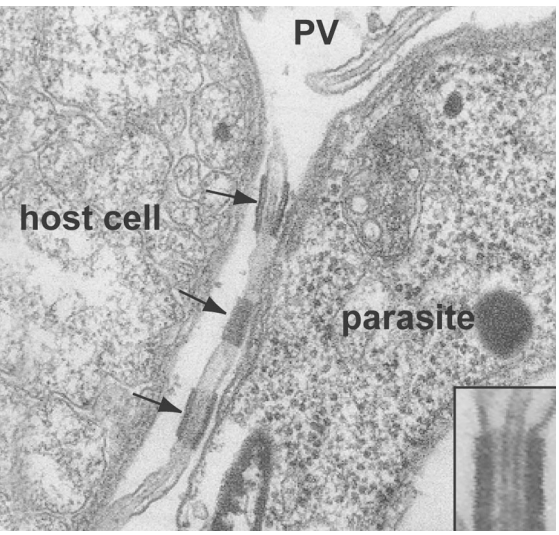
Research Roundup

Lysosomes for lunch

If an intracellular parasite is too successful at hiding itself, it can end up starving. *Toxoplasma gondii* gets around this potential problem by munching nutrient-filled host lysosomes, say Isabelle Coppens (Johns Hopkins University, Baltimore, MD), Keith Joiner (University of Arizona, Tucson, AZ), and colleagues.

T. gondii has an extraordinary mechanism for creating a parasitophorous vacuole (PV) around itself—it motors into a host mammalian cell and uses a ring-shaped moving junction to exclude almost all host-derived proteins from the nascent PV membrane. But this leaves the parasite responsible for importing all nutrients including, as Coppens and Joiner found previously, cholesterol.



COPPENS/ELSEVIER

***T. gondii* wraps an invagination that delivers a lysosomal meal.**

The group therefore looked at whether endocytosed cholesterol found its way to the PV. They found that it did, and that a wholesale rearrangement of the cytoskeleton was involved. Host microtubule-organizing centers (MTOCs) detached from the nucleus and reattached to the PV. Endolysosomes followed them, clustering around the PV. Finally, the microtubules nucleated by MTOCs surrounded and poked into the PV.

But "the invagination is not a V like you would get from poking a balloon," says Coppens. A parasite protein called GRA7 forms a collar around the invagination (which can be over 1-μm deep) that keeps it narrow. In most of the invaginations, a central microtubule allows host membranous compartments to be drawn inside. These compartments are eventually surrounded by PV-derived membrane as they are taken into the PV. The ingested lysosomes stay intact and thus do not spill their digestive contents.

This reorganization of the cytoskeleton keeps the parasite fed. Further experiments will help determine whether the parasite also makes attractants to lure lysosomes into the PV's mouth. JCB

Reference: Coppens, I., et al. 2006. *Cell.* 125:261–274.

Twisting endocytosis

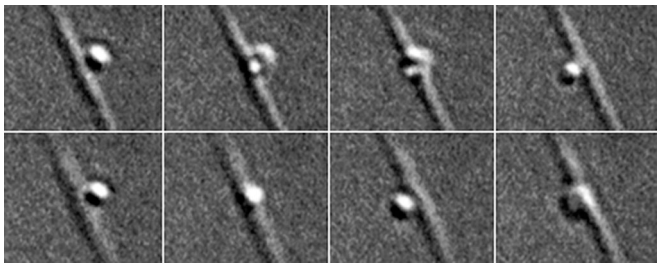
Aurélien Roux, Pietro de Camilli, and colleagues (Yale University, New Haven, CT) report the best evidence yet that dynamin uses mechanochemical activity—specifically a twisting action—to pinch off endocytic vesicles.

Dynamin was, early on, localized to the collar around the neck of forming endocytic vesicles. This suggested that dynamin may use the energy of GTP hydrolysis to directly pinch a membranous neck. Indeed, dynamin could tubulate lipids and break apart the tubules in vitro, although later it seemed that the breaking apart was happening as the samples dried on EM grids.

Meanwhile, Sandy Schmid (Scripps Research Institute, La Jolla, CA) had come up with a "regulatory GTPase" model: that dynamin was active not as it hydrolyzed GTP but in its GTP-bound form, which recruited other proteins to do the pinching. This theory was controversial, and now the Yale group has more evidence for the earlier "pinchase" model.

They used light microscopy rather than EM to follow tubulation and fission directed by dynamin in vitro. Addition of GTP caused twisting, retraction, and, if both ends were anchored, straightening and breakage. Thus longitudinal tension was needed with constriction to achieve fission.

Twisting was evident because tubules became supercoiled and, most strikingly, attached beads rotated around the tubules. "You can't look at that movie and say there isn't something mechanochemical going on," says Schmid. Previous work had established the importance of a conformational change in dynamin, but "that doesn't distinguish between mechanochemical and regulatory functions," says Schmid. "Spinning around a liposome sure does."


DE CAMILLI/MACMILLAN

A spinning bead betrays the twisting action of dynamin.

Not that Schmid is throwing out the regulatory model. "The two models are not mutually exclusive," she says. "This is a smarter molecule than we give it credit for. There is a lot more to this molecule than a pinchase." She thinks the regulatory model may be operative early on, as dynamin assesses curvature, coated pit formation, or cargo loading before giving the go-ahead.

The "pinchase" function of dynamin would then take over. In vivo, say Roux and de Camilli, the dynamin collars are relatively short, so cortical actin is the most likely source of tension that would help the dynamin to wrench an endocytic vesicle free. JCB

Reference: Roux, A., et al. 2006. *Nature.* doi:10.1038/nature04718.

