Heat shock protein that facilitates myelination of regenerating axons

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Peripheral Nerve Regeneration

It is widely recognized that neurons in the peripheral nervous system (PNS) are capable of regeneration after injury. In contrast, those neurons that are entirely located within the CNS (i.e., the brain and spinal cord) largely do not regenerate. What is often not emphasized, however, is that the extent of PNS regeneration is limited and that this results in incomplete functional recovery (1). A great deal of attention has been focused recently on the cellular and molecular mechanisms underlying degeneration and regeneration in the PNS, with an eye toward enhancing this regeneration.

Regeneration is not a neuron-autonomous process, but depends heavily on interactions between neurons, glial cells (i.e., Schwann cells in the nerve and satellite cells in peripheral ganglia), and immune cells, particularly macrophages (2, 3). Among the several reasons that have been postulated for the incomplete regeneration in the PNS, is the extremely slow rate of this regeneration (about 1–2 mm per day), leading perhaps to an eventual decrease in the intrinsic growth capacity of the injured axons and the limited period after injury during which Schwann cells actively promote axonal elongation (4).

Once an axon is transected or crushed (referred to as axotomy), the distal segment fragments and then is cleared by phagocytic cells by a process called Wallerian degeneration. Advances in our understanding of the mechanism of Wallerian degeneration have come from genetic studies on the mutant gene *WLD*^{s} and its normal counterpart *NMNAT1*, both of which temporarily protect the axon from degeneration (5), and *SARM1*, which is involved in triggering axon degeneration (6, 7). Schwann cells upregulate their expression and release of the macrophage chemokine CCL2, which draws in inflammatory monocytes that then differentiate into macrophages and are involved in phagocytosis of myelin and axonal debris (2). Although it was previously believed that these macrophages played an essential role in Wallerian degeneration, studies with a mouse strain in which the CCL2 receptor CCR2 is knocked out indicate that compensatory phagocytic mechanisms are possible (8).

Two types of adult Schwann cells exist: myelinating and nonmyelinating. Nonmyelinating Schwann cells are also the developmental precursor of the myelinating cell. During Wallerian degeneration, the myelinating Schwann cell dedifferentiates and ceases to synthesize myelin proteins (3). This dedifferentiation process is controlled by "a balance between two opposing transcriptional programs": the myelinating program involves transcription factors like Krox20, whereas the nonmyelinating involves factors like cJun (9). Recently, it was shown that these Schwann cell changes can be mimicked by the activation of an inducible Raf kinase transgene (10). What might trigger this induction in situ after injury is not yet known.

Axons in the proximal stump of the severed nerve exhibit an initial period of dieback (11) but then form growth cones and begin to elongate. These initial axonal sprouts are not myelinated; however, given that myelination is crucial to controlling the conduction velocity of an axon, recovery of normal axonal function requires that the formerly myelinating Schwann cells "redifferentiate" so that the new axonal segments can become myelinated. Ultimately the physiological significance of regeneration is the restoration of normal function. For this to occur, the growing axons must reach their original targets, form endings capable of releasing neurotransmitters, and make effective synaptic connections with these targets.

Role of AlphaB-Crystallin in PNS Regeneration

A paper by Lim et al. in PNAS (12) examines the effects of the protein alphaB-crystallin (αBC) on peripheral nerve regeneration, providing a thorough investigation of the effects of this protein on the various processes outlined above. αBC was first identified as one of the three major proteins in the mammalian lens, which are α-, β-, and γ-crystallin (13). αBC is made up of two subunits, αA and αB. Although αBC was first thought to be found only as a structural protein in the lens, it was later identified as a small (~22 kDa) heat shock protein (also known as HspB5 and CRYAB) and was present in other tissues in addition to the lens, including the sciatic nerve (14, 15). In culture, αBC is expressed in both myelinating and nonmyelinating Schwann cells from the rat, and it is present both during development and

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after axotomy (16). This finding led Lim et al. (12) to speculate that the protein was involved in myelination in the PNS. αBC was also reported to be present in the CNS in multiple sclerosis lesions and in the corresponding animal model of multiple sclerosis, experimental autoimmune encephalomyelitis. Experiments on experimental autoimmune encephalomyelitis revealed more severe inflammation and demyelination in the CNS in mice in which αBC had been knocked out than in WT animals (17).

