Main Research Interests

Central nervous systems range in complexity from the few hundred neurons of nematode worms and hydroid coelenterates to the 100,000,000,000,000 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 mechanism by which positive 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. 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 (Hanz et al., 2003).

Identification of a mechanism for retrograde signaling in lesioned neurons focused our attention on the need to characterize the signaling molecules trafficked after injury. Initial leads came from studies wherein we utilized a molluscan model system in a differential proteomics approach to identify components of the retrograde signaling complex. We used a nerve lesion/ligation model in the mollusk Lymnaea and compared retrogradely concentrated axoplasm from lesioned and control Lymnaea nerves by 2D-PAGE. Mass spectrometric sequencing of 128 differential spots allowed their assignment to over 40 different proteins, some belonging to a vesicular ensemble blocked by the lesion, and others comprising an upregulated ensemble highly enriched in calpain cleavage products of a type III intermediate filament (Perlson et al., 2004). Truncation mutants of the closely related mammalian type III IF vimentin can translocate from cytoplasm to nucleus in transfected cells and vimentin is known to interact with signaling molecules. We therefore examined potential roles of mammalian type III intermediate filaments in retrograde injury signaling in the sciatic nerve and in neurons of the dorsal root ganglia (DRG), and showed that soluble forms of vimentin are elevated by local translation and calpain-mediated cleavage in sciatic nerve axoplasm after injury (Perlson et al., 2005). Vimentin binds phosphorylated Erk (pErk) in a calcium dependent manner, and links...
the activated MAP kinase to the retrograde transport system via direct binding of vimentin to importin beta. Strikingly, pErk is protected from phosphatases while bound to vimentin, and since calcium is elevated in the axon after injury this enables long distance translocation of an activated kinase from the lesion site to the cell body (Fig. 1). Upon arrival in the cell body pErk activates the ETS domain transcription factor Elk1, thus demonstrating a link between axonal injury and a specific transcriptional response. These findings demonstrate a way for signaling proteins lacking NLS to traffic by importin-mediated mechanisms, and suggest that retrograde injury signaling is dependent on a number of distinct molecular signals and their associated scaffolding and regulatory molecules.

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

**How is formation of the retrograde injury signaling complex regulated?** Here we are analyzing the role of calcium in the regulation of local protein translation in the axon, and axonal occurrence and roles of additional regulators of nuclear import mechanisms.

**What are the signaling molecules (in addition to pErk) and how are they modified?** Here we are employing advanced proteomics and mass spectrometry approaches to address these issues.

**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.

For more details on these and other on going projects please see our group web page.

**Selected publications**


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