (bottom).

Based on the localization results, Pellman suggests that "the microtubule brings its own tethering and motor device out to the membrane." He proposes that dynein is inactive on plus ends until it is activated by local recruitment to membrane domains, as has been shown for at least one class of kinesins. Local transfer to the membrane (and thus activation) could be done by Num1p, a pleckstrin homology–containing protein, as dynein again accumulated at plus ends in the absence of Num1p. ■

Reference: Sheeman, B., et al. 2003. *Curr. Biol.* 10.1016/S00960982203000137.