

# Research Roundup

## Elimination by shedding

Synapse elimination occurs via a combination of axon retraction and the shedding of vesicles dubbed "axosomes," according to Derron Bishop, Thomas Misgeld, Jeff Lichtman (Harvard University, Cambridge, MA), and colleagues. The axosomes appear to end up inside glia, and may help carry both signals and essential functional materials to this supporting cell type.

Terrestrial vertebrates need synapse elimination because their nervous systems are not dedicated, hard-wired circuits but redundant. Overconnection in uncoordinated neonates gradually gives way, via synapse elimination, to single, point-to-point neural connections and fine motor control.

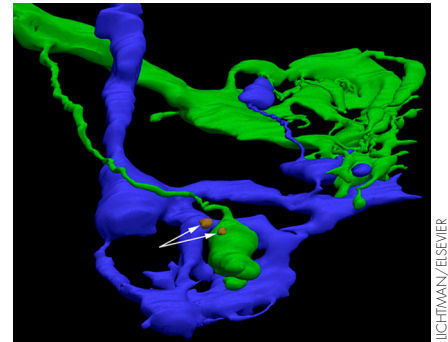
Lichtman's group got a new look at the process at the mouse neuromuscular

junction by correlating light and serial electron microscopy (EM). The resolution of the EM allowed them to show that axosomes were derived from but distinct from axon tips. Axosome formation may be cell autonomous, but glia may also help. Axon remnants were deformed as if they were being pinched off by another cell, and axosomes ended up inside the ensheathing Schwann cells.

Lichtman and colleagues hope to confirm glial involvement using mice with fluorescent markers in both their glia and neural organelles. Any transfer from axon to glia may serve two functions. The axosomes contain synaptic vesicles and the cytoplasmic constituents for delivering them; this machinery may help glia to release small amounts of neurotransmitter and thus maintain the

temporary viability of deinnervated muscles. Second, the axosomes may carry a signal to tell the glia that, with the axon gone, the glia are no longer needed and would be better off dead. **JCB**

Reference: Bishop, D.L., et al. 2004. *Neuron*. 44:651–661.



**Axosomes (arrow) are shed by an axon (green) as it is eliminated.**

## Rocking on Pins

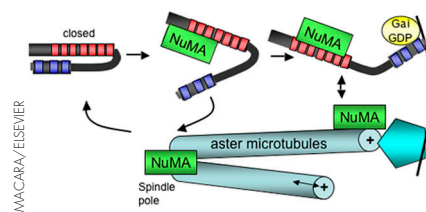
Mitotic spindle positioning involves a conformational switch in LGN, the mammalian version of the invertebrate Pins protein, according to Quansheng Du and Ian Macara (University of Virginia, Charlottesville, VA). Interference with the switch induces a spindle rocking that represents the first genetically manipulable model for studying spindle positioning in vertebrates.

The switch operates via the opening of LGN, which can normally fold back onto itself. The unfolding is triggered by binding to either membrane-bound  $G\alpha_i$  or NuMA, a protein that localizes to and stabilizes spindle poles. Unfolding was detected both as lack of self-binding and a mitotic reduction in FRET between fluorophores placed on either end of LGN.

In the cell, binding of NuMA to LGN appears to be a prerequisite for further interactions, as in cells lacking NuMA the LGN never made it to the cortex. NuMA and its microtubule-bundling activity are nuclear during interphase. During mitosis, NuMA is liberated when the nuclear envelope is dissolved and can now pry apart cytoplasmic LGN. This makes the LGN–NuMA complex available for binding to  $G\alpha_i$  on the membrane.

Overexpressed  $G\alpha_i$  or LGN induced rocking of the spindle, perhaps thanks to unequal pulling forces on the spindle. Macara hopes to sort out the basis for this rocking by visualizing the behaviors of individual microtubules near the cortex. Meanwhile, the rocking should be invaluable as a readout of spindle–cortex attachment. "It's artificial," says Macara, "but it gives us a really powerful tool to look at forces on astral microtubules during mitosis." **JCB**

Reference: Du, Q., et al. 2004. *Cell*. 119:503–516.



**LGN opens to link NuMA and  $G\alpha_i$  at the cortex.**

## Destroy and localize

Localization normally results from specific binding. Kimberly Collins, Suzanne Furuyama, and Sue Biggins (Fred Hutchinson Cancer Research Center, Seattle, WA) now report that localization can also be driven by degradation at every location but the target site.

The protein in question is Cse4. This budding yeast homologue of CENP-A is localized specifically to kinetochores, where it incorporates into nucleosomes. It may be one of the initial (although not necessarily sufficient) building blocks of a kinetochore.

Biggins noticed that Cse4 was marked for degradation by ubiquitin tags. A stable mutant of Cse4 was lethal and localized all over chromosome arms. By contrast, adding another degradation tag to Cse4 did not induce instability of the protein that had localized to the kinetochore.

The Seattle group plans to delete various kinetochore proteins to see if any one of them is responsible for protecting kinetochore-localized Cse4 from degradation. They will also use the more abundant nondegradable Cse4 mutant to continue searching for a loading factor—a factor that probably complements the degradation system to achieve Cse4 localization. **JCB**

Reference: Collins, K.A., et al. 2004. *Curr. Biol*. 14: 1968–1972.