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Tracks for cellulose

Cellulose synthase (CESA) tracks along paths coincident with microtubules, say Alex Paredez, Chris Somerville, and David Ehrhardt (Carnegie Institution, Stanford, CA). The resulting parallel cellulose fibrils constrain cell expansion so that plants elongate primarily along a single axis.

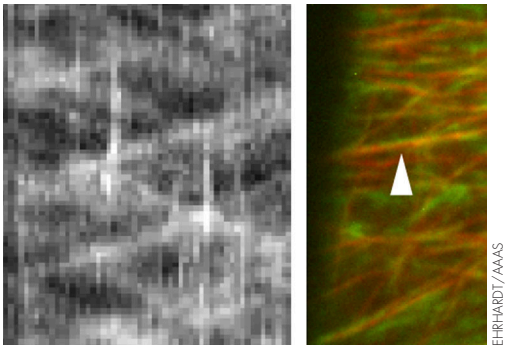
A transmembrane CESA complex takes cytoplasmic substrates and turns them into 36 extracellular glycan chains. At some distance from the complex, the extruded chains crystallize into a cellulose microfibril.

CESA's relationship to plant cortical microtubules has been difficult to determine given the microtubules' dynamic nature. Through rapid treadmilling and turnover, the microtubules bump into each other and realign, thus helping create a parallel array that is perpendicular to the axial direction of plant growth. In static pictures CESA was often nowhere near a microtubule, leading some to suggest that CESA was channeled between microtubule tracks rather than interacting with them directly.

Using live, single-particle imaging, however, the Stanford group saw that CESA was often coincident with microtubules, tracked along the microtubules, and reoriented in response to reorientation of the microtubule arrays by light.

When a microtubule treadmilled away from CESA, the CESA complex kept going in a straight line as defined by the now-absent microtubule. This is consistent with the team's belief that most if not all of the motive force comes from cellulose polymerization rather than a cytoskeletal motor. Extruded cellulose microfibrils bond to other cell wall polymers, so it is the CESA that must move forward as more cellulose is created. Any link between CESA and microtubules is yet to be determined. JCB

Reference: Paredez, A.R., et al. 2006. *Science*. doi:10.1126/science.1126551.



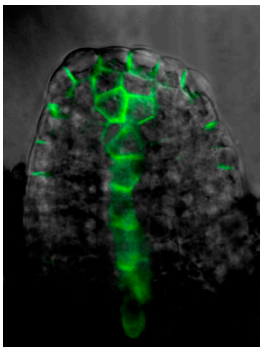
EHRHARDT/AAAS

Moving in straight lines (left), CESA (green) is coincident with microtubules (red).

Flow makes vein

Leaf veins, just like roots and shoots, use flows of the plant hormone auxin to drive their patterning, say Enrico Scarpella (University of Alberta, Canada), Thomas Berleth (University of Toronto, Canada), and colleagues.

Models for leaf vein patterning have been based on either auxin flows or reaction–diffusion systems. The flow models had a hard time explaining how loops would arise. But "all the modeling so far has been based on the final pattern," says Berleth. "Now flow can be reconciled with closed networks, although other types of cellular interactions may also play a role."



BERLETH/CSH

Auxin flows from margin inwards to create leaf veins.

His team used the auxin efflux protein AtPIN1 as an early marker of vein formation. Expression was found at a series of "convergence points" at the leaf margin, from where it led down paths that foreshadowed first the leaf's main central vein and then other major veins connected to the main vein.

Auxin is presumed to flow from leaf margin to the interior, consistent with AtPIN1's polarization to the parts of the cells closest to the main vein. Loops could also form, however, because certain cells had bipolar AtPIN1, allowing auxin flow to the main vein via two alternate routes. Auxin turns on AtPIN1 expression, thus refining and reinforcing the pattern.

Interfering with auxin flow disturbs vein formation and spacing. Vein placement seems to depend on the positions of convergence points, but those may be defined both by auxin's positive feedback on its own flow and on undefined genetic circuits. JCB

Reference: Scarpella, E., et al. 2006. *Genes Dev.* 20:1015–1027.

Lamins as youth elixir

Aging looks and feels like it is multifactorial: everything falls apart independently. But now Paola Scaffidi and Tom Misteli (National Cancer Institute, Bethesda, MD) report that multiple hallmarks of cellular aging can be reversed by eliminating one aberrant splicing product of lamin A.

The lamins form a structural cage on the interior surface of the nucleus. Lamin A has a long tail that is first farnesylated and then chopped off. In the 50 or so patients known to have the premature aging syndrome Hutchinson-Gilford Progeria (HGPS), an aberrant splicing event creates a lamin A that gets farnesylated but not cleaved.

The NCI team now shows that normal cells also have a small amount of this aberrant splice product. Although neither the splice product nor its protein product accumulate to higher levels with age, their effects do. As in HGPS cells, older cells have decreased heterochromatin and other nuclear markers, and increased markers of unrepaired DNA damage. Many of these changes were reversed by an oligonucleotide that eliminated the aberrant splice product.

Normal lamin A is found both at the nuclear periphery and within the nucleoplasm. But the aberrant splice product retains its farnesylation, and therefore gloms itself, and normal lamin A, onto the nuclear envelope. It is not clear how this leads to the many problems, although another group has suggested that lamin defects trigger a checkpoint that assesses nuclear envelope integrity. This, or some other mechanism that deals with the presence of the aberrant lamin product, must somehow be more sensitive in older cells. JCB

Reference: Scaffidi, P., and T. Misteli. 2006. *Science*. doi:10.1126/science.1127168.