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Trophic signalling in the nervous system

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Research Interests

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 allowed elaboration of increasing complexity? 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? Our research addresses specific aspects of these overall questions, as detailed below.

Evolving better brains: A need for neurotrophins?

The NGF family of neurotrophins has a crucial role in regulating neuron numbers during vertebrate development. Six years ago the prediction was made that invertebrates with simple nervous systems, such as C. elegans, would lack neurotrophins. Surprisingly, it now appears that not only C. elegans but also Drosophila melanogaster, lack homologs of the neurotrophins or their trk receptors (Jaaro et al., 2001). In contrast, we and others have identified a recognizable trk homolog in the mollusc Lymnaea, a phylum that includes species with the most complex nervous systems in the invertebrate kingdom (Van Kesteren et al., 1998). This suggests that neurotrophic signaling mechanisms might be one of the prerequisites for evolution of complex nervous systems. Our current efforts in this line are focused on analysis of the functional roles of invertebrate trks, and on using novel functional genomics screens developed in the group to identify secreted trophic ligands across phyla.

Retrograde information flow along the axon

Neuronal responses to trophic factors entail a transcription/translation dependent process wherein the neuronal cell body changes patterns of macromolecular synthesis in response to a trophic signal emanating from the nerve terminal. Similarily, neuronal cell bodies can activate regeneration programs in response to retrogradely transported injury-signaling proteins emanating from axonal lesion sites. The identity of these injury signals are largely unknown, however there is evidence that some of them are targeted to the nucleus. We use fluorescently labeled ligands to study retrograde transport and its role in mediating trophic signals. In addition, we are using proteomic approaches to identify retrograde protein ensembles activated after lesion. Axoplasm from lesioned and ligated Lymnaea nerves was compared to ligated controls by 2D-PAGE and high resolution mass spectrometry. A comprehensive series of silver-stained gels at different pI ranges revealed approximately 60 differential spots, including both expected and unexpected protein families (Perlson et al., 2002). The more interesting candidates have a wide phylogenetic distribution in vertebrates and invertebrates, and their functional characterization is currently in progress.

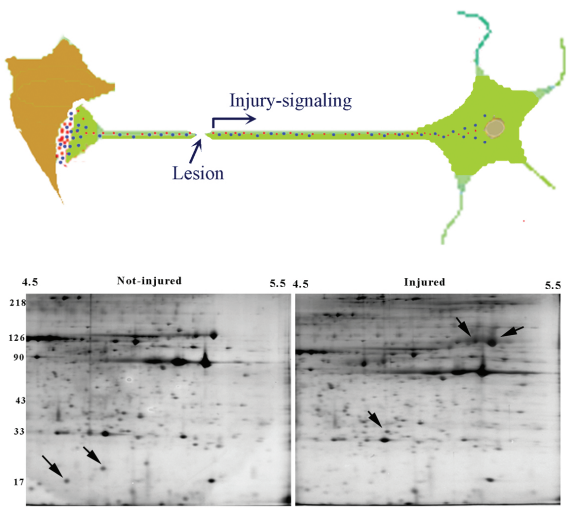

Fig. 1 *2D-PAGE of axoplasm from ligated nerves, comparing control and lesioned preparations 18 hr after injury. Arrows indicate examples of differential spots.*

Life and death downstream of the p75 neurotrophin receptor

All neurotrophins interact with two receptor types, the shared p75 neurotrophin receptor (p75), and discriminative receptor tyrosine kinases of the trk family. Whereas it is well established that the trk receptors are crucial in mediating the survival role

of neurotrophins, the roles of p75 are a still unfolding story, and can be broadly divided between regulation of phenotype, versus regulation of cell death. A central aim of the laboratory is to obtain comprehensive insight on p75 signaling mechanisms in individual neurons. Recent work has focused on characterization of the protein interaction network for p75 in neurons, and on analysis of the mechanisms of internalization and retrograde transport of p75. We have used RRS, the Ras rescue system for protein interaction cloning to identify new interactors for p75. One of the first clones obtained in our screen represented a protein belonging to the MAGE domain family. Subsequent assays revealed that a range of MAGE family members interact with p75, and modulate differentiation or apoptosis of p75-expressing cells (Tcherpakov et al., 2002). Specific internalization and retrograde trafficking of p75-ligand complexes has been visualized with a fluorescein-labeled monoclonal antibody. P75 is internalized with slower kinetics than trk receptor complexes, although both appear to follow the clathrin coated pits pathway. We are currently interested in identifying the retrograde trafficking mechanisms and organelles that carry p75 signaling complexes, in order to integrate the cell biology and signal transduction approaches to a comprehensive understanding of p75 functions in neurons.

