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Retrograde and trophic signaling mechanisms in healthy and injured nerves

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How do nervous systems build themselves, and what are the molecules or mechanisms that might allow their repair after injury? How do the "simple" nervous systems of invertebrates repair themselves after injury, whereas lesions in mammalian brain have such debilitating consequences? What are the cellular mechanisms regulating survival or regeneration signaling in neurons? All these questions fascinate us, but currently the main focus is on retrograde signaling in healthy and in injured neurons. Axons are extremely long in relation to the size of neuronal cell bodies, and highly sophisticated mechanisms are required for the transmission of macromolecular signals from terminals or lesion sites to cell bodies. We seek to understand the molecular basis of these mechanisms.

Retrograde injury signaling in lesioned nerve.

The cell body of a lesioned neuron must receive accurate and timely information on the site and extent of axonal damage, in order to mount an appropriate response. Specific mechanisms must therefore exist to transmit such information along the length of the axon from the lesion site to the cell body. These include retrogradely transported activated proteins emanating from the injury site, termed positive injury signals. Over the past four years we have been working on the positive retrograde injury signal hypothesis, starting from observations that axoplasmic proteins containing nuclear localization signals (NLS) signal retrogradely in injured nerve. In most recent work we have demonstrated that the importin/karyopherin alpha and beta families underlie this process. We found importins in axons at significant distances from the cell body and demonstrated that importin beta protein is increased after nerve lesion by local translation of axonal mRNA. This leads to formation of a high-affinity NLS binding complex that traffics retrogradely with the motor protein dynein. Trituration of synthetic NLS peptide at the injury site of axotomized dorsal root ganglion (DRG) neurons delays their regenerative outgrowth, and NLS introduction to sciatic nerve concomitantly with a crush injury suppresses

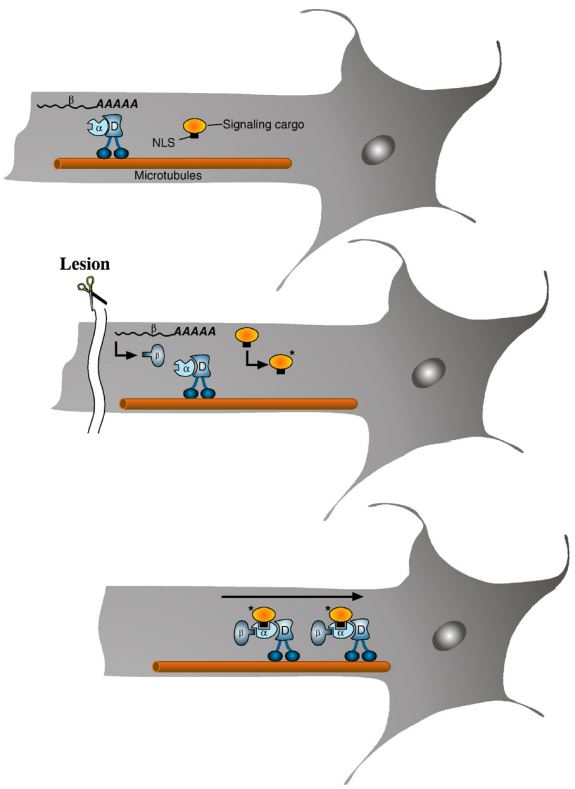

Fig. 1 *Schematic model of the retrograde injury-signaling mechanism in lesioned nerve. Importin-beta mRNA is found in axons, and is translated locally to protein upon lesion, concomitantly with activation of NLS-containing signaling proteins. Interaction of the newly synthesized importin-beta protein with importin-alpha complexed with dynein forms a retrogradely transported high-affinity binding site for NLS, thus enabling retrograde injury signaling from the axonal lesion site to the cell body and the nucleus.*

the conditioning lesion induced transition from arborizing to elongating growth in L4/L5 DRG neurons. These data suggest a model whereby lesion-induced upregulation of axonal importin beta enables retrograde transport of signals that modulate the regeneration of injured neurons. This model is currently under examination in a number of paradigms in the laboratory. We would like to have a detailed understanding of the regulation of axonal importin-beta, since this is a critical regulation point for the whole system. Proteomic approaches are being applied to determine the identities of the signaling proteins carried by the complex, and microarray systems are being used to define specific transciptional responses to these injury signals.

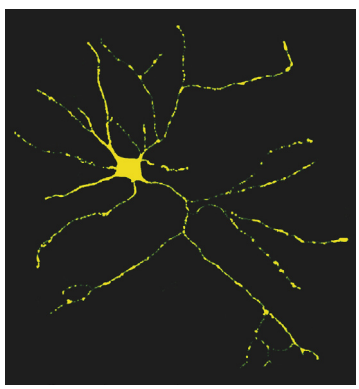

Fig. 2 *Embryonic hippocampal neuron immunostained with antibodies for importin-alpha and importin-beta. Yellow indicates co-localization, note that staining is found in all processes, including all the dendrites and the axon.*

Retrograde signaling by the p75 receptor.

The NGF family of neurotrophins has crucial roles in development and maintenance of the nervous system. The p75 neurotrophin receptor (p75) binds all known neurotrophins, as well as other ligands. A large number of intracellular molecules have been found to interact with p75, thus this receptor integrates multiple extracellular signals with a range of intracellular signaling pathways, leading to diverse biological consequences. Although retrograde signaling from trk receptors has been intensively studied in recent years, p75 retrograde signaling in neurons has remained enigmatic. We are developing a number of fluorescent and biotinylated probes that allow monitoring of p75 internalization and transport. Our studies so far revealed that p75 is internalized together with its ligand. Intracellular interactors are then recruited to the complex, thus creating a potential signaling endosome. How does this endosome escape the recycling pathway and connect to the retrograde transport machinery? Strikingly, many intracellular p75 interactors contain an NLS. Thus, if p75 signaling somehow also induces local formation of an importins complex, the signaling endosome may be transported in a similar manner as described above. Current efforts are geared to examination of this hypothesis.

Selected Publications

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