

Meeting report

Towards understanding neural survival, differentiation and death

Karen Bailey and Andrew Gibbons

Address: School of Biological Sciences, Monash University, Victoria 3800, Australia.

Correspondence: Karen Bailey. E-mail: karen.bailey@sci.monash.edu.au

Published: 14 June 2002

Genome Biology 2002, **3(7)**:reports4018.1–4018.3

The electronic version of this article is the complete one and can be found online at <http://genomebiology.com/2002/3/7/reports/4018>

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A report from the 14th Biennial Meeting of the International Society for Developmental Neuroscience, Sydney, Australia, 31 January to 4 February 2002.

The Biennial meeting of the International Society for Developmental Neuroscience was held in the beautiful surrounds of Darling Harbour, Sydney. It preceded the Australian Neuroscience conference, allowing delegates to listen to renowned workers from both the international and local community. The conference covered a wide range of topics from molecular studies to cell biology; here, we highlight some of the talks about neurotrophic factors, neuronal cell death, neuronal patterning, and axonal development.

Neurotrophic factors

During the past ten years there has been a wealth of information generated about neurotrophic factors and their mechanisms of action. While the intracellular pathways utilized by these factors and their receptors have been extensively studied, there is little known about the molecular events following signal transduction. Lloyd Greene (Columbia University, New York, USA) has started to address these issues using serial analysis of gene expression (SAGE). This method has allowed Greene's group to identify transcripts that are regulated following treatment with nerve growth factor (NGF) of the PC12 rat pheochromocytoma cell line, a widely used model of growth and differentiation. Many of the regulated genes were identified as transcription factors and included the expected genes such as *Fos* and *Jun*, which encode the immediate-early transcription factors, but there were also some unexpected genes. One of these was *MAFK*, which encodes a basic zipper (bZIP) transcription factor that is broadly expressed in neurons. The *MAFK* mRNA and protein levels are elevated in response to NGF, and the mRNA levels remain elevated for 2-3 days. The studies

suggest that *MAFK* regulates neurite outgrowth, a process that occurs in PC12 cells when they are treated with NGF. Another gene that was identified, *ATF5*, was shown to be downregulated in response to NGF. *ATF5* mRNA levels drop rapidly in response to NGF and the ensuing neurite outgrowth, whereas exogenous expression of *ATF5* inhibits NGF-promoted neurite outgrowth. This study, among others, indicates that there is still much we do not know about neurotrophic factor signaling and the events involved in neuronal survival, maturation, and differentiation.

The studies presented by Lloyd Greene provided a backdrop for a session on trophic regulation of synaptic plasticity. Ira Black (Robert Wood Johnson Medical School, Piscataway, USA) discussed recent studies aimed at identifying genes associated with trophic regulation of synaptic plasticity. Black used differential gene expression to identify transcripts that are regulated by brain-derived neurotrophic factor (BDNF) in populations of cells, as well as at the single-cell level. The genes for the Ras-like GTPase Rab3A and guanylate cyclase were two of the genes identified that showed increased transcription and translation as identified by western blot analysis. Black and his colleagues are now using similar types of cells and treatments with microarray analysis to identify other genes that are either upregulated or downregulated in response to trophic factors. Bai Lu (National Institutes of Health, Bethesda, USA) continued the focus on neurotrophic actions on synaptic plasticity, describing studies of both acute and long-term effects on the expression of synaptic proteins. Acute application of neurotrophin-3 (NT3) to the neuromuscular junction potentiates neurotransmitter release, whereas long-term effects include structural and functional changes. They observed that inhibition of internalization of NT3 in complex with its tyrosine kinase receptor, TrkC, abolished the long-term effects but not the acute effects. Conversely, blocking the action of the phosphoinositide 3-kinase (PI3K) activity affected both acute and long-term effects of NT3, yet

overexpression of a dominant-negative form of the Akt protein kinase (a downstream target of PI3K) affected the long-term but not acute effects of NT3. These studies suggest that the acute and long-term effects on synaptic transmission may use distinct signaling mechanisms.

