It takes two to tango:
Bi-directional axoglial signalling is required for effective nerve repair

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Schwann cells are the glia of the peripheral nervous system, ensheathing and myelinating large axons and grouping smaller diameter axons within Remak bundles. Bi-directional signalling between axons and Schwann cells has long been known to be essential for the development of the peripheral nervous system. More recently, (and the focus of this review) it has been shown that axo-glial signalling in neural injury is essential for effective repair and is distinct from signalling events during development.

Injury to the peripheral nervous system can be caused by many insults, from metabolic diseases such as diabetes, inherited genetic disorders such as Charcot-Marie-Tooth disease (CMT), infectious and inflammatory disorders including Guillan-Barré syndrome and traumatic injury which alone affects up to 300,000 people in Europe per year.[1] Traumatic nerve injury in rodents is very commonly used as a model to study the process of peripheral nerve repair and functional recovery. Following an injury to the peripheral nervous system, axon and myelin fragments are broken down by a process termed Wallerian degeneration. Axons then regenerate, are remyelinated and eventually reinnervate target organs. How complete this process is and the extent to which target organs are innervated by the correct axons is related to the degree of functional recovery. Signalling between Schwann cells and axons is essential throughout the phases of this process and disruption of this signalling has severe consequences for nerve repair.

How do Schwann cells respond to nerve injury/support axons following nerve injury?

Schwann cells are essential for nerve repair. Following injury, they re-enter the cell cycle and activate Raf/MEK/ERK signalling.[2] This drives their differentiation into a phenotype which actively phagocytoses myelin and axonal fragments, promotes recruitment of macrophages, enhances axon growth and increases neuronal survival. The alignment of Schwann cells into bands of Büngner guides regenerating axons back to their synaptic targets (Figure 1).[3,4] Schwann cells undergo this phenotypic transformation as a direct response to axon derived signals (the identity of which are not established), which trigger a transcriptional program driven by the transcription factor c-Jun. This results in a down regulation of genes that regulate myelination and an upregulation of genes involved in macrophage recruitment, axon growth and survival. If this phenotypic switch is disrupted by inactivating c-Jun in Schwann cells, axon regeneration, functional recovery and neuronal survival is severely impaired following peripheral nerve injury.[5] This phenotypic plasticity of Schwann cells may in rare instances be detrimental. *Mycobacterium leprae*, the causative agent of leprosy utilises this property of Schwann cells. Having infected Schwann cells the bacterium triggers reprogramming of these cells to aid bacterial dissemination.[6]

As axons regenerate through bands of Büngner, in addition to providing guidance towards their targets, Schwann cells must provide other supportive roles (Figure 1 C). Local translation in axons is likely to be necessary during growth cone formation and axon elongation, indeed, *in vitro* inhibition of local translation results in retraction of growth cones.[7] Polyribosomes have been visualised to transfer from Schwann cells to regenerated axons, raising the possibility that Schwann cells may not only provide translational machinery to support axons but also mRNA and therefore could modify axonal translational products.[8]

The very long distances between axons and their cell bodies has long implicated a need for Schwann cells to provide a metabolic supportive role.[9] In mutant mice in which Schwann cell mitochondria are dysfunctional, development occurs normally, but in adulthood mice develop a severe axonal neuropathy despite axonal mitochondria being unaffected.[10] Schwann cell mitochondria function plays a role in repair, as although regeneration is unaffected in mutant mice remyelination fails.[10] Furthermore mice with Schwann cells lacking functional peroxisomes, organelles housing oxidative metabolic reactions, present in non-compact myelin membranes, develop an adult-onset neuropathy.[11] Recently it has been demonstrated that myelinating Schwann cells contain glycogen granules which are likely to provide a source of glycogen derived lactate to axons in order to metabolically support axons particularly in hypoglycaemic conditions.[12] The long narrow channels of glial cytoplasm connecting to the periaxonal space including Cajal bands, Schmidt-Lanterman incisures and the lumina of paranodal loops are likely to provide a means for Schwann cells to transfer metabolites to axons in order to provide metabolic support necessary for maintenance and more than likely essential for the metabolically expensive process of repair of the peripheral nerve.