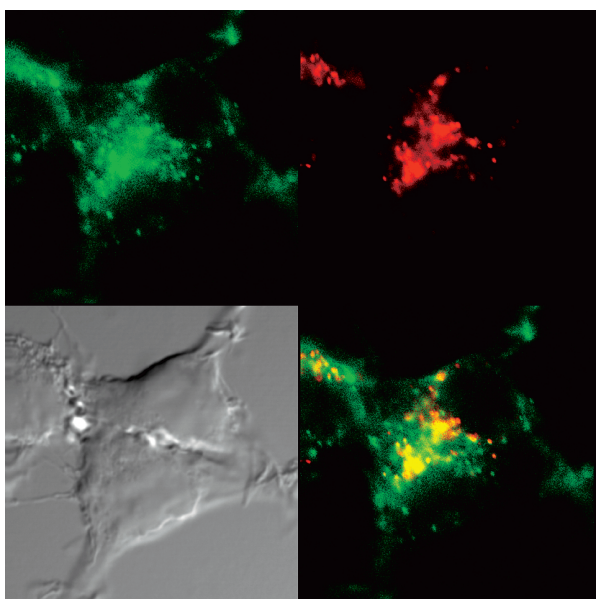

Fig. 2 *P75 (green) and transferrin (red) internalization in PC12 cells. The observed co-localization (yellow) indicates that p75 accumulates in the recycling endosome.*

Selected Publications

van Kesteren, R.E., Fainzilber, M., Hauser, G., van Minnen, J., Vreugdenhil, E., Smit, A.B., Ibanez, C.F., Geraerts, W.P.M., & Bulloch, A.G.M. (1998) Early evolutionary origin of the neurotrophin receptor family. EMBO. J. 17, 2534-2542.

Brann, A.B., Scott, R., Neuberger, Y., Boldin, S., Fainzilber, M., & Futerman, A.H. (1999) Ceramide signaling downstream of the p75 neurotrophin receptor mediates the effects of nerve growth factor on outgrowth of cultured hippocampal neurons. J. Neurosci. 19, 8199-8206.

Conticello, S.G., Pilpel, Y., Glusman, G., & Fainzilber, M. (2000) Position-specific codon conservation in hypervariable gene families. Trends Genet. 16, 57-59.

Conticello, S.G., Gilad, Y., Avidan, N., Ben-Asher, E., Levy, Z., & Fainzilber, M. (2001) Mechanisms for evolving hypervariability: the case of conopeptides. Mol. Biol. Evolut. 18, 120-131.

Jaaro, H., Beck, G., Conticello, S.G., & Fainzilber, M. (2001) Evolving better brains: A need for neurotrophins? Trends in Neurosci. 24, 79-85.

Brann, A.B., Tcherpakov, M., Williams, I.M., Futerman, A.H., & Fainzilber, M. (2002) NGF-induced p75-mediated death of cultured hippocampal neurons is age-dependent and transduced through ceramide generated by neutral sphingomyelinase. J. Biol. Chem. (in press).

Conticello, S.G., Kowalsman, N.D., Gerrits, A.J., Sato, K., Petersen, C.M., Aronheim, A., & Fainzilber M. (2002) The propeptide of a cysteine-rich mini-protein enhances its secretion via an interaction with sorting receptors from the sortilin family. Submitted for publication.

Tcherpakov, M., Bronfman, F.C., Conticello, S.G., Levy, Z., Niinobe, M., Pinon, L., Davies, A.L., Arenas, E., & Fainzilber, M. (2002) The p75 neurotrophin receptor interacts with multiple members of the MAGE gene family to modulate neuronal differentiation and cell death. Submitted for publication.

Perlson, E., Medzihradszky, K.F., Darula, S., Burlingame, A.L. and Fainzilber, M. Cleaved and modifield intermediate filament is a major functional component of the injury-signaling retrograde protein ensemble in lesioned nerve. Submitted for publication.

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For additional information see:

www.weizmann.ac.il/Biological_Chemistry/scientists/Fainzilber/Fainzilber.html