Regulation of neuronal cell death

Although neuronal survival has long been of intense interest to developmental neurobiologists, extensive research is now being undertaken to understand the cellular and molecular events controlling neuronal cell death. David Kaplan (McGill University, Montreal, Canada) discussed some of the recent advances towards understanding the regulation of apoptosis in two signal transduction pathways: one involving TrkA, the NGF tyrosine kinase receptor, and the other the p75 low-affinity receptor that is capable of binding all the neurotrophins. Survival signals mediated by the TrkA receptors primarily use the PI3K/Akt signaling pathway, with the MEK/MAP kinase pathway playing a secondary role. These pathways function to suppress the apoptotic signals induced by p75. The Akt protein has been found to downregulate a number of molecules upstream of the tumor suppressor p53, including mixed-lineage kinase (MLK), apoptosis signal-regulating kinase 1 (ASK1) and MAP kinase kinase kinase 1 (MEKK1). In addition to Akt regulation of cell death, truncated forms of p63 and p75 (two p53 homologs) act to directly control p53 activity. In the presence of NGF, expression of these truncated proteins is upregulated and they bind to p53, inhibiting its activity. Downstream of p53 inhibition, the XIAP inhibitor of apoptosis acts to directly inhibit the activity of the caspases, key components of the apoptotic machinery. The TrkA molecule also downregulates its own pro-survival signals by binding to the SHP1 phosphotyrosine phosphatase. SHP1 dephosphorylates the Trk receptor, thus inactivating the receptor's signaling potential in the presence of NGF. It was evident from the research presented how the TrkA and p75 signal transduction pathways are controlled at various levels.

The mechanism underlying the dual roles of p75, as a potentiator of both survival and apoptosis, is an area of intense research interest. In his overview of the bifunctionality of this receptor, Ralph Bradshaw (University of California, Irvine, USA) concluded that p75 seems to mediate cell death and survival through independent pathways. For example, the signal transduction molecule NF κ B can promote survival in response to both p75 and Trk, through the activity of the I κ B kinase (IKK), or it can be pro-apoptotic, acting through Jun. Elizabeth Coulson (Walter and Eliza Hall Institute, Melbourne, Australia) discussed the role of an intracellular 'death domain' within the p75 receptor, which has been named Chopper. The Chopper domain appears to act through a putative second messenger to regulate the activity of an as-yet unspecified K⁺ channel. The subsequent decrease in intracellular K⁺ leads to caspase activation.

Graham Barrett (University of Melbourne, Australia) presented data showing that, as a result of p75 forming a receptor complex with the TrkA dimer in the presence of NGF, p75 enhances phosphorylation of the SHC adaptor molecule by either stabilizing SHC or presenting the molecule to TrkA. In a related session in the Australian Neuroscience Meeting, Simon Murray (New York University School of Medicine, USA) described the action of p75 in the Schwann cell, where TrkA is not expressed but truncated forms of the related receptor TrkB are. The neurotrophin BDNF does exhibit activity on the cells, in the absence of the full-length TrkB receptor, yet the resulting signal transduction is quite different from that of NGF. Adaptor proteins such as TRAF and RIP2 mediate the dual roles of p75. The RIP2 protein, for example, interacts with p75 to upregulate NF κ B, leading to the inhibition of NGF-induced death by blocking the death signals.

Neuronal plasticity, patterning and repair

The neural crest gives rise to an array of different cell types, although it has been a matter of contention whether cell fate is specified within the neural tube or after multipotent neural crest progenitor cells have emigrated out of the neural tube. Within one neural crest cell lineage, the skin melanocytes, it seems that specification of cell fate occurs within the neural tube. Yvette Wilson (University of Melbourne, Australia) used the receptor tyrosine kinase c-Kit, a neural crest cell marker that is exclusively expressed in melanocyte progenitors, to determine the origin of melanocytes. A population of c-Kit-positive cells arises from the dorsal midline of the mouse embryo at embryonic day 10 before migrating into the ectoderm, where the cells express other proteins specific to melanocytes, providing evidence that melanocytes are specified in the neural tube. Yet neural crest patterning does not seem to be as preprogrammed as current models suggest. Paul Trainor (The Stowers Institute, Kansas City, USA) has been investigating the effects of transplanting cells from different rhombomeres - segmented areas of the developing hindbrain - to other sections of the neural tube. Contrary to expectation based on previous models of craniofacial development, cranial neural crest cells seem to be very plastic, with cells of the mesoderm appearing to be important for maintaining neural crest cell identity. Addressing classic studies that transplanted isthmus cells to other regions of the neural tube, and which gave support to models of preprogrammed cell fate, Trainor suggested that such effects were due to the inhibition of the activity of the homeobox transcription factor Hox2 by fibroblast growth factor 8 (FGF8), which is expressed in the isthmus.

