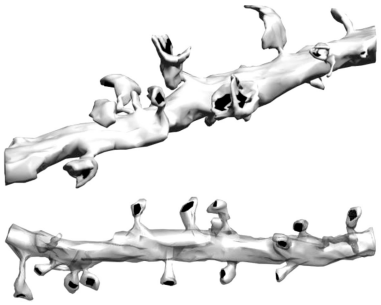
In This Issue



More PSD-95 means bigger spines (top), and multiple axon connections.

Multiple axons and actions with PSD-95

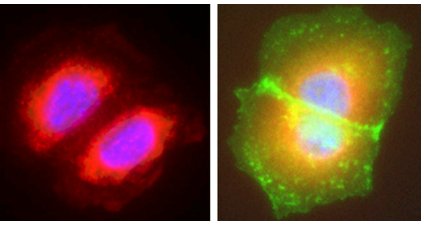
Nitric oxide gets neurons together. And it seems to do it backward. Work by Nikonenko et al. suggests that a protein called PSD-95 prompts nitric oxide release from postsynaptic dendritic spines, prompting nearby presynaptic axons to lock on, and develop new synapses.

It is becoming increasingly clear that synaptogenesis is not solely axon driven. PSD-95 is a major component of postsynaptic densities—a conglomeration of scaffolding proteins, neurotransmitter receptors, and signaling proteins that are thought to shape dendritic spines—and reduced levels of PSD-95 impair synapse development. How PSD-95 works, however, was unknown.

Nikonenko et al. overexpressed PSD-95 in cultured hippocampal neurons and found that the cells' dendritic spines grew two to three times their normal size and were often contacted by multiple axons—a rare occurrence in the adult brain. By mutating different parts of PSD-95, the team discovered that the region responsible for prompting multi-axon connections was also required for binding nitrogen oxide synthase. The team cut to the chase, bathed neurons in nitric oxide, and showed this was sufficient to promote the extra axon connections. Since bathing cells in nitric oxide and overexpressing proteins do not reflect normal physiological conditions, the team also inhibited nitric oxide synthase in wild-type neurons and confirmed that synapse density was reduced.

Overexpressing PSD-95 increased the amount of nitric oxide synthase at postsynaptic densities, suggesting PSD-95 recruits the synthase to its required locale. Interestingly, PSD-95 that lacked its synthase interaction domain still induced super-sized dendritic spines, suggesting PSD-95 wears more than one hat at the synapse construction site. **RW**

Nikonenko, I., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200805132.



Without α–E-catenin (green), dynactin (red) and its associated organelles remain on the microtubule highways (left).

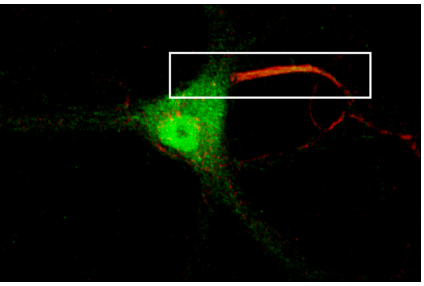
Junction protein goes on the road

If microtubules are the highways of the cell, then actin filaments are the local roads. Lien et al. suggest that α–E-catenin might sit at the junction between the two and help organelles transit from one to the other—a curious discovery for a protein commonly known to operate at an entirely different type of junction.

α–E-catenin is a major component of adherens junctions, binding via β-catenin to transmembrane protein E-cadherin to hold neighboring cells together. However, α–E-catenin is also suspected to control cell proliferation. Lien et al. set out to discover how the protein performs this alternate function, by searching for new α–E-catenin interaction partners. Much to the authors' surprise they identified dynamitin—a crucial piece of microtubule motor machinery, and not an immediately obvious candidate in proliferation control.

The microtubule motors traffic organelles around the cell, and in cells that lacked α–E-catenin this trafficking was faster. This might be caused by a reduced connection between microtubules and actin; α–E-catenin is a known actin binder, and similarly increased organelle speeds are seen in cells with disrupted actin filaments. Indeed, when the team replaced endogenous α–E-catenin with a version of the protein that could not bind actin (but that still bound dynamitin), organelle speeds matched that of cells that lacked α–E-catenin entirely. Thus, α–E-catenin might slow organelles' passage along microtubules by continually tempting them to take the scenic actin route. **RW**

Lien, W.-H., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200805041.



CK2 (green) localizes with ankyrin G (red) at the axon initial segment.

CK2: channel controller

In neurons, the kinase CK2 ensures that sodium channels are positioned for maximum potential, report Bréchet et al.

Action potentials shoot down axons thanks to a wave of membrane depolarization that triggers specialized membrane regions at the start of the axon—the axon initial segment (AIS)—and at regular intervals along the axon corresponding to gaps in the myelin sheath—the nodes of Ranvier. These specialized regions are characterized by a local clustering of sodium channels.