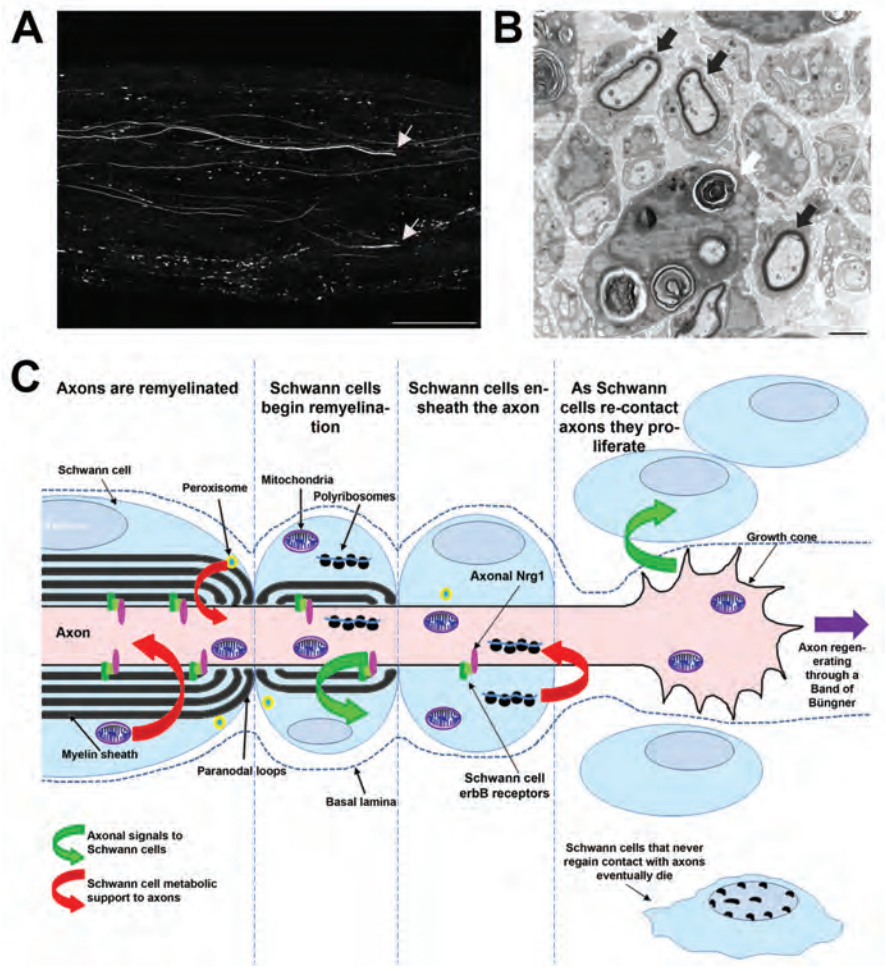

Figure 1: The bidirectional relationship between axons and Schwann cells during neural repair. A) A photomicrograph of YFP labelled axons regenerating in the tibial nerve following a Sciatic nerve crush. Arrows label leading growth cones. Scale bar 500µm. B) An electron micrograph of a transverse Sciatic nerve 10 days following a crush injury. Black arrows label axons with a diameter greater than 1µm where Schwann cells have begun the process of remyelination. Note that the myelin sheath at this stage is much thinner than in the uninjured state. White arrows label myelin debris still being degraded by macrophages. Scale bar 2µm. C) Schematic diagram showing an axon regenerating, the phases of repair and how bidirectional communication and metabolic support between Schwann cells and axons regulates this process.

How do axons regulate SC health and phenotype following nerve injury?

