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Center Stage for NGF in Peripheral (but Not Central) Sensory Neuron Outgrowth

Nerve growth factor (NGF) has long been known to be required for the survival, axonal growth, and differentiation of cultured sensory and sympathetic neurons. Similarly, data from the NGF and the *trkA/NGF* receptor knockout mice demonstrated a key role for NGF in the survival of nociceptive and sympathetic neurons in vivo. However, because these neurons die without NGF, it has not been possible to study the role of NGF and TrkA signaling in axon growth and differentiation in vivo. To overcome this problem, Snider and colleagues (Patel et al., 2000 [this issue of *Neuron*]) have generated mice that lack not only NGF or Trk but also the proapoptotic BAX protein. The Snider group, in collaboration with Korsmeyer's and Johnson's groups, previously showed that naturally occurring dorsal root ganglia (DRG) and sympathetic neuron cell death is eliminated in mice deficient in BAX, and that these neurons survive indefinitely in culture without trophic factors. The prediction therefore is that the lack of BAX in *NGF/Bax* or *trkA/Bax* double null mice should prevent the sensory neurons deprived of TrkA signaling from undergoing apoptosis. The $NGF^{-/-}/Bax^{-/-}$ and $trkA^{-/-}/Bax^{-/-}$ mice thus provide an ideal system to study the requirements for neurotrophin and TrkA in axon growth and differentiation in the absence of the requirement for these proteins for neuronal survival.

Patel and colleagues (2000) first showed that in the *NGF/Bax* and *trkA/Bax* double null mice, all sensory neurons of the DRG that normally die in the absence of TrkA signaling survived. DRG axons in the double null mice extended centrally into the dorsal roots and established collatorals in the superficial laminae of the spinal cord. In contrast, peripheral sensory innervation and terminal arborization, in particular superficial skin and whisker pad innervation, were absent. Furthermore, the DRG neurons failed to express the nociceptive markers calcitonin gene-related peptide (CGRP) and substance P or the GDNF receptor Ret. This failure to express biochemical markers characteristic of nociceptive neurons appeared to be a direct action of NGF rather than a result of a failure to innervate targets, since cultured DRG explants from $Bax^{-/-}$ mice expressed CGRP in the presence of NGF, even though no target was present. These results demonstrate a requirement for TrkA/NGF signaling for sensory innervation into peripheral but not central targets, and indicate that NGF is necessary for biochemical differentiation of DRG neurons.

The approach of using the double null mice is both clever and elegant, and the data definitively demonstrate a developmentally important role for NGF in regulating sensory axon growth and differentiation, a result that confirms the predictions of numerous earlier in vitro studies. In addition, several fundamental questions about the roles of NGF were addressed for the first time in vivo, including whether NGF may function early to promote axon initiation and elongation or later as a chemoattractant or guidance factor. Snider's, Johnson's and Korsmeyer's groups previously showed that $Bax^{-/-}$ neurons survive in vitro and in vivo in the absence of trophic factor support, although the neurons were smaller and extended only rudimentary projections with short branches (Derkwerth et al., 1996; White et al., 1998; Lentz et al., 1999). Neurotrophin addition to the $Bax^{-/-}$ sensory neurons induced a bipolar phenotype and much more elaborate branching (Lentz et al., 1999). This result suggested that neurotrophins are required for extensive axon branching and guidance but not for neurite initiation or extension. In support of these data, neurotrophins have been shown to function as guidance factors in vitro (Gallo et al., 1997; Ming et al., 1997) and in vivo (O'Conner and Tessier-Lavigne, 1999), and axon extension from peripheral sensory ganglia has been suggested to be neurotrophin independent (e.g., see Lumsden and Davies, 1983; Wang et al, 1999). Patel et al. (2000) found that NGF is in fact required for initial axon elongation, since TrkA-labeled neurons from the $Bax^{-/-}/NGF^{-/-}$ mice were not found in the major nerve trunks of the hindlimb, and axon counts in the cutaneous nerve were markedly reduced. It is possible, however, that the axons could have extended short projections that then retracted during development. Patel et al. (2000) do definitively show that neurotrophin and Trk signaling are required for the maintenance of axon projections and terminal innervation, while strongly suggesting a role in axon elongation.

A key finding of Patel et al. (2000) was the observation that DRG neurons extended central axon projections to the spinal cord in the absence of TrkA signaling. Central projections were grossly normal in double null mice, suggesting that the absence of NGF signaling does not affect the ability of neurons to respond to different guidance and branching cues. Indeed, branching of these central projections may depend upon the Slit family of guidance factors (Wang et al., 1999), which function in an NGF-independent manner. It will also be critical to determine whether correct synaptogenesis occurs or if functional connections were made in the double null mice. In this regard, both NGF and brain-derived neurotrophic factor (BDNF) have been implicated in modulating synaptic density and innervation in vivo (Causing et al., 1997; Davis et al., 1997).

