This Week in The Journal

CD2AP Facilitates TrkA Interactions

Benjamin J. Harrison, Gayathri Venkat, James L. Lamb, Tom H. Hutson, Cassa Drury, et al.

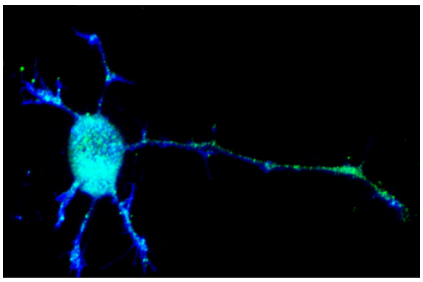
(see pages 4259 – 4275)

Sprouting of uninjured axons helps to restore function after nerve damage, but it can also cause pathological conditions such as neuropathic pain and autonomic dysreflexia after spinal cord injury. Because relatively little is known about the molecular mechanisms underlying axonal sprouting in the mature nervous system, Harrison et al. previously used microarray analyses to identify proteins upregulated in sprouting sensory axons after adjacent nerves were transected. One of the most strongly upregulated genes encoded the scaffolding/adaptor protein CD2-associated protein (CD2AP). The authors have now elucidated the role of CD2AP in collateral sprouting.

Axons of spared nerves sprouted and reinnervated denervated skin within 4 weeks of injury. Throughout this period, CD2AP levels were increased in the sprouting axons. This increase may have been triggered by nerve growth factor (NGF), which is released by denervated skin and induces sprouting of spared sensory neurons. Consistent with this hypothesis, treating neuron-like PC12 cells with NGF to induce neurite outgrowth also increased CD2AP expression. In these cells, CD2AP accumulated in F-actin-rich regions, including branch points and the tips of growth cones, where it colocalized with the NGF receptor TrkA. CD2AP also associated with RAB5-expressing early endosomes, which are thought to transport activated TrkA to the cell body, where it can activate transcription factors to regulate gene expression.

Knocking down CD2AP reduced the number of filopodia on PC12 growth cones and reduced localization of TrkA with RAB5. In addition, CD2AP knockdown decreased the association between TrkA and one of its local effectors, the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K), and it reduced NGF-induced activation of AKT kinase, an indirect target of PI3K. Notably, however, CD2AP knockdown did not affect NGF-induced activation of ERK kinase.

These results suggest that NGF increases expression of CD2AP, which in turn facilitates interactions between the NGF receptor and specific effectors in growth cones and endosomes. Targeting these interactions therapeutically might help promote optimal levels of sprouting of spared axons after peripheral nerve injury. Interestingly, CD2AP mutations and dysfunction of NGF signaling have been linked to Alzheimer's disease, so these results might lead to therapies for that disease as well.



CD2AP (green) colocalizes with actin (blue) in PC12 cells. See Harrison et al. for details.

Ascl1 Helps Specify Enteric Neuron Subtypes

Fatima Memic, Viktoria Knoflach, Rebecca Sadler, Gunilla Tegerstedt, Erik Sundström, et al.

(see pages 4339 – 4350)

The gastrointestinal tract has its own nervous system, the enteric nervous system (ENS), which controls gastrointestinal muscle contractions, fluid flux across the lining of the gut, blood flow, and nutrient uptake. ENS neurons are generated from neural crest stem cells, most of which migrate caudally from the vagal neural crest to populate the developing gut wall. Enteric neuronal stem cells differentiate into several classes of neurons, including sensory neurons, interneurons, and excitatory and inhibitory motor neurons, which express a variety of neurotransmitters. Although the transcriptional programs that drive neuronal subtype specification in the ENS are poorly understood, the transcription factor Ascl1 has been proposed to specify serotonergic and other neuronal fates.

Memic et al. report that Ascl1 is first expressed in mouse neural crest stem cells as they migrate into the foregut, and its expression declines as stem cells differentiate into neurons. Ascl1-expressing stem cells generated all types of ENS neurons, as well as glial cells. Knocking out Ascl1 delayed enteric neurogenesis and reduced the number of neurons present around birth. Loss of neurons was not uniform, however: the reduction was greatest in the esophagus and became progressively less pronounced along the rostral–caudal extent of the gastrointestinal tract. Furthermore, while knocking out Ascl1 reduced the proportion of neurons expressing Calbindin (Calb1), tyrosine hydroxylase (TH), or vasoactive intestinal peptide (VIP) in some parts of the gastrointestinal tract, the percentage of neurons expressing nitric oxide synthase increased, and the proportion of serotonin-expressing neurons was unchanged. Importantly, rescuing neurogenesis by expressing neurogenin2 in Ascl1-null mice did not rescue the generation of Calb1-, TH-, and VIP-expressing neurons, indicating these deficits were not simply the result of decreased neurogenesis.

These results confirm that Ascl1 is involved in enteric neurogenesis and specification of some enteric neuron types. Contrary to previous results, however, they suggest that Ascl1 does not specify serotonergic fate. The reason for this discrepancy remains unclear, but it likely relates to the use of different serotonergic markers in this and previous studies. Regardless, these findings may inform attempts to generate enteric neurons *in vitro* for use in treating Hirschsprung disease, in which caudal enteric neurons fail to develop, or chronic Chagas disease, in which *Trypanosoma* infection leads to degeneration of ENS neurons.

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