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Research Roundup

EATON/MACMILAN

Hh (red) catches a ride with lipophorin (green).

A fat ride

The Wingless (Wg) and Hedgehog (Hh) morphogens spread beween cells by catching a ride on lipoprotein particles, say Daniela Panáková, Hein Sprong, Christoph Thiele, Suzanne Eaton, and colleagues (Max Planck, Dresden, Germany).

The group had earlier spotted unidentified intercellular signaling particles that they dubbed argosomes. Argosomes are now shown to be distinct from exosomes, a set of intercellular travelers derived from the exocytosis of multivesicular bodies. Instead, the argosome passengers Wg, Hh, and assorted glycophosphatidylinositol (GPI)-linked proteins colocalize with the fly protein lipophorin. This is the fly homologue of vertebrate lipoproteins, which scaffold low- and high-density lipoprotein (LDL and HDL) particles.

Getting rid of lipophorin with RNAi caused Wg and Hh to pile up near the cells that synthesize them in wing imaginal discs. The morphogens failed to spread as far as they normally do, although a nonargosome, nonexosome mechanism seems to ensure continued short-range spreading.

All these signaling proteins have lipid anchors that could insert into the glycolipid monolayer that surrounds lipoprotein particles, but how that insertion event might be controlled or promoted is unclear. When HDL particles pick up lipids from peripheral tissues they dock at scavenger receptors and rely on ABC transporters to spit lipids out of the cell. Eaton's group plans to test proteins that might carry out similar functions in delivering signaling proteins.

Lipoproteins may provide the morphogens with a vector that has just the right mobility; perhaps other proteins associated with the particles provide adhesive properties that ensure the particles do not spread throughout the body. The lipoprotein carriers might also "provide a link coordinating the morphogen gradient with nutritional status," says Eaton. Starved flies grow up smaller, perhaps because they have less lipoprotein carriers to transport Wg and Hh morphogens such long distances. JCB

Reference: Panáková, D., et al. 2005. *Nature.* 435:58–65.

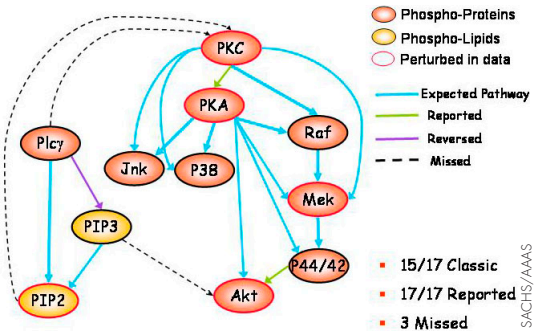
Make your own network

For systems biologists desperate to get beyond transcriptional analysis directly into the world of signal transduction, hope comes from a Stanford/MIT collaboration. The team has successfully derived the structure of a signaling network in primary human cells from simultaneous measurements of multiple labeled proteins and lipids. The method detects a web of interactions, rather than the linear pathways considered in smaller scale experiments.

Karen Sachs and Douglas Lauffenburger, computational biologists at MIT (Cambridge, MA), wanted to use Bayesian networks to model biological systems. These graphical models have been used to predict transcriptional network structures based on mRNA levels.

The problem for those interested in signaling, says Sachs, was that the method is probabilistic, and thus requires many independent samples. This is what Omar Perez and Garry Nolan (Stanford University, Stanford, CA) could provide, with the help of a very fancy flow cytometer. They were able to label 11 reagents (antibodies recognizing phosphorylated proteins and lipids) with fluorophores that could be detected simultaneously in single cells. Having battled through that "tremendous challenge," says Sachs, "in the blink of an eye you can get thousands of data points."

The team measured the response to a number of perturbations, and used the results to build their model. The Bayesian



SACHS/AAAS

The model predicts 17 linkages and misses only 3 (dotted lines).

algorithm threw out redundant linkages and assessed candidate models for simplicity and accuracy. The final model of T cell signaling had 17 linkages, all of which were reported previously, although two of those with little evidence. Three known linkages were missing from the new model, probably because of a lack of feedback in the Bayesian model.

Future challenges will include detecting how the model changes under different conditions, especially in response to diseases and drugs. In tackling less well-characterized pathways, the Bayesian system will be able to deduce connections successfully even if the linkages are indirect. JCB

Reference: Sachs, K., et al. 2005. *Science.* 308:523–529.

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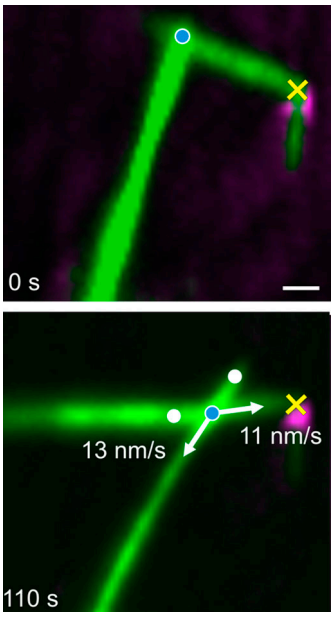
Walk this (and that) way



PETERMAN/MACMILLAN

Eg5 simultaneously walks along both microtubules that it cross-links.

