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Review

A conditioning lesion induces changes in gene expression and axonal transport that enhance regeneration by increasing the intrinsic growth state of axons

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ABSTRACT

Injury of axons in the peripheral nervous system (PNS) induces transcription-dependent changes in gene expression and axonal transport that promote effective regeneration by increasing the intrinsic growth state of axons. Regeneration is enhanced in axons re-injured 1–2 weeks after the intrinsic growth state has been increased by such a prior conditioning lesion (CL). The intrinsic growth state does not increase after axons are injured in the mammalian central nervous system (CNS), where they lack the capacity for effective regeneration.

Sensory neurons in the dorsal root ganglion (DRG) have two axonal branches that respond differently to injury. Peripheral branches, which are located entirely in the PNS, are capable of effective regeneration. Central branches regenerate in the PNS (i.e., in the dorsal root, which extends from the DRG to the spinal cord), but not in the CNS (i.e., the spinal cord). A CL of peripheral branches increases the intrinsic growth state of central branches in the dorsal columns of the spinal cord, enabling these axons to undergo lengthy regeneration in a segment of peripheral nerve transplanted into the spinal cord (i.e., a peripheral nerve graft). This regeneration does not occur in the absence of a CL. We will examine how changes in gene expression and axonal transport induced by a CL may promote this regeneration.

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Contents

A conditioning lesion increases the intrinsic growth state of axons . . . 11
Gene expression during axonal growth . . . 12
Changes in gene expression induced by a conditioning lesion . . . 12
Elevated cyclic AMP overcomes CNS inhibition of elongation . . . 13
Transport along axonal microtubules . . . 14
Slow axonal transport results from rapid, but intermittent movement punctuated by long pauses . . . 14
The axonal transport of mRNAs . . . 14
Changes in axonal transport induced by axonal injury . . . 15
Conclusion . . . 16
Acknowledgments . . . 16
References . . . 16

A conditioning lesion increases the intrinsic growth state of axons

Injury of axons in the peripheral nervous system (PNS) induces transcription-dependent changes in gene expression and axonal transport that promote effective regeneration by increasing the intrinsic growth state of axons (Lasek and Hoffman, 1976; Smith and Skene, 1997). Regeneration is enhanced in axons re-injured 1–2 weeks after the intrinsic growth state has been increased by such a prior conditioning lesion (CL) (McQuarrie et al., 1977, 1978). Enhanced regeneration after a CL is characterized by accelerated outgrowth, a reduction in the lag time (latent interval) between the time of injury and the onset of elongation, and an increase in the number of regenerating sprouts (McQuarrie et al., 1977; Forman et al., 1980; McQuarrie, 1985; Richardson and Verge, 1987; Jenq et al., 1988; Smith and Skene, 1997). The intrinsic growth state does not increase after axons are

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injured in the mammalian central nervous system (CNS), where they lack the capacity for effective regeneration (Ramon y Cajal, 1928).

Sensory neurons in the dorsal root ganglion (DRG) have two axonal branches that respond differently to injury (Carmel and Stein, 1969). Peripheral branches, which are located entirely in the PNS, are capable of effective regeneration. Central branches regenerate in the PNS (i.e., in the dorsal root, which extends from the DRG to the spinal cord), but not in the CNS (i.e., the spinal cord) (Ramon y Cajal, 1928). This correlates with differences in the environment of central branches, which are myelinated by Schwann cells in the PNS and oligodendrocytes in the CNS (Carlstedt, 1985; Liuzzi and Lasek, 1987). The intrinsic growth state of both branches increases substantially after the injury of peripheral branches, increases much less after central branches are injured in the PNS (i.e., in the dorsal roots near the DRG), and does not increase at all after central branches are injured in the spinal cord (Richardson and Issa, 1984; Chong et al., 1994; Smith and Skene, 1997).

A CL of peripheral branches increases the intrinsic growth state of central branches in the dorsal columns of the spinal cord, enabling these axons to undergo lengthy regeneration in a segment of peripheral nerve transplanted into the spinal cord (i.e., a peripheral nerve graft), which provides a permissive environment for axon elongation (Fig. 1, green axon on left) (Richardson and Issa, 1984). This regeneration does not occur in the absence of a CL (Fig. 1, red axon on right). We will examine how changes in gene expression and axonal transport induced by a CL may promote this regeneration.

