Clearing a path for nerve growth

Lars Olson

After spinal-cord injury, severed nerve fibres face a thicket of obstacles as they try to regenerate. Researchers have used a bacterial enzyme to help prune these obstacles in rats.

Spinal-cord damage is tragically common, affecting three to five people out of every 100,000 in the United States and similar numbers in other countries. The consequences for both the injured person and their families and friends can be devastating. Injuries typically occur in young adults, and of the 11,000 or so new cases each year in the United States, 80% are men who were involved in driving accidents, violence or falls. Most victims survive, but are completely or partially paralysed for the rest of their lives.

It has long been thought that such traumatic injuries are incurable, but intense experimental work is beginning to suggest that they might one day be treatable at least. In the latest advance, Bradbury and colleagues[1] provide evidence (page 636 of this issue) that infusion of chondroitinase ABC — a bacterial enzyme that trims the carbohydrate side chains off large extracellular proteins — enables severed nerve fibres to regenerate after the spinal cord has been crushed in rats. The authors used forceps to squeeze the nerve fibres in the so-called dorsal columns of the spinal cord until nerve fibres carrying sensory information from the limbs to the central nervous system, and fibres conveying motor (movement) information to the limbs, had been cut. This severely impairs limb function. In animals that were then treated with chondroitinase ABC, there was convincing evidence of nerve-fibre regeneration at the cut nerve stumps. Although the growth of sensory nerve fibres was not enough to lead to a clear recovery of sensation, the authors have electrophysiological and behavioural evidence of remarkable motor recovery.

Imagine that you are as small as the tip of a nerve fibre, a few thousandths of a millimetre in diameter, and have to advance in the narrow regions between cells — the extracellular space — in a recently damaged tissue. You would find that these spaces are by no means open territory, but rather like an endless overgrown shrubbery, consisting of many different long, and sometimes branched, molecules that form an intertwined network, the extracellular matrix (Fig. 1). Some of the molecules are anchored to cells, some hold adjacent cells together, and many are simply deposited outside the cells. The terrain becomes particularly hostile after injury because new classes of cells

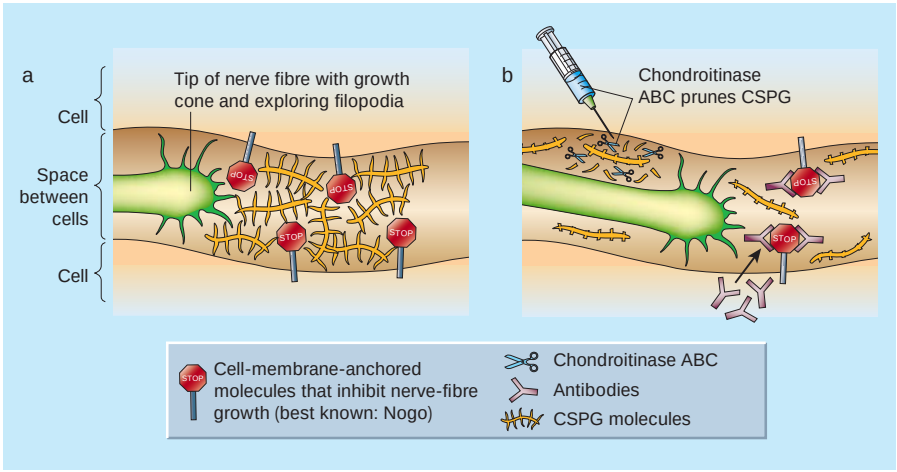

Figure 1 **Obstacles to nerve regeneration.** a, **A severed nerve is shown on the left, in the extracellular space between cells in a wounded spinal cord. The nerve end has just formed a 'growth cone', which is exploring the territory by constantly forming and retracting minute processes (filopodia). The extracellular space contains two obstacles to nerve growth: chondroitin sulphate proteoglycan (CSPG) molecules, which have a backbone and many side branches that block the way; and special cell-membrane-anchored proteins that actively stop growing nerve fibres. The best known of these is Nogo.** b, **Bradbury *et al.*[1] show that the bacterial enzyme chondroitinase ABC can prune the side chains of CSPGs, clearing the way for growing nerve fibres. It has previously been shown that Nogo can be blocked by specific antibodies. The antibodies and enzyme could potentially be used in combination, along with proteins that stimulate nerve growth.**

arrive, and a scar that is burdened by increasing deposition of matrix molecules builds up[2,3]. To advance, you would need a machete.