As axons re-contact Schwann cells after nerve injury, signals from the axolemma are critical in directing the differentiation of Schwann cells back into a mature state in which they ensheath and in the case of large diameter axons myelinate axons (Figure 1). There are a few receptor-ligand signalling pairs known to regulate axo-glial signalling, the most characterised of which is the protein Neuregulin-1 type III expressed on the surface of axons which signals through binding to erbB2/erbB3 heterodimer receptors expressed on the Schwann cell. Although essential for peripheral nerve development Neuregulin-1/erbB signalling is dispensable for peripheral nerve maintenance. In contrast, in the early phases following peripheral nerve injury Neuregulin-1/erbB axo-glial signalling drives a transcriptional programme which enhances the rate of remyelination, and regeneration of peripheral axons as well as functional recovery. Interestingly at delayed time points after injury axons remyelinate and function is restored in the absence of axonal Neuregulin-1 implying the presence of alternative signalling systems instructive in determining myelination fate of axons following injury.[13,14] It has recently been shown that Schwann cell-derived Neuregulin-1 can also promote remyelination.[15] Other axoglial receptor-ligand signalling pairs which are known to regulate myelination during development and potentially mediate nerve repair include axonal adam22 signalling through Schwann cell Lgi4,[16] Necl-1 on axons signalling through Schwann cell Necl-4[17-19] and the as yet unidentified axonal ligand to Schwann cell G-protein-coupled receptor gpr126.[20]

Ultimately, Schwann cells need axonal contact to survive, this is shown in chronically denervated nerve stumps where, as time progresses, in the absence of axonal contact survival of Schwann cells declines. Importantly the Schwann cells that survive are much less able to support any axons that do eventually regenerate into such a stump.[21] This is likely to be caused by transcriptional changes caused by a lack of axo-glial signalling. It is known that the expression of erbB receptors and of the growth factor glial cell-line derived neurotrophic factor (GDNF) is greatly reduced in such chronically denervated Schwann cells.[21,22] This decline in Schwann cell capability and survival is clinically very important as the rate of axon regeneration is slow at 1-3mm per day resulting in Schwann cells distal to the injury being denervated for prolonged periods contributing to the poor functional outcomes particularly seen following proximal injuries for instance following brachial plexus avulsion.

We have concentrated on traumatic neuropathy as an exemplar. The critical nature of axo-glial signalling to nerve injury and repair is virtually ubiquitous to all forms of neuropathy. An example is CMT1A which is due to an excessive gene dosage of PMP22 in Schwann cells, resulting in demyelination. However the level of disability in patients relates to the degree of secondary axonal loss and not the degree of conduction velocity slowing.[23]

Axo-glial signalling may also be usurped by infective agents. Neuregulin-1 is normally presented to Schwann cells on the axolemma however high doses of exogenous soluble Neuregulin-1 have been reported to cause demyelination by triggering Schwann cell proliferation. The leprosy causing *Mycobacterium leprae* directly binds to and activates the erbB2 receptor activating the downstream MEK–ERK pathway and causing pathological demyelination.[24]

Therapeutic opportunities

There is currently no pharmacological intervention to promote peripheral nerve repair. Greater understanding of how Schwann cells provide metabolic and trophic support to axons may provide means to provide axonal protection given that axonal loss is a major determinant of progressive disability. Manipulation of Neuregulin-1/erbB signalling may provide a means to promote remyelination. It is still not clear as to whether administration of exogenous soluble Neuregulin-1 *in vivo* can substitute for juxtacrine Neuregulin-1 which is presented on the axolemma. A more tractable means of manipulating this pathway may be to inhibit enzymes such as TACE (ADAM17) which process Neuregulin-1 into an inactive form.[25] In the problematic situation of chronic denervation substituting signals that Schwann cells would normally receive from axons could promote their survival so that when regenerating axons finally reach distal regions of nerve they enter a much more hospitable environment. Using these approaches we hope that greater knowledge of axo-glial communication can ultimately be used to choreograph effective nerve repair in patients. ◆

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