nature neuroscience gateway

NEUROTECHNIQUES

Cell bodies get the shaft

Neuroscience Gateway (February 2008) | doi:10.1038/ngw1807

Researchers physically separate neuron cell bodies and neurites to determine signaling pathways important in process extension.

What does it take for one neuron to reach out and touch another? In neuritogenesis, neurons develop the long, thin projections that eventually become axons and dendrites. Because these neurites are so small, it has been difficult to differentiate them biochemically from neuron cell bodies. Now Pertz *et al*. report proteomic analysis of separate populations of neurites and somas in a recent article in *Proceedings of the National Academy of Sciences*.

During neuritogenesis, cell bodies develop microtubule-rich projections capped by an actin-rich growth cone. This process involves chemical gradient sensation, integrin-mediated adhesion, membrane trafficking and cytoskeletal reorganization.

Neurites receive directional cues from extracellular matrix proteins and soluble factors. The authors plated neuroblastoma cells on porous filters covering the bottom of the culture dish, which was coated with the extracellular matrix protein laminin. More than 80% of neuroblastoma cells extended neurites through the filters onto the laminin-coated surface. The authors mechanically separated and lysed the cell bodies and neurites on the tops and bottoms of the filters, respectively. They characterized neurite and soma proteomes by two-dimensional liquid chromatography-coupled tandem mass spectrometry and confirmed their findings by western and gene ontology analysis. Of the 4855 proteins identified, 1229 were enriched in neurites, 1676 were enriched in somas, and 1950 were equally distributed.

Signaling pathway analysis showed differences between the samples. The neurite, soma and equally distributed proteomes contained 39, 7 and 5 pathways, respectively. Pathways that regulate axon guidance and the actin cytoskeleton were enriched in the neurite proteome, whereas pathways that regulate cell cycle and steroid hormone signaling were enriched in the soma proteome. Although the authors expected pathways involved in apoptosis and growth factor signaling to be enriched in the soma, these pathways were enriched in the neurite instead, suggesting that these processes may be important in neurite extension or guidance.

Previous research indicated important roles for the Rho family GTPases Rac and Cdc42, which regulate the actin cytoskeleton in response to extracellular cues, in neuritogenesis. Guanine-nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) regulate Rac and Cdc42. The majority of GEFs and GAPs localized to the neurite sample. However, of the 16 regulators downstream of Rac and Cdc42, 10 were equally distributed.

What do GEFs and GAPs do in neuritogenesis? Normally, neuroblastoma neurites extend and retract repeatedly. However, short interfering RNA (siRNA) directed against the GAPs ArhGAP30, SrGAP2 and BCR or the GEFs Dock4 and Dock10 reduced extension and retraction, resulting in longer, straighter neurites. In contrast, siRNA directed against the GEF Trio resulted in growth cone defects.

The authors hope to similarly analyze neurite and soma proteomes from primary neurons. Perhaps different culturing conditions will allow the analysis of signaling pathways important in neurite maturation and guidance.

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1. Pertz, O. C. *et al.* Spatial mapping of the neurite and soma proteomes reveals a functional Cdc42/Rac regulatory network. *Proceedings of the National Academy of Sciences* **105**, 1931–1936 (2008). | Article | ChemPort |