In one model of spindle extension, a cross-linking motor walks outwards simultaneously on two antiparallel microtubules, thus shoving them apart. The Eg5 kinesin fits that bill, according to Lukas Kapitein, Erwin Peterman, Christoph Schmidt (Vrije Universiteit, Amsterdam, Netherlands), Tarun Kapoor (Rockefeller University, New York, NY), and colleagues. This homotetrameric, bipolar motor walks toward the plus ends of both microtubules that it cross-links.

To visualize the hand-over-hand and foot-over-foot process, the group manipulated surface chemistry to ensure the formation of microtubule–motor–microtubule sandwiches. But 20-nm/s movement in two directions looks like 40-nm/s in one direction. The solution was to drag Eg5-linked microtubules into X-shaped conformations using antibody-coated beads and a laser trap. Motors were then seen to be translocating toward the plus ends of both linked microtubules.

This confirms a "mitotic muscle" model of spindle extension. The model has been around for a long time, and has had candidate mediators in budding yeast, but until now had not been directly visualized. Kapoor says that, for him and his collaborators, "this is just a starting point." They hope to add opposing, minus-end–directed motors and more complex microtubule arrays to the in vitro assays, with the eventual aim of reconstructing a functioning spindle from known components. JCB

Reference: Kapitein, L.C., et al. 2005. *Nature.* 435:114–118.

Getting ahead by staying still

Migrating cells send the nucleus backward rather than the microtubule-organizing center (MTOC) forward, say Edgar Gomes, Shantanu Jani, and Gregg Gundersen (Columbia University, New York, NY).

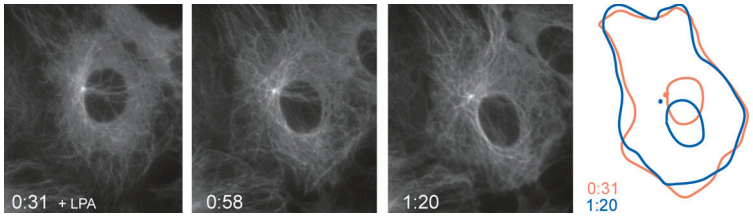
As cells begin to migrate, they spin their internal contents around to orient in the direction of overall cell movement. Movement of a MTOC was thought to lead the way in this process. Consistent with this idea, dynein tugged on MTOCs in other settings, and was concentrated at the leading edge of moving cells. The MTOC moving toward the front of a migrating cell "had been our model forever," says Gundersen.

The trouble is, he says, "people weren't looking early enough." They had seen the final result but not the movement itself. Now, this team gets reorientation going with LPA before initiating migration with serum. That allows them to catch the nucleus moving backward even as the MTOC stays put.

The GTPase Cdc42 and its target MRCK were necessary and sufficient for nuclear movement. These proteins prompt actin polymerization at the front of the cell and thus actin retrograde flow, whose timing and speed match that of nuclear movement. The team favors an actin conveyor-belt model for nuclear movement, but has not ruled out a bulldozer model.

The MTOC stayed fixed in the center of the cell thanks to a group of proteins including dynein, Par6 and PKCζ; without them it wandered backward with the nucleus. How the MTOC is centered is a mystery—some have suggested that the pull of motors on microtubules is proportional to the length of the microtubules. JCB

Reference: Gomes, E.R., et al. 2005. *Cell.* 121:451–463.



GUNDERSEN/ELSEVIER

The centrosome is fixed even as the nucleus moves to the back of the cell.

RNA in the machine

Spindle assembly requires a large complex that includes essential RNAs, say Michael Blower, Maxence Nachury, Rebecca Heald, and Karsten Weis (University of California, Berkeley, CA).

The team was searching in frog egg extracts for spindle assembly factors—specifically those downstream of the Ran GTPase. Ran activated by a chromatin-bound GTP exchange factor displaces importin β, an inhibitor of spindle assembly. Known factors downstream of Ran all bind importin β indirectly, via its partner importin α.

Depleting extracts using a mutant importin β gave a nonfunctional extract even though proteins that bind importin α remained. What was missing was Rae1. This importin β-binding protein was previously associated with mRNA export in yeast, and full spindle-promoting activity of Rae1 required a complex of 10 or more proteins and a host of RNAs. Without the RNA, the complex lost several of its proteins and some of its activity. Clusters of RNA were visible in the spindle, and RNase treatment of frog egg extracts inhibited spindle assembly.

"Everyone believed that the spindle was a purely protein-based machine," says Weis. Identifying the new RNAs will help determine whether they simply hold together a complex or are more active, as in ribosomes and spliceosomes. JCB

Reference: Blower, M.D., et al. 2005. *Cell.* 121: 223–234.