The chemistry of the extracellular matrix is complex, but one key class of molecules are the chondroitin sulphate proteoglycans (CSPGs). These are made up of a protein core — the chondroitin sulphate — that is equipped with many side chains consisting of carbohydrate building-blocks such as glycosaminoglycans. Bacteria such as *Proteus vulgaris* have evolved enzymes, including chondroitinase ABC, that can prune CSPGs by removing glycosaminoglycans. Perhaps this helps the bacteria to invade their animal hosts.

Knowing all this, Bradbury *et al.*[1] wondered whether chrondroitinase ABC could be used as a molecular machete to clear the way for nerve regeneration in injured rat spinal cords. After crushing the dorsal columns of rats at the level of the fourth cervical vertebra, the authors repeatedly infused either active chondroitinase ABC or a control protein, delivered through a thin tube, to the site of injury. Then, using an antibody that binds to the CSPG core but not the intact molecule, they showed that chondroitinase ABC effectively prunes CSPGs in the spinal cord (Fig. 1). CSPGs remained intact in controls.

In the treated rats, sensory neurons in the dorsal root ganglia corresponding to the fourth cervical vertebra upregulated a nerve-growth marker protein, GAP-43, and the cut extensions (axons) of these neurons in the spinal cord regenerated by up to 4 millimetres towards the brain[1]. Similarly, the important dorsal corticospinal tract, completely severed by the crush, regenerated downwards from the site of injury in the spinal cord. This was parallelled by a return of electrical signalling between the brain's cerebral cortex and the spinal cord, albeit at a reduced strength and somewhat slower speed. Bradbury *et al.* also showed that if they cut the spinal cord again, stimulation of the cerebral cortex no longer elicited signals in the cord.

In attempting to repair experimentally damaged spinal cords, it is crucial to see how well the animals recover. Bradbury *et al.* went to great lengths to test this. They show that rats that had been treated with chondroitinase ABC recovered normal or near-normal walking behaviour in two tests — beam and grid walking. But sensorimotor functions — becoming aware

of and removing a piece of adhesive tape — were not significantly recovered.

The results[1] are promising nonetheless, and it might prove possible to integrate the use of chondroitinase ABC with other strategies (compare with ref. 4). Let's consider the worst-case scenario, in which the spinal cord has become completely divided, leaving a gap between two stumps. While scar tissue invades the gap, initial oedemas (swellings) in the stumps themselves may transform into growing, fluid-filled cysts[5]. Yet both stumps contain severed axons with an intrinsic ability to regenerate — if the conditions are right. The problem can be likened to opening up traffic on a blocked-off, overgrown and partly demolished road. We may need to remove scar tissue, empty cysts, build physical bridges such as nerve grafts across a void[6], and perhaps provide cellular surfaces that prompt nerves to grow[7–9].

To clear the road, chondroitinase ABC could be delivered to prune back the extracellular-matrix shrubbery. Chemical stop signs — such as those composed of the nerve-growth-inhibitory protein Nogo, expressed by supportive glial cells — could be covered over by antibodies and thus neutralized[10]. Chemical gradients of nerve-growth-activating molecules may need to be established to stimulate and to direct growing nerve fibres. Finally, to protect newly regenerated nerve fibres and increase signalling speed, cells that provide fibres with a new insulating sheath (composed of a lipid, myelin) might be needed. It may prove easier to induce long axon growth in white matter than in grey matter. Grey matter is made up mainly of nerve-cell bodies, with some other cells, and the complex network of contacts between nerve-fibre branches. White matter consists largely of myelin-coated nerve fibres. Any fibres in grey matter tend to branch greatly, so the neuron's need for growth factors from underlying cells may be satisfied long before it reaches its target area. But fibres in white matter branch less, so can perhaps grow further before they are adequately supported.