Lim et al. (12) examined the role of αBC on the PNS in adult mice after crushing the sciatic nerve unilaterally. In vivo in the mouse, αBC was found to colocalize with both myelinating Schwann cells and axons, but not nonmyelinating Schwann cells. Levels of the protein decrease after nerve crush and appear to partially recover around 4 wk later. Lim et al. focused on comparing the response to injury in WT and $\alpha BC^{-/-}$ mice, and also examined the effect of administration of recombinant αBC. In all tests, the researchers studied the two genotypes before injury to make sure the animals all started from the same baseline. The only difference found, which is not commented on, is a lower level of neurofilament heavy-chain protein levels found in the mutants (in figure S4 of ref. 12). The authors then performed a number of motor and sensory behavioral tests on which performance declines after sciatic nerve injury. In general, the WT mice recovered well, whereas the knockout animals recovered more slowly and to a lesser extent. Next, the conduction velocity was measured in the sciatic nerve. The amplitude of the compound action potential was similar in the two genotypes (suggesting that the same number of axons regenerated) but the velocity was reduced in the knockout. A possible reason for a change in this velocity is a difference in axonal myelination. This possibility was assessed by measuring the axons' g-ratios (i.e., the ratio of the diameter of the axon divided by the diameter of the axon plus myelin). A small g-ratio is characteristic of an extensively myelinated axon. In the $\alpha BC^{-/-}$ mice after axotomy, the g-ratio was larger than in WT mice, suggesting hypomyelination. This conclusion is significantly strengthened by the finding that there were no differences between the two groups of animals in the number of myelinated axons or in the axonal cross-sectional area. Also consistent with the interpretation that the groups did not differ in their regenerative ability were the findings of no differences in the expression of the prototypic regeneration-associated gene, growth associated-gene 43 (GAP-43), or in the extent of neurite outgrowth observed in sensory neurons examined in culture. Incidentally, no changes were found between the two genotypes in Wallerian degeneration or macrophage accumulation.

One question raised by these loss-of-function studies is whether exogenous αBC is capable of enhancing certain aspects of regeneration. WT mice had their sciatic nerves crushed unilaterally and were then injected (presumably systemically) with either saline or recombinant human αBC every other day for 4 wk. The animals were next assessed for g-ratio, walking, and mechanical sensitivity. With respect to g-ratio, the αBC-treated animals showed more axons with low g-ratios (<0.65) and fewer axons with high g-ratios (>0.65), consistent with an increase in myelination. In addition, both walking and mechanical sensitivity were closer to control values for the treated animals.

As part of an attempt to determine what might be responsible for the decrease in axon myelination, Lim et al. (12) examined the ratio of myelinating and nonmyelinating Schwann cells in animals after axotomy. They found that the total number of Schwann cells did not differ between genotypes nor did the ratio of myelinating to nonmyelinating cells at early times after axotomy. However, this ratio was lower at later times in the mutant animals, suggesting that the redifferentiation of the dedifferentiated Schwann cells was impaired in these animals. Attempts to uncover the molecular causes

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of this effect on redifferentiation yielded less clear results. For example, measurements were made of neuregulin 1 types I and III expression (the Schwann cell and the axonal forms of the growth factor, respectively). Neuregulin 1 type III is a key signal between intact axons and Schwann cells and has a number of effects, including the promotion of myelination (18, 19). Lim et al. (12) report that axonal neuregulin decreases dramatically in the distal nerve segment after a sciatic nerve crush in WT mice and then rebounds, whereas the decrease in $\alpha BC^{-/-}$ mice is much smaller. However, the relationship between this difference and the hypomyelination seen in the mutant is not examined. The researchers also measured the phosphorylation state of a variety of kinases in the mutant animals. No changes in MAP kinase pathways were observed between WT and knockout animals before or after injury. On the other hand, increased phosphorylated AKT was found in $\alpha BC^{-/-}$ mice before and at 1 and 3 wk after injury. Again, however, the functional importance of this difference is unclear, such as whether it is involved in the hypomyelination in the mutant animals. It would have been interesting if the time course of remyelination after sciatic nerve crush had been compared with these molecular changes.

Some other questions not addressed in the Lim et al. (12) paper concern the relative importance of axonal and Schwann cell αBC in regulating myelination, the extent to which αBC acts intracellularly or extracellularly, and the receptors on which exogenous and endogenous αBC act. One might expect that axonal αBC is cleared fairly rapidly during Wallerian degeneration and, therefore, might not account for the delayed effects attributed to αBC after injury. On the other hand, it is possible that the axonal protein only becomes accessible to Schwann cells during axonal degeneration. Interestingly, in retinal pigment epithelial cells, αBC has been shown to be secreted in exosomes (20). One point of caution that should be kept in mind in terms of the therapeutic usefulness of αBC is that increased expression of α-crystallins has been reported in a number of neurodegenerative diseases, including Alzheimer's and Parkinson's disease (21, 22). To what extent this expression is neuroprotective and to what extent it is pathological still needs to be clarified.

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