PERSPECTIVES: NEUROBIOLOGY

Regeneration in the Nogo Zone

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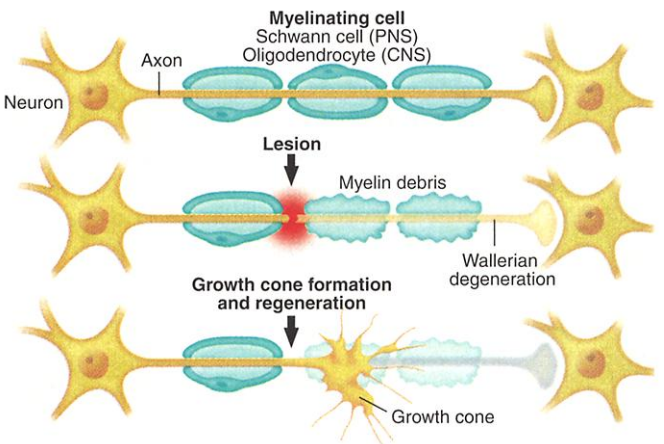
Neurons make connections with other neurons or muscles through axons that transmit electrical impulses (see the figure, this page). Traumatic injuries to the spinal cord cause paralysis because axons of neurons with cell bodies in the brain are cut and disconnected from their target neurons in the spinal cord. Finding a cure for paralysis requires developing therapies that encourage these severed axons to regenerate.

Three important papers in a recent issue of *Nature* (*1*–*3*) identify and characterize the Nogo-A protein, previously implicated in the inhibition of axon regeneration, and show that it is a member of the reticulon protein family. These studies provide essential tools to assess whether Nogo-A will be a useful target for therapies aimed at stimulating spinal cord regeneration. The papers also provide interesting data that may challenge the simplest models of how Nogo-A functions.

Regenerating axons, like developing axons, are tipped by motile structures called growth cones. In the adult peripheral nervous system (PNS), cut axons will reseal at their ends and re-form growth cones that can sometimes regenerate back to their targets (see the figure, this page). In the central nervous system (CNS; the brain and spinal cord), cut axons will also re-form growth cones but, tragically, they fail to regrow to any significant extent.

Why are the regenerative potentials of the CNS and PNS so different? In the 1980s, Aguayo and colleagues showed that CNS axons are able to regenerate when they are exposed to the PNS but not the CNS environment (*4*). The CNS environment is hostile to regrowth partly because of the presence of structures and molecules that actively suppress axon regrowth. For example, scar tissue made by glial cells (support cells) at the site of the injury expresses molecules, including proteoglycans, that inhibit axon regeneration (*5*). Other proteins, including members of the Semaphorin, Netrin, Ephrin, and Slit axon guidance protein families, have been identified that inhibit axon growth during development (*6*) and are candidates for contributing to inhibition in the adult.

Schwab and colleagues showed that another source of inhibition is provided by myelin, the insulating sheath that surrounds axons—but only in the CNS, not the PNS (*7*). This led to the search for molecules that contribute to this source of inhibition by biochemical purification of growth-inhibitory activities in myelin. Fractionation studies led to the molecular identification of the first of these myelin inhibitors, myelin associated glycoprotein (MAG), in 1994 (*8*, *9*). Analysis of knockout mice deficient in MAG showed, however, that in the absence of MAG, other inhibitors still block extensive regeneration (*10*). Earlier fractionation of myelin extracts by Schwab and co-workers demonstrated the presence of two additional inhibitors, NI35 and NI250, of molecular mass 35 and 250 kD, respectively (*11*). Subsequent purification by Schwab's group (*12*) of NI250, now called Nogo-A, made it possible to determine peptide sequences that were used by the laboratories of Schwab, Strittmatter, and Walsh to clone the *Nogo* gene (*1*–*3*).



