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Slit holds a strange attraction for filopodia

The guidance cue Slit induces axonal repulsion by directing the extension of growth cone filopodia.

Growth cones are dynamic, actin-rich structures at the tips of developing axons that lead the axon to its correct target by responding to extracellular guidance cues. In general, attractive cues are thought to stimulate growth cone actin assembly, while repulsive cues induce actin depolymerization. McConnell et al. find, however, that Slit ligands repel dorsal root ganglion (DRG) axons by inducing the extension of actin-rich filopodia (1).

During development, DRG axons grow into the spinal cord. When they encounter Slit ligands secreted from the spinal cord midline, the axons bifurcate and extend branches along the rostral-caudal axis of the body. Surprisingly, this Slit-induced change in axon direction requires the activity of Ena/VASP proteins (2, 3), which stimulate the assembly of unbranched actin filaments to form rod-like membrane protrusions called filopodia. "Because the general model is that repulsive cues cause actin depolymerization in the growth cone, we wanted to understand how proteins that promote actin assembly could mediate the repulsive response to Slit," explains Frank Gertler, from the Massachusetts Institute of Technology.

"The downstream signals from repulsive receptors can be distinct."

In vitro, high concentrations of Slit cause DRG growth cones to collapse, but when Gertler and colleagues, led by postdoc Russell McConnell, imaged the process at high temporal resolution, they saw that, before collapsing, the growth cones formed numerous, long filopodia (1). Moreover, when the researchers exposed DRG axons to gradients of Slit, the growth cones extended filopodia toward the repulsive cue's source, before turning away to grow in a different direction.

McConnell et al. wondered whether filopodia were required for Slit-induced repulsion. When the researchers selectively blocked filopodia formation with low doses of the actin polymerization inhibitor cytochalasin D, DRG axons were no longer repelled by Slit gradients. "The growth cones didn't even respond to concentrations of Slit that would normally cause collapse," Gertler says.

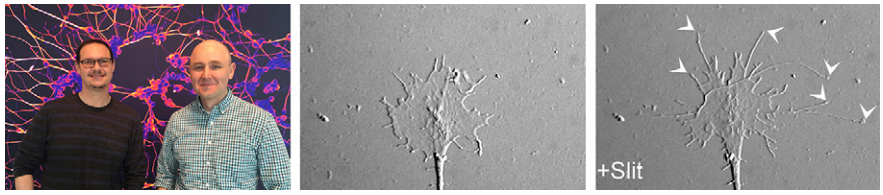
The growth cones of DRG axons isolated from mouse embryos lacking all three members of the Ena/VASP family also failed to form filopodia, and were not repelled by Slit gradients in vitro. In vivo, Ena/VASP-deficient DRG axons often crossed the spinal cord midline instead of bifurcating along the rostral–caudal axis, a similar phenotype to that seen in embryos lacking Slit ligands or their cognate Robo receptors (4).

Ena/VASP proteins might therefore mediate Slit-induced repulsion by stimulating filopodia elongation in response to the guidance cue. Indeed, Ena/VASP family members can bind to the intracellular domain of Robo receptors (2, 3), and McConnell et al. found that this interaction was enhanced by Slit. To investigate whether this association was required for Slit-induced axonal repulsion, the researchers used chimeric receptors consisting of the intracellular domain of Robo fused to the extracellular domain of the HGF receptor Met (5). The growth cones of DRG neurons expressing this chimera extended filopodia toward a source of HGF, and were subsequently repelled as if they were encountering Slit. But a point mutation that disrupts the Robo-Ena/VASP interaction abolished this response to HGF, indicating that the ligand-induced recruitment of Ena/VASP to Robo receptors promotes the directional elongation of filopodia and the subsequent repulsion of axonal growth cones.

Not all repulsive cues work this way, however. McConnell et al. found that Semaphorin3A induced DRG growth cone collapse without stimulating filopodial extension. "So the downstream signals from repulsive receptors can be distinct," Gertler says, adding that this mechanistic diversity may help fine-tune axonal responses to combinations of guidance cues in vivo.

Filopodia formed in response to the attractive guidance cue netrin are adhesive and promote growth cone motility. Slit-induced filopodia likely have a more sensory role, potentially sampling the growth cone's environment to better detect shallow gradients of the guidance cue before somehow inducing a change in growth cone direction. "We're interested in figuring out how the filopodia growing toward Slit differ from other filopodia," says McConnell. "They may have a unique molecular composition or biochemical function."

FOCAL POINT



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Frank Gertler (left), Russell McConnell (right), and colleagues examine how the repulsive guidance cue Slit affects the cytoskeletal dynamics of axonal growth cones, and find that it induces the formation of filopodia (white arrowheads) that extend toward the ligand's source. These oriented filopodia are required for axonal repulsion, and their elongation depends on the Ena/VASP family of actin polymerization factors, which are recruited to Slit's receptor, Robo, upon ligand stimulation.

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