This Week in The Journal

Distinct Roles of Tropomodulins in Dendrites and Spines

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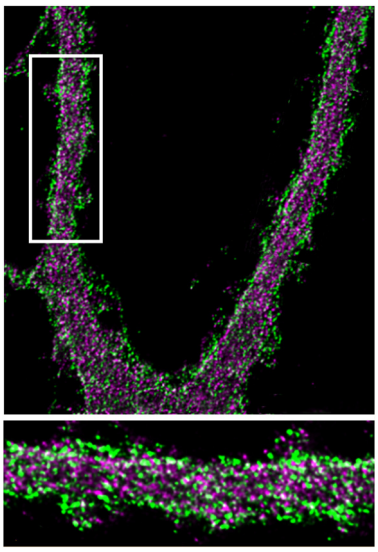
(see pages 10271–10285)

Actin filaments have an essential role in neuronal morphogenesis. Elongation of axons and dendrites begins with exploratory protrusions of actin-based filopodia from growth cones. Similar protrusions are involved in dendritic branching and spinogenesis. Subsequent rearrangement of actin filaments in these structures stabilizes neurite shafts and forms mature, mushroom-shaped spines. Throughout these developmental processes, assembly, rearrangement, and stabilization of actin filaments depend on actin-binding proteins. These include members of the tropomodulin family, which bind to the slowly growing end of actin filaments and inhibit disassembly. Omotade et al. now report that two tropomodulins, Tmod1 and Tmod2, have distinct roles in the development of dendritic shafts and spines in rat hippocampal neurons.

Both *in vivo* and *in vitro*, Tmod2 appeared before Tmod1; the latter began to be expressed when dendritic spines started forming. In cultured neurons, both Tmod1 and Tmod2 were present in dendritic shafts, as well as spines. Within spines, both tropomodulins were restricted to the neck and center of the head, where actin filaments are relatively stable; they were largely absent from the perimeter of spine heads, where actin filaments are dynamic. Tmod1 and Tmod2 puncta rarely overlapped, however.

Knocking down Tmod2 reduced dendritic length and branching in cultured neurons, but knocking down Tmod1 did not. Knocking down either tropomodulin reduced the stability of actin filaments in spines, but the effects on spine and synapse density differed. Whereas knocking down Tmod2 increased the density of spines and the frequency of miniature EPSCs, knocking down Tmod1 reduced the density of mushroom-shaped spines, increased the density of filamentous protrusions, decreased the proportion of spines apposed to presynaptic markers, and reduced the frequency and amplitude of miniature EPSCs.

These results suggest that Tmod2 stabilizes actin filaments in dendritic shafts to support dendritic growth and branching, whereas Tmod1 primarily promotes spine maturation and stabilization leading to synapse formation. Why knocking down Tmod2 enhances spine and synapse formation is unclear, but it might allow enhanced binding of Tmod1 to actin filaments in these structures. Future work should investigate this question and determine whether and how tropomodulin is regulated during activity-dependent remodeling of dendrites and spines.



Tmod1 (purple) and Tmod2 (green) are present in non-overlapping patterns in dendritic shafts and spines of cultured hippocampal neurons. See Omotade et al. for details.

Retrograde Transport and Central Effects of Botulinum Toxin

Matteo Caleo, Matteo Spinelli, Francesca Colosimo, Ivica Matak, Ornella Rossetto, et al.

(see pages 10329–10337)

The ability of botulinum neurotoxin type A (BoNT/A) to reduce muscle contraction has been exploited not only for reducing wrinkles—for which it is widely known—but also for treating spasticity, focal dystonia, and other conditions involving excessive muscle contraction. When injected into muscles, BoNT/A is taken up by the most active motor neuron terminals, where it cleaves SNAP-25, a membrane protein required for synaptic vesicle fusion. Consequently, BoNT/A reduces acetylcholine release and thus weakens contractions. But accumulating evidence suggests that BoNT/A can have effects beyond motor terminals. Indeed, BoNT/A is transported retrogradely in motor axons *in vitro*, and when BoNT/A is injected into rodent whisker pads, cleaved SNAP-25 appears in the facial nucleus. Similarly, when BoNT/A is injected into the superior colliculus, it is retrogradely transported to the retina, after which SNAP-25 fragments appear in cholinergic amacrine cells. Whether SNAP-25 fragments detected in these experiments are the products of local cleavage or are instead generated in motor terminals and then retrogradely transported has been unclear, however.

To address this question, Caleo, Spinelli, et al. injected BoNT/A into whisker pads, waited several hours for toxin transport to occur, then severed the facial nerve. SNAP-25 cleavage products continued to accumulate in the facial nucleus after axotomy, indicating that SNAP-25 was cleaved locally by previously transported active toxin. Consistent with this, injecting BoNT/A antitoxin into the facial nucleus greatly reduced accumulation of SNAP-25 fragments. Notably, double-labeling experiments indicated that although GABAergic and glutamatergic terminals were more numerous than cholinergic terminals in the facial nucleus, cholinergic terminals were more likely to contain SNAP-25 fragments after peripheral BoNT/A injection.

These data demonstrate that active BoNT/A is retrogradely transported in motor axons *in vivo* and that it is released centrally and taken up by presynaptic neurons, with a particular affinity for cholinergic terminals. Because cholinergic inputs excite motor neurons, this central effect is expected to reduce motor neuron activity and thus help to reduce muscle spasticity. Why BoNT/A is preferentially taken up by cholinergic terminals in the facial nucleus, as well as in the retina and in the periphery remains a mystery to be solved in the future.

This Week in The Journal was written by Teresa Esch, Ph.D.