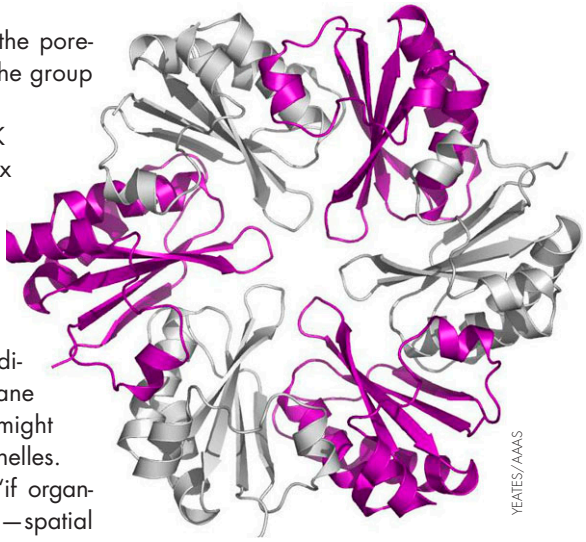
Research Roundup

A view of primitive organelles

Though the textbook view of bacteria is one of a homogenous mixed bag of materials, many bacteria contain large polyhedral protein shells believed to serve as primitive organelles. The first of these bodies to be isolated, called a carboxysome, holds large amounts of RuBisCO, which uses CO_2 to form small sugars. Now, the first high resolution views of carboxysomes, from Cheryl Kerfeld, Todd Yeates (University of California, Los Angeles, CA), and colleagues, suggest that these structures do have many of the functional aspects of organelles.

Carboxysome proteins CcmK2 and CcmK4 crystallized in tightly packed hexameric units that assembled, also tightly, into sheets. "The six subunits leave very little space down the middle," says Yeates. "And the space left is highly [positively] charged." The proximity of so many charges is more than coincidence. For instance, the pore might regulate ion passage, allowing through small negatively charged molecules such as bicarbonate (which is converted to CO_2) but blocking oxygen (which inhibits RuBisCO). Mutagenesis of the pore-lining residues should allow the group to test this hypothesis.

Shells formed by CcmK homologues do more than fix carbon. The breakdown of propanediol in *Salmonella*, for instance, occurs inside polyhedral bodies, which might confine a toxic pathway intermediate. As they are not membrane bound, the compartments might not be considered true organelles. But they are, Yeates says, "if organelles are defined by purpose—spatial separation and control of what comes in and out. The bacteria are trying to achieve the same level of sophistication [found in eukaryotic organelles]."



YEATES/AAAS

CcmK subunits (purple and silver) pack into a hexamer with a small central pore.

Structurally, carboxysomes and other polyhedral bodies resemble viral particles, suggesting that perhaps bacteria co-opted viruses in the same way that led eukaryotic cells to contain bacteria-like organelles such as mitochondria. But small proteins with CcmK-like architectures seem to have evolved independently multiple times. There is, therefore, no sure way to know whether the primitive bacterial organelles were originally viruses or arose on their own. JCB

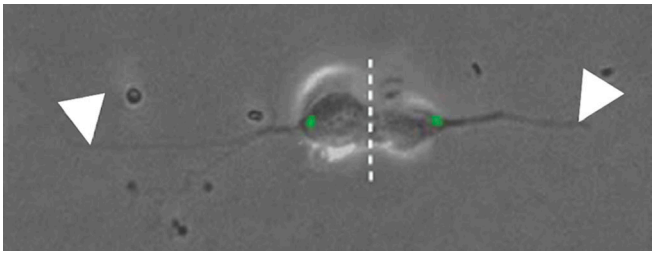
Reference: Kerfeld, C.A., et al. 2005. *Science.* 309:936–938.

The early neurite gets the axon

A developing neuron sprouts many neurites, only one of which will become the axon, while the rest form dendrites. Axon choice has been thought to result from competition among neurites, possibly for a growth promoter. But now, Froylan Calderon de Anda, Guilia Pollarolo, Jorge Santos Da Silva, Carlos Dotti, and colleagues (Universita degli Studi di Torino, Italy) show that the location of the axon is determined before most neurites form.

By the time the first neurite forms, the group finds, it already marks the location of the axon. No one has seen this association before because they have been looking at stage 2 neurons, which have already formed many neurites. "To find the specific molecular events that are instructions for polarity," says Dotti, "we should look at the immediate postmitotic stage, even before neurites form."

At this stage, the centrosome and Golgi apparatus still sit on the opposite end of the cell from the site of cytokinesis. It was here, near the centrosome, where the first lamellipod (and thus the first neurite) formed. Polarized membrane trafficking was aimed at the plasma membrane near the centrosome. This polarization might be a result of mechanical deformation of the membrane by centrosome-organized microtubules (perhaps as a continuation of cytokinetic forces). Or it might be due to the local destabilization of actin filaments (which promotes membrane fusion events) by pericentrosomal proteins.



DOTTI/MACMILIAN

The centrosome (green), which sits opposite the division plane (dotted line), determines where the axon forms.

The first neurite is expected to receive the most external growth cues, given a uniform environment, and thus would be the first to reach any threshold that leads to axon commitment. Cells with two centrosomes—and therefore two early neurites—formed two axons. Loss of centrosomal function, in contrast, blocked axon formation and led only to dendrites.

Not long after mitosis, the centrosome changes its position, so it is easy to imagine why scientists have missed the centrosome–axon correlation. Given the centrosome's ability to polarize membrane growth, Dotti wonders whether its rotation within the cell is actually required to make the later, dendrite-forming neurites. JCB

Reference: de Anda, F.C., et al. 2005. *Nature.* 436:704–707.

