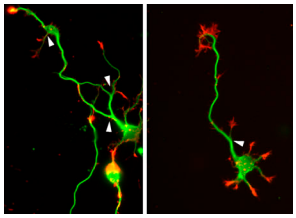
In This Issue

Text by Mitch Leslie
mitchleslie@comcast.net

TRIM9 cuts back on axon branching



Netrin-1 spurs neurons to branch (left) but not if SNAP25 is blocked (right).

Netrin-1 promotes axon branching by stimulating exocytosis, Winkle et al. show.

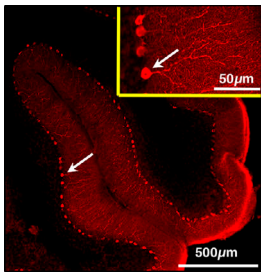
During development, glia and other cells secrete Netrin-1, which induces neurons to sprout extensions and make new connections. How Netrin-1 induces a neuron's plasma membrane to obtain the new membrane material necessary for growth is unclear. A likely source is exocytosis, in which formation of the SNARE complex prompts vesicles to fuse with the plasma membrane.

By blocking two SNARE complex proteins, Winkle et al. showed that Netrin-1 requires exocytosis to incite axon branching. The authors proposed that the ubiquitin ligase TRIM9 linked exocytosis to the Netrin-1 pathway. Consistent with this, TRIM9 was active in the brains of embryonic mice, and loss of the protein triggered excessive axon branching.

TRIM9 makes two important connections that enable it to control exocytosis and branching, the researchers found. It fastens to the cytoplasmic tail of the Netrin-1 receptor DCC, and it binds to SNAP25, a member of the SNARE complex. Winkle et al. conclude that TRIM9 normally clings to DCC and SNAP25, preventing the latter from joining the SNARE complex and thus blocking exocytosis and branching. When Netrin-1 binds to DCC, TRIM9 releases SNAP25, allowing exocytosis and branching. In the absence of TRIM9, nothing restrains SNAP25 and SNARE complex formation, so axon branching increases. TRIM9's ubiquitin ligase activity was essential for exocytosis and branching, and future work will aim to identify its specific ubiquitylation targets.

Winkle, C.C., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201311003.

Closing the calcium floodgates



In the cerebellum, BARP (red) is prevalent in cell bodies and dendrites.

Béguin et al. identify a new protein that curbs the activity of voltage-gated calcium channels, which prompt everything from muscle contraction to hormone release.

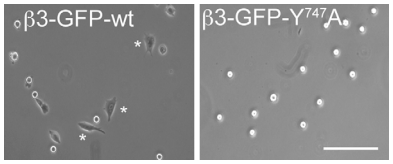
By allowing calcium to pour into cells, voltage-gated calcium channels trigger a variety of responses. The calcium surge spurs neurons to release neurotransmitters, for instance, and induces pancreatic islet cells to secrete insulin. A core component, the $Ca_v\alpha1$ subunit, combines with several other subunits, including $Ca_v\beta$, to form the channels. Keeping the channels under tight control is crucial because excess calcium can kill cells. Researchers have identified a number of proteins that adjust the activity of the channels by latching onto $Ca_v\alpha1$ and $Ca_v\beta$.

Béguin et al. uncovered another regulator, which they dubbed BARP, when they searched for proteins that attach to one variant of $Ca_v\beta$. BARP is expressed in several tissues, including the brain and pancreas, and two sections of the protein, domains I and II, can bind to $Ca_v\beta$.

The researchers discovered that BARP dials down the activity of calcium channels but doesn't affect channel abundance at the cell surface. When they dosed adrenal gland cells with siRNAs that target BARP, they found that the treatment almost doubled release of the neurotransmitter acetylcholine. The researchers think that BARP works by separating the $Ca_v\beta$ and $Ca_v\alpha1$ subunits, thereby shutting down the channels. One question they want to pursue is whether BARP is mutated in channelopathies, diseases in which calcium channels malfunction.

Béguin, P., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201304101.

Talin holds tight during cell spreading



Control cells rapidly spread (left), whereas cells remain compact if they carry an integrin that can't bind talin (right).

Pinon et al. clarify the sequence of events during integrin-mediated cell spreading.

When a moving cell finds itself on an unfamiliar substrate, integrin receptors in the plasma membrane first get a grip on the surface. Then the integrins begin to signal, spurring the cell to spread out and make itself at home. Analyzing the connection between the two functions has been difficult because integrin mutations that disrupt one process usually thwart the other. One important protein in these processes is talin, which latches onto integrins and helps to lock them in an active posture. Other proteins such as paxillin and focal adhesion kinase (FAK) arrive at cell surface junctions to trigger spreading and detect forces acting on the cell. Researchers have been unsure about whether these proteins are present at the same time and how they interact, however.

Pinon et al. were able to isolate the two processes—holding on and spreading out—using engineered integrins that bind talin tightly. Cells carrying these integrins could fasten to a surface and settle down. But when the researchers analyzed mutations in the engineered integrins, they identified cells that were only capable of attachment, not spreading. Mutations that thwarted cell spreading also prevented integrins from binding to paxillin. Furthermore, cells didn't stretch out if they harbored altered integrins that couldn't bind to kindlin, a protein that helps activate the receptors.

The results suggest that talin doesn't have to disconnect from integrins before paxillin and FAK can bind. Instead, talin and kindlin first grab onto integrins, forming a platform that allows paxillin and FAK to join them. The findings might provide new insights into how the control of cell attachment and spreading goes wrong in cancer.

Pinon, P., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201308136.