Growth cones direct nerve regeneration. After a lesion, the cut axon reseals and then re-forms a growth cone, which attempts to regenerate a new axon segment. In the PNS, extensive regeneration is seen, but the CNS regeneration is severely limited in part because of inhibitory factors associated with CNS myelin. [Adapted from (*18*)]

As expected, recombinant Nogo-A has the properties of purified NI250 (*1*–*3*): It inhibits fibroblast spreading and neurite extension from cerebellar neurons and mature (but not immature) sensory neurons; it is expressed at high levels in myelinating cells from the CNS (oligodendrocytes) but not from the PNS (Schwann cells); and it is recognized by a monoclonal antibody, IN-1, raised against NI250 and NI35.

One property of Nogo-A is, however, surprising. It was predicted that Nogo-A would be prominently expressed on the surface of oligodendrocytes, the source of CNS myelin. However, Nogo-A is largely concentrated in the endoplasmic reticulum (ER), with only small amounts reaching the surface of oligodendrocytes, at least in cell culture (*2*). This finding, while unexpected given the proposed inhibitory function of Nogo-A, is, however, consistent with the finding that Nogo-A is a member of the reticulon (Rtn) family, initially characterized as ER-associated proteins of unknown function (*13*).

Furthermore, although the three groups agree that the Nogo-A protein is inhibitory (*1*–*3*), they disagree on which part of the protein causes the inhibition (see the figure, next page). Like the *Rtn1* gene, the *Nogo* gene actually codes for three distinct proteins, Nogo-A, -B, and -C, which share a conserved carboxyl (C)-terminal region and arise by alternative splicing and/or promoter usage. This C-terminal region has two transmembrane domains which, in other Rtn proteins, are believed to anchor the proteins to the ER membrane (see the figure, next page) (*13*). Nogo-A is characterized by a region (>1000 amino acids long) that is amino (N)-terminal to these transmembrane domains. Epitope mapping studies showed that a short, 66–amino acid linker between the two C-terminal transmembrane domains is on the lumenal/extracellular side of the membrane, whereas the N and C termini are cytoplasmic (*2*) (see the figure, next page). The entire N-terminal region of Nogo-A has been proposed to be in the same cellular compartment (that is, the cytoplasm) (*2*), consistent with the fact that this domain, when fused to a heterologous signal sequence, does not get trapped in membranes and is secreted (*3*). However, the presence of additional short hydrophobic stretches in this domain leaves open the possibility that part of the domain might normally be on the lumenal/extracellular side of the membrane.

The point of apparent disagreement is that Strittmatter and colleagues (*2*) find that the 66–amino acid linker is strongly in-

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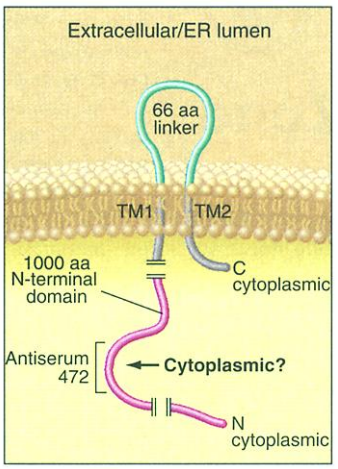
hibitory—which would imply that Nogo-A, -B, and -C should all be inhibitory. In contrast, Schwab and co-workers (*1*) report that the long N-terminal region of Nogo-A is its main inhibitory domain—which would imply that Nogo-B and -C are not inhibitory. Walsh and colleagues (*3*) agree that the isolated N-terminal region is inhibitory (and as potent as MAG) (*2*), and Schwab's group show that an antiserum against a peptide sequence in the N-terminal region (antiserum 472) can block the inhibitory action of myelin extracts (*1*).



Proposed topology of Nogo-A. The 66–amino acid (aa) linker is on the extracellular/lumenal side of the membrane, whereas the extreme N and C termini are cytoplasmic. It remains to be determined whether the entire N-terminal domain, including the region recognized by blocking antiserum 472, is completely cytoplasmic.

What accounts for these apparently divergent results? Does Nogo-A have two distinct inhibitory domains? If so, why does one antibody block both effects? Or do the differences reflect the varied assays used? No doubt these apparent contradictions will be resolved soon.