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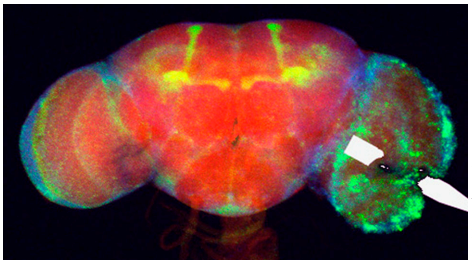
APP for injury repair

The cleavage of the amyloid precursor protein (APP) by γ-secretase releases the Aβ peptide, which forms plaques that are associated with Alzheimer's. Because APP is induced by injury, boxers and head trauma sufferers are prone to developing Alzheimer's. But new findings suggest that, without APP, the initial injuries might be lethal. Maarten Leyssen, Bassem Hassan, and colleagues (VIB and the University of Leuven, Belgium) find that APP helps mature axons grow and branch.



HASSAN/EMBO

A fly with a head wound (white) is more likely to survive if it expresses APP (green).

The function of APP has been elusive, as mice and flies lacking APP develop relatively normally. So to look for post-developmental functions, Hassan's group induced neuronal APP expression in adult flies. The result was a strong arborization of mature axons.

Recalling that APP is induced by injury, Leyssen devised a strategy to produce head wounds in flies. "The APP mutants," says Hassan, "had a lower chance of survival after their injuries." Survival probably depends on restoring severed synaptic connections, which would be helped along by axonal arborization.

APP works by activating Abl, a kinase that destabilizes actin filaments. The authors find that the JNK pathway is also activated by injury and is required for APP-induced arborization. Although the Abl pathway probably produces immediate responses needed for axon outgrowth, JNK-induced transcriptional changes might be longer lasting, perhaps even returning the mature neuron to growth mode. JCB

Reference: Leyssen, M., et al. 2005. *EMBO J.* doi:10.1038/sj.emboj.7600757.

A new mRNA polymerase

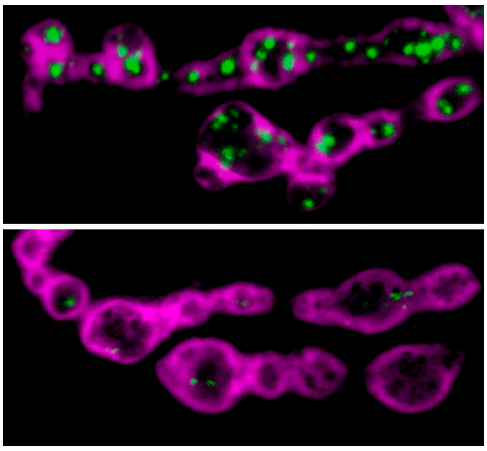
Mitochondria share a version of their RNA polymerase with the nucleus, as shown by Julia Kravchenko, Igor Rogozin, Eugene Koonin (NIH, Bethesda, MD), and Peter Chumakov (Cleveland Clinic Foundation, Cleveland, OH). This new fourth mammalian nuclear RNA polymerase adds a new dimension to mRNA transcription.

Until now, mRNA transcription was considered the sole domain of RNA polymerase II (RNAP-II). While trying to determine why some transcripts were still abundant when RNAP-II was inhibited, Kravchenko et al. found that an alternatively spliced version of the mitochondrial RNAP (mtRNAP) was responsible. Although nuclear DNA–encoded, mtRNAP is used solely by mitochondria. But the new spliced version (spRNAP-IV) was instead imported into the nucleus and was shown to transcribe several nuclear mRNAs whose genes share a short putative promoter sequence not recognized by RNAP-II.

The authors estimate that the number of genes transcribed by spRNAP-IV is on the order of a thousand (although some might be co-regulated by RNAP-II). At least some of these genes encode vital proteins, as loss of spRNAP-IV slows proliferation and leads to cell death. Koonin hypothesizes that spRNAP-IV "somehow allows the cell to simultaneously regulate mitochondrial function and other aspects of cell behavior," possibly including apoptosis. JCB

Reference: Kravchenko, J.E., et al. 2005. *Nature.* 436:735–739.

Local power for synapses



BELLEN/ELSEVIER

Mitochondria (green) in axon terminals (top) make energy for reserve pool mobilization, which is blocked in *drp1* mutants (bottom).

New results from Patrik Verstreken, Hugo Bellen, and colleagues (Baylor College of Medicine, Houston, TX) show that mitochondria in axon terminals provide energy to overwrought synapses.

Being far from the cell body, synapses have their own reserve of cellular machinery, including ER and mitochondria. Bellen's group analyzed the contribution of local mitochondria to synaptic function using a fly dynamin-like mutant called *drp1*, which lacks mitochondria at the synapse but retains them in the cell body.

Although synaptic endo- and exocytosis are thought to depend on local ATP, neurotransmission in response to slow stimulation was fairly normal in *drp1* synapses. But under high frequency stimulation—such as occurs during muscle contractions—synapses at *drp1* neuromuscular junctions (NMJs) ran out of steam.

The failure stemmed from an inability of *drp1* synapses to mobilize reserve pool (RP) vesicles, which sit back from the membrane until they are called upon during strong stimulations. "It's a surprisingly specific defect," says Bellen. "[Cytoplasmic] glycolysis must be sufficient, except when energy is in high demand." By providing this energy in the form of ATP, the authors were able to partially restore *drp1* neurotransmission.

RP vesicles might be moved by myosin, since myosin inhibitors blocked this ATP-mediated rescue. "A vesicle," says Bellen, "is a pretty large object to drag through the cytoplasm at high speeds. It makes sense in retrospect that this requires the most energy." JCB

Reference: Verstreken, P., et al. 2005. *Neuron.* 47:365–378.