The Patel et al. (2000) study demonstrates a requirement for NGF signaling in sensory growth and differentiation, which prompts an important question about the nature of the intracellular signals used by TrkA to regulate these responses in vivo. This question is not an easy one, since several of the major mediators of NGF signaling may be multifunctional, promoting both survival and axonal growth and differentiation. For example, Ras, MEK/MAP kinase, and SH2-B each regulate NGF-mediated axonal growth and survival (Kaplan and Miller, 2000). Mice whose neurons are deficient in both BAX and Ras or MAP kinase activity will provide a powerful

reagent to elucidate the growth and differentiation functions of the Trk signaling proteins.

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Genetic Evidence for a Nova Regulator of Alternative Splicing in the Brain

How do genetic programs direct the development and physiology of distinct cell types in such complex systems as brain, muscle, liver, and blood? In the brain, regulated alternative splicing is a major mechanism by which neuronal cells acquire specialized molecular structures and regulatory pathways needed to receive and transmit informational signals. New biological functions are generated by subtle alterations in protein structure as a consequence of tissue-specific splicing patterns that occur in an impressive variety of morphological patterns. In contrast to its biological utility, there is a dark side to tissue-specific splicing, since its misregulation is closely associated with some forms of malignant transformation, tumor metastasis, neurodegenerative disease, and myotonic dystrophy (Cooper and Mattox, 1997; Grabowski, 1998).

In the simplest variation, protein isoforms involved in synapse formation, neurotransmitter reception, and ion channel function are synthesized in neurons with an additional (usually small) protein segment not found in other cell types. This means that the corresponding pre-mRNA is spliced in an identical manner in the different cell types, except that a subset of splicing events is activated exclusively in neurons or in a subpopulation of neurons. This suggests that in addition to the general splicing machinery, which is found ubiquitously (small nuclear ribonucleoprotein particles, RNA binding proteins, and related factors), there must be specialized molecular machineries to direct regulation in a corresponding cell type–specific manner. What are these specialized machineries, how do they recognize their specific intron targets, and how do they promote neuron-specific splicing? In the brain as well as in other tissues, splicing regulators with strict, cell-specific expression patterns have been an enigma. Now, a report by Jensen et al. (2000) in this issue of *Neuron* presents genetic evidence for the role of the neuron-specific protein Nova-1 as a tissue-specific splicing regulator in the brain.

Nova-1 and its close relative Nova-2 are RNA binding proteins first identified as autoimmune antigens in patients with certain forms of paraneoplastic neurological disorders (Darnell, 1996). RNA binding proteins are abundant in the cell nucleus where they package nascent transcripts and participate in RNA processing or nuclear export of mRNA, and in the cytoplasm where they regulate mRNA stability, translation efficiency, or mRNA localization (Dreyfuss et al., 1993; Ostareck-Lederer et al., 1998). Biochemical and molecular genetics approaches have shown that a variety of RNA binding proteins of the SR (serine-arginine-rich) and hnRNP (heterogeneous nuclear RNA–associated protein) family can function as splicing regulators for specific sets of alternatively spliced pre-mRNAs. It has been most provocative to note that Nova proteins are found exclusively in neuronal cells of the brain where they are localized to the cell nucleus—hence the prediction that these proteins might be tissue-specific regulators of alternative splicing.

What jobs do neuron-specific RNA binding proteins perform in the nucleus, and how important are these molecules for the survival and/or development of neurons? The study of Jensen et al. (2000) sheds new light on these questions by generating *Nova-1* knockout mice. The *Nova-1* null mice appear normal at birth, but die within about 2 weeks. The hallmark pathological defect is neuronal cell death in precisely the regions of brainstem and spinal cord where Nova-1 is normally expressed. Thus, Nova-1 is important for neuronal cell survival in the postnatal stage of development.

Is the physiological importance of Nova-1 due to it's role as a splicing regulator in brain? The Jensen et al. (2000) study makes a strong case for this by demonstrating significant splicing defects in the brains of *Nova-1* null mice for neuron-specific exons of the inhibitory glycine receptor (GlyRα2) and the γ2 subunit of the $GABA_A$ receptor. There is no indication that general splicing is disrupted, but these particular neuron-specific splicing events are decreased by as much as 3-fold compared to healthy littermates. In several respects the defect in regulation appears to be quite specific, since four additional neuron-specific splicing events tested show no apparent change upon loss of Nova-1 expression. Control experiments that probe in more detail show that regulation of GlyRα2 and γ2 pre-mRNA splicing is not