The precise membrane organization of Nogo-A also affects the interpretation of other experiments. The lumenal/extracellular 66–amino acid linker should be exposed on the surface of oligodendrocytes, but if the long N-terminal region of Nogo-A is cytoplasmic, it would not normally be accessible to extending axons. However, Schwab and colleagues report that the antibody to this domain facilitates the entry of regenerating axons into explanted optic nerves in vitro (*1*). If the epitope that is blocked is intracellular, how can the antibody promote regeneration?

One possibility is that Nogo-A contributes to inhibition when released from intracellular stores of damaged oligodendrocytes. Thus, at one extreme, if the N-terminal region is cytoplasmic and is the only inhibitory domain, then Nogo-A would contribute to inhibition only in pathological states. At the other extreme, if the membrane organization of Nogo-A is such that part of the N-terminal inhibitory domain is extracellular, and if this region and/or the 66–amino acid linker are inhibitory to regenerating axons in vivo, then Nogo-A may provide an ongoing source of inhibition in intact myelin. Either way, given the abundance of intracellular Nogo-A, one might expect damaged oligodendrocytes to be more inhibitory. Interestingly, some recent studies suggest that intact (as opposed to damaged) myelin may be less inhibitory than originally thought (*14*).

The most important question is whether inhibition of Nogo-A in vivo allows axon regeneration. The IN-1 antibody, which blocks the inhibitory action of Nogo-A in in vitro assays, has been shown to allow modest but clear axon regeneration after spinal cord injury (*15*). It is reasonable to attribute this important effect of IN-1 to inhibition of Nogo-A, although some of the effect could be contributed by blockade of NI35 or other cross-reactive proteins. The ability to make blocking reagents specific for Nogo-A and to generate Nogo-A knockout mice will now make it possible to establish the precise contribution of Nogo-A to the inhibition of regeneration.

If, however, the results with MAG are any guide, one might expect that blockade of any single inhibitory system might not permit more than a modest amount of axon regrowth. It is just as important, therefore, that this work will make it possible to determine how much more regeneration can result from blockade of multiple systems simultaneously—for example, by removal of both MAG and Nogo-A. A recent study showed that a vaccine against crude myelin extracts allowed much more regeneration than has been seen when any one protein alone is blocked (*16*). Although blocking multiple inhibitory systems may therefore be necessary, it may also be that all of these systems converge on a common signal transduction pathway (*17*). The identification of Nogo-A provides the tools to identify its signaling mechanism and to assess whether it converges on a common inhibitory transduction pathway, which might provide an even more effective target for regeneration therapies.

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PERSPECTIVES: GEOPHYSICS

The Strength of a Continent

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We can measure the strength of a small piece of rock by putting it in a press and watching it strain (deform) when we apply stress (force). But how can we measure the strength of an object that is 100 km or more thick and extends over a whole continent? On page 834 of this issue, Flesch *et al.* (*1*) attempt just that kind of measurement for the southwestern part of the United States.

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In their approach, the strength of the lithosphere—the rigid outer layer of Earth including the crust and the part of the upper mantle that sustains plate tectonics—is described by an effective viscosity, the ratio of applied stress difference to resulting strain rate. This may seem counterintuitive because the lithosphere is not obviously a viscous fluid. It does, however, slowly deform by multiple deformation mechanisms, including faulting, plasticity, and dislocation creep. Earthquakes are one sign of that deformation, but the major deformation mechanism in the lithosphere is probably viscous creep. Relatively low lithospheric viscosities might be caused by high local temperatures or high strain rates, because geological materials generally obey a nonlinear constitutive law. We are talking here about viscosities at least 20 orders of magnitude greater than that of water—effectively rigid on the time scale of human perception but flowing freely on the geological time scale. Describing the lithosphere's resistance to deformation by a viscosity parameter remains a great simplification but can give valuable insights in the context of an assumed level of applied stress difference. At any point the

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