# Spatial Dynamics of Intracellular Communication

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Central nervous systems range in complexity from the few hundred neurons of nematode worms and hydroid coelenterates to the 10000000000000 or so neurons comprising the brain of the reader of this page. How do these 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 our 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. Three distinct types of signals have been postulated to underlie this process, starting with injury-induced discharge of axon potentials, and continuing with two distinct types of retrogradely transported macromolecular signals. The latter include, on the one hand, an interruption of the normal supply of retrogradely transported trophic factors from the target; and on the other hand activated proteins emanating from the injury site. Over the past five years we have combined proteomics and cell biology approaches to characterize the mechanisms by which injury signals traffic retrogradely in injured nerve. We 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 nuclear localization signal (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 the conditioning lesion induced transition from arborizing to elongating growth in L4/L5 DRG neurons. In most recent work we have

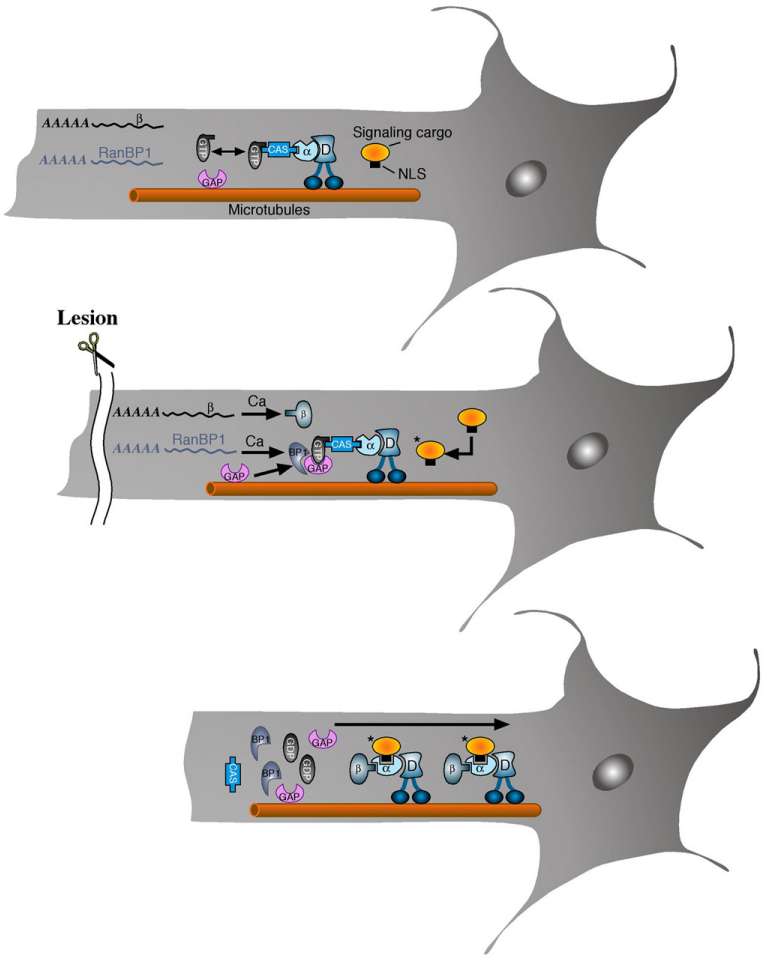


***Fig. 1*** *Schematic model of Ran regulation of axonal retrograde signaling after nerve lesion. Under normal conditions (upper panel), Ran-GTP bound to axonal CAS and importins will prevent importin α and β interaction and binding of cargo proteins. Importin β1 and RanBP1 are found in the axon as mRNAs. Following lesion (middle panel), localized translation of these mRNAs leads to upregulation of the corresponding proteins. The newly synthesized RanBP1 stimulates disassociation of RanGTP and RanGAP synergized hydrolysis, thus allowing formation of a cargo-binding complex of importin α with de novo synthesized importin β (lower panel).*

shown that the system is regulated by the small GTPase Ran in axons (Figure 1). These data suggest a model whereby lesion-induced regulation of axonal importins enables retrograde transport of signals that modulate the regeneration of injured neurons.

**Our current efforts in this project are focused on the following three questions**

1. How is formation of the retrograde injury-signaling complex regulated? Here we are analyzing UTR motifs in localization of specific transcripts to axons, the regulation of their local protein translation, and roles of additional regulators of nuclear import mechanisms.
2. What are the signaling molecules and how are they modified? Here we are employing proteomics and mass spectrometry approaches, as well as targeted analyses of specific candidates.
3. What is the transcriptional response to the retrograde injury signal? Microarray-based approaches are being used in conjunction with specific perturbations of the signal in order to focus on transcriptional responses important for regeneration.

**Retrograde signaling by neurotrophic factors**

The NGF family of neurotrophins has crucial roles in development and maintenance of the nervous system. Trk receptors are receptor tyrosine kinases with specificity for individual neurotrophins, while the p75 neurotrophin receptor (p75) binds all known neurotrophins, as well as other ligands. In addition to activating independent signaling pathways, p75 can function as part of a receptor complex with the trks or with other receptors. 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 have shown that p75 is internalized to the recycling endosome upon neurotrophin binding, but at much slower kinetics than trk-neurotrophin complexes in the same cells. Nonetheless p75 is internalized together with its ligand, and intracellular interactors from the MAGE gene family are recruited to the complex, thus creating a potential signaling endosome or proteolytically cleaved signaling complex. How might this entity connect with the retrograde transport machinery? Strikingly, many of the intracellular p75 interactors contain nuclear localization signals (NLS). Thus, if p75 signaling somehow also induces local formation of an importins complex, the signaling endosome/complex may be transported in a similar manner to the injury-signaling mechanism described above. Current efforts are geared to examination of this hypothesis.

**Differentiation/Survival Signaling in neurons or in tumors of neural origin**

Over the past decade we have carried out a number of protein-protein interaction screens to study neurotrophic signaling networks. A yeast RRS screen using the p75 intracellular domain as bait pulled out necdin, a member of the MAGE gene family known primarily as a facilitator of cell cycle exit and neuronal differentiation. The necdin gene is located in the chromosomal region affected in Prader-Willi syndrome (PWS), and necdin-deficient mice display phenotypes mimicking some aspects of PWS. Necdin has been shown to be present in different subcellular compartments ranging from membrane receptor association to cytoplasmic and nuclear localization, raising the question whether its functional importance is as a signal transduction protein or an effector of gene expression, or both. Additional RRS screens with necdin as bait suggest that it is involved in two interaction networks – one primarily nuclear and one including cytoplasmic and plasma membrane interactors. Current efforts are focusing on the mode of translocation of necdin between cytoplasmic and nuclear compartments, and the role of necdin in each of these compartments for differentiation and survival. We are also studying a new necdin interactor that we call Karet, which is involved in TrkA-dependent cell death in transfected cells. TrkA-dependent cell death is an atypical phenomenon that has been described in very few reports in the literature, primarily in pediatric tumor cells of neural origin, underscoring the interest in elucidating the functions and mechanisms of action of karet.

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