In a shift to a direct study of human disease, Richard Faull (University of Auckland, New Zealand) discussed Huntington's chorea, which results from the degeneration of the striatal basal ganglia. Although progenitor cells are usually

associated with embryonic development, the presence of neural stem cells in the subependymal region, found adjacent to the striatal basal ganglia, has potential for Huntington's therapy. By labeling with an antibody against proliferative cell nuclear antigen (PCNA), Faull showed an increase in the thickness of the subependymal region and an associated increase in PCNA-reactive cells correlating with the severity of Huntington's chorea. It was suggested that this may be a proliferative cellular response to neuronal loss and it may be possible to provide therapy by turning on neurogenesis in this population of stem cells during the onset of the disease.

Many neurotrophic factors have been shown to promote neuronal survival and repair following injury or degeneration, often being expressed by supportive cells, such as glia, following trauma to the central nervous system (CNS). Repair of the damaged neurons does not normally occur, however. Axonal sprouting, at the wound site, is seen to occur after traumatic injury to the CNS. The sprouting axons appear to associate with microglial cells before innervating the wound site and associating with macrophages; however, axon regeneration across the wound site fails to occur. Research carried out by Peter Batchelor (University of Melbourne, Australia) suggests that a gradient of expression of BDNF and glial cell-line derived neurotrophic factor (GDNF) is established by the microglia and macrophages, respectively; this gradient does not extend beyond the wound site, however. Thus, axonal sprouting is halted in an area of high trophic support before it can enter the wound site. In an attempt to improve regeneration in the spinal cord, Giles Plant (University of Miami, USA) has used transgenic olfactory-ensheathing glia (OEG) to overexpress BDNF and NT3 in the rat rubrospinal tract following severing of the spine. The transgenic OEG were transplanted to either side of the lesion. Overexpression of BDNF and NT3 at the lesion site rescued 20-25% of the nerve fibers and motor skills were also seen to increase.

Neuronal migration and axonal development

Classic studies of neurogenesis concluded that all neurons in the cerebral cortex arise from the germinal ventricular zone at the surface of the lateral ventricles. Recent evidence suggests, however, that pyramidal cells and non-pyramidal cells are generated in distinct proliferative zones. Although pyramidal cells are derived from the neuroepithelium of the cortical ventricular zone, it is now thought that most non-pyramidal cells are generated in the ventral telencephalon. John Parnavelas (University College London, UK) has mapped a population of non-pyramidal cells migrating from the ventral telencephalon to the ventricular zone. He proposed that the cells migrate into the cortex before receiving positional cues from the ventricular zone, after which they migrate along radial glia to their destination in the developing cortex.

An understanding of the mechanism of neuronal layering requires an understanding of how the cells finish migration. As there has been no convenient method to identify migrating cells in the cortical plate, however, most research into neuronal migration has addressed migration in the intermediate zone itself, rather than the termination of migration. Using a new gene transfer technique, Kazunori Nakajima (Soka University, Tokyo, Japan) has now been able to visualize the migrating cells specifically. As well as displaying neurons migrating by locomotion and somal translocation, migrating cells were seen to undergo multipolar locomotion, jumping across radial processes.

In a final presentation on axon migration and navigation, Helen Cooper (University of Melbourne, Australia) presented results obtained from analyzing loss-of-function mutants in zebrafish that affect the netrin system. Netrin, a chemoattractive axonal navigation cue, is highly conserved among both vertebrates and invertebrates. Whereas the guidance potential of netrin and its receptor DCC (deleted in colon cancer) in axonal guidance has been studied extensively, little is known about the function of a second DCC-like receptor, neogenin, and its ligand netrin-3. DCC and neogenin are similar immunoglobulin-like proteins that differ predominantly in their intracellular regions. Netrin-3 is localized in motor neurons and sensory neurons but, unlike netrin, it does not form chemotactic gradients. Disruption of neogenin expression in fish led to aberrant brain development with a loss of defined structures. Within the spinal cord, major axon tracts were lost and netrin-3's removal uncovered chemorepulsive systems that led to axonal growth in the wrong direction. Within the brain, sensory neuron and motor neuron populations were also lost. It seems that neogenin may have a more complex role than that of simple axon guidance and its loss may lead to a possible disruption of stem cells or differentiation.

This report covers only a few of the studies presented at the meeting. Developmental neuroscience is still a growing field, and the recent advances in genomics and molecular techniques have opened up new avenues in several key areas of research. We are slowly starting to understand both the molecular and cellular events involved in the development of the nervous system and how these interact.