The clustering requires binding of the sodium channels to a cytoskeletal protein called ankyrin G, also spatially restricted to the AIS and nodes of Ranvier. However, ankyrin G has a homologous protein called ankyrin B that localizes to different parts of the neuron. Given that sodium channels

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can bind to either ankyrin in vitro, what makes the channels preferentially bind to ankyrin G in vivo?

To answer this question, Bréchet et al. homed in on the precise residues in sodium channels that are required for the interaction with ankyrin G. They pinpointed a sequence of residues that looked like a target for phosphorylation by CK2, and sure enough, it was. CK2 phosphorylated the sodium channels and this was required for the channels' correct positioning along the axon membrane.

In vitro, however, phosphorylation by CK2 prompted the channels to bind both ankyrin G and B. The clue to the sodium channels' specificity for ankyrin G came when the team looked at the distribution of CK2 in neurons and found that it was also restricted to the AIS and the nodes of Ranvier. The big question now is, what targets CK2? **RW**

Bréchet, A., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200805169.

The actin flow paradox

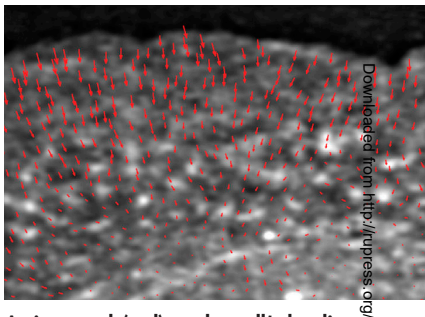
Gardel et al. have discovered a perplexing anomaly regarding actin dynamics and cell traction on its extracellular matrix.

In a migrating cell, actin filaments polymerize at the leading edge, and flow back into the body of the cell (retrograde flow). It has been proposed that focal adhesions (FAs)—the cell's tether points to the extracellular matrix (ECM)—forming at the cell's leading edge provide handholds for the flowing actin that impede actin's retrograde movement, and in so doing create the traction needed to push the cell forward. The situation might be likened to a crowd of people being washed downriver and certain individuals gripping rocks (FAs) on the riverbed to work their way back against the flow.

Gardel et al. hypothesized that the speed of actin retrograde flow should be inversely correlated to traction force—the slower the river flows, the easier it is to grip the rocks and work one's way upstream—and indeed, this is exactly what they discovered. However, this was only true for the actin speeds seen at the front end of the cell (10–30 nm per second). At the frontal tip (where FAs are small), actin retrograde flow was rapid and traction was minimal. A little farther back in the leading edge (where FAs are bigger), traction increased and actin speeds slowed. Back toward the cell body, however, despite actin speeds dropping below 8–10 nm per second, the FAs exerted less traction.

Clare Waterman, who led the study, explains that the switch occurs roughly in the area where the lamellipodia (leading edge) ends, and the cell body begins. There must therefore be something different about the way the FAs and actin combine to generate tension in this region. **RW**

Gardel, M.L., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200810060.



Actin speed (red) at the cell's leading edge has a biphasic relationship with cell traction.

FHL1 adds some muscle

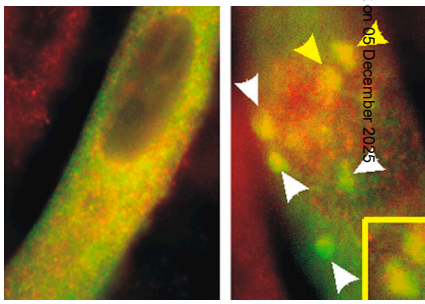
Cowling et al. report how to build muscle mass with FHL1. The protein partners with and activates the transcription factor, NFATc1. Encouraging this partnership might provide a possible treatment for muscle wasting disorders.

Mutations in FHL1 are present in several myopathies, including reducing-body myopathy (RBM), but until now, both the molecular mechanisms causing the disease, and the regular function of FHL1 in healthy tissue, remained unknown.

To address this, Cowling et al. overexpressed FHL1 in both transgenic mice and cultured myoblasts. The mice developed skeletal muscle hypertrophy, and showed increased strength and endurance. Overexpression in myoblasts also increased cell fusion, resulting in hypertrophic myotubes. These phenotypes are similar to those caused by the calcineurin/NFAT pathway and, indeed, inhibiting calcineurin blocked the effects of FHL1 overexpression in vitro. The authors showed that FHL1 binds to and enhances the transcriptional activity of NFATc1 in vitro and in vivo.

So what goes wrong when FHL1 is mutated? In RBM, mutant FHL1 accumulates in cytoplasmic aggregates called reducing bodies, probably as a result of misfolding. When these mutants were expressed in cultured myoblasts, they also aggregated, and did not induce hypertrophy. Cowling and colleagues found that NFATc1 was sequestered to the aggregates, and was therefore unable to activate its target genes. **BS**

Cowling, B.S., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200804077.



Unlike the wild-type protein (left), mutant FHL1 (right, green) forms cytosolic aggregates (arrows) that sequester NFATc1 (red).