What could go wrong by using chondroitinase ABC? Not much, it seems. Highly purified preparations have no protein-degrading activity and so would not destroy crucial proteins in the body. However, CSPGs come in many forms, and some might have specific roles in the central nervous system, for example to guide growing axons. In grey matter, many nerve-cell bodies are surrounded by nets of particular CSPGs, which might be important in neuronal contacts. These nets would degrade upon enzyme treatment, and take a long time to rebuild.

That said, treatment with chondroitinase ABC has also proved effective in treating brain injuries[11], and constitutes one hopeful avenue to promoting nerve regeneration. Several other treatment strategies (see, for example, ref. 12) have also shown promise in different experimental models of spinal-cord injury. The bad news is that none of these techniques has led to complete recovery. Fortunately, however, many of them can be combined, perhaps in ways discussed above. In the short term, the prognosis for people with complete spinal-cord injury remains grim. Yet, looking further into the future, we can perhaps allow ourselves to be a bit more optimistic. ■

Lars Olson is in the Department of Neuroscience, Karolinska Institute, 171 77 Stockholm, Sweden.
e-mail: lars.olson@neuro.ki.se

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Plant biology

The first harvest of crop genes

Michael Bevan

Draft sequences of the rice genome have been produced by two groups. The drafts will be an invaluable resource for research on the genomes of other plants, the cereals in particular.

Last week's publication in *Science*[1,2] of two draft sequences of the rice genome is another milestone in the inexorable progress of genome sequencing. The work is significant on three counts. Rice (*Oryza sativa*) is the staple in the diet of nearly a third of the world's population; the sequences provide a reference for all the other cereals, which collectively account for an overwhelming proportion of human and domesticated animal feed; and this is the first representative of the monocotyledonous plant lineage to be sequenced. Together with the dicotyledonous plants, the monocots (grasses, palms, rushes, and so on) constitute the flowering plants, the most diverse and numerous of all plants.

One of the draft sequences has been produced by Syngenta (a Swiss agricultural biotech company)[1]; the other comes from a collaboration between the Beijing Genomics Institute and other Chinese academies[2]. Both reports are initial analyses of assembled whole-genome shotgun sequences, involving random sequencing of the entire genome and assembly of the sequence into contiguous regions, or contigs. The Syngenta group worked with the subspecies *japonica* Nipponbare (a popular rice cultivar in Japan), whereas the Chinese team sequenced a hybrid *indica* variety, the major subspecies grown in China and most other regions. Draft DNA sequence is generally accurate enough to identify genes and to estimate genome size. In this case, the sequences reveal a genome of 362–389 million base pairs (Mbp), and a conservative number of between 33,000 and 44,000 genes. Two other important studies of the rice genome provide independent evidence, based on the construction of a near-complete physical map[3] and a map of expressed genes[4], that the gene-rich regions of the rice genome total about 400 Mbp and contain about 44,700 genes.

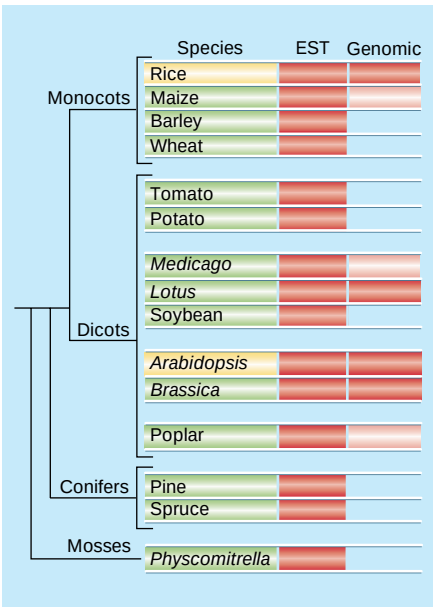

Figure 1 **Progress in plant-genome sequencing. Large-scale sequencing of representatives of several of the major groups of plants is underway (red bars). Mostly this is through analysis of expressed sequence tags (ESTs), as with pine and moss (*Physcomitrella*). But genome sequencing of *Brassica* species and *Lotus* (a model legume) is ongoing, with various approaches being used, and work on maize, *Medicago* (alfalfa) and poplar will start imminently (pale red bars). The evolutionary relationships shown here are figurative only.**

The rice genome is much smaller than that of maize (which, at 2,500 Mbp, is about the same size as the human genome), and is tiny compared to the monster barley (twice