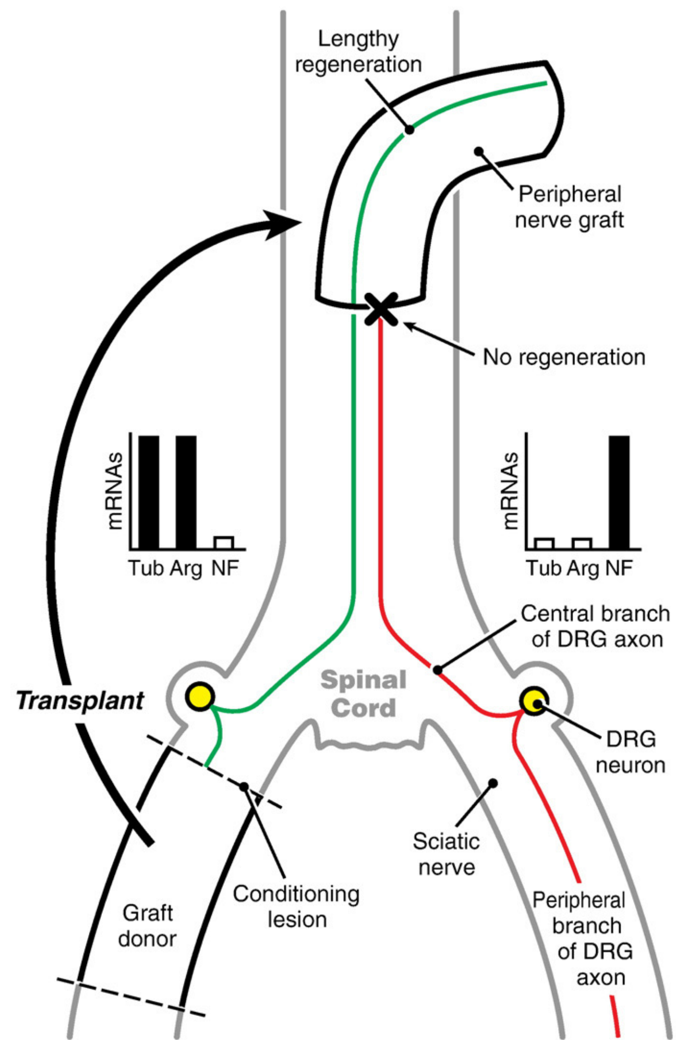

Fig. 1. A CL of peripheral branches, which was induced by harvesting a segment of sciatic nerve for transplantation into the spinal cord, increases the intrinsic growth state of central branches and promotes their lengthy regeneration in a peripheral nerve graft (green axon on left). This regeneration does not occur in the absence of a CL (red axon on right). Changes in gene expression induced by the CL include the elevated expression of growth-associated tubulin isotypes (Tub) and arginase 1 (Arg) and reduced NF expression (left histogram). The expression of these proteins is not altered by cutting central branches in the dorsal columns of the spinal cord (right histogram).

Gene expression during axonal growth

Regenerating sprouts resemble embryonic axons, which also elongate at a rate of several millimeters per day (Ramon y Cajal, 1928). Both are relatively thin (0.2–0.3 μm in diameter) and have cytoskeletons composed almost entirely of microtubules (MTs), cytoskeletal polymers composed of tubulin (Peters and Vaughn, 1967; Weisenberg et al., 1968).

Elongating axons destined to acquire myelin sheaths begin to grow in diameter after they have innervated targets (Weiss et al., 1945). This growth correlates with a massive increase in the number of axonal neurofilaments (NFs), intermediate filaments (i.e., 10 nm in diameter) composed of three distinct protein subunits (NF-L, NF-M, and NF-H) (Hoffman and Lasek, 1975). NFs are intrinsic determinants of axonal caliber that outnumber MTs by more than 10:1 in large-caliber myelinated axons. Axonal cross-sectional area, which may increase more than 2500-fold during postnatal development (e.g., as an axon grows from 0.2 to 10 μm in diameter), is directly proportional to NF number (Friede and Samorajski, 1970; Berthold, 1978). Since axonal diameter is the primary determinant of conduction velocity in myelinated nerve fibers (Hursh, 1939), NFs also play a major role in regulating this important physiological parameter.

The high intrinsic growth state of embryonic axons correlates with the elevated expression of proteins that support axon elongation, including actin, the growth-associated tubulin isotypes, and GAP-43, a protein enriched in growth cone membranes (Skene and Willard, 1981; Hoffman, 1989; Miller et al., 1989). In contrast, NF expression is barely detectable in embryonic neurons (Hoffman and Cleveland, 1988). The radial growth of myelinated axons, which begins during early postnatal development, correlates with a dramatic rise in NF expression that is accompanied by the reduced expression of actin, the growth-associated tubulin isotypes, and GAP-43 (Hoffman, 1989). However, unlike GAP-43, which is generally undetectable in mature neurons, actin and tubulin (i.e., constitutive isotypes) are expressed at significant levels in mature neurons (but at lower levels than in embryonic neurons) (Hoffman, 1989). NF expression is high in mature neurons with thick myelinated axons, which contain many NFs, and low in neurons with unmyelinated axons, which are thin and contain few NFs (Hoffman et al., 1987). Target-derived trophic factors may be responsible for the high levels of NF expression in neurons with large-caliber myelinated axons (Munson et al., 1997).

Changes in gene expression induced by a conditioning lesion

Injury of axons in the PNS (e.g., a CL of peripheral branches) triggers a recapitulation of the developmental pattern of expression for growth-associated proteins (Hoffman and Cleveland, 1988; Hoffman, 1989). Expression increases for actin, the growth-associated tubulin isotypes, and GAP-43, and decreases for the NF proteins (Skene and Willard, 1981; Hoffman et al., 1987; Hoffman and Cleveland, 1988). Other changes include the elevated expression of transcription factors (i.e., ATF-3, c-jun, and Sox11), regulators of translation, and arginase 1, a rate-limiting enzyme in polyamine synthesis, and the reduced expression of ion channels and proteins involved in neurotransmitter synthesis (Fig. 1, left histogram) (Tsujino et al., 2000; Costigan et al., 2002; Cai et al., 2002; Jankowski et al., 2009).

These changes in gene expression, which are much greater after injury of peripheral than central branches at the same distance from the DRG, do not occur after central branches are injured in the spinal cord (Fig. 1, right histogram) (Greenberg and Lasek, 1988; Chong et al., 1994; Smith and Skene, 1997; Seijffers et al., 2007; Jankowski et al., 2009). This reflects a general tendency to induce progressively smaller changes in gene expression as axons are injured at increasing distances from neuron cell bodies (Richardson and Verge, 1986; Doster et al., 1991; Mathew and Miller, 1993; Fernandes et al., 1999).

We can only speculate why changes in gene expression are greater after injury of peripheral than central branches. Schwann cells exert a trophic influence on axons in the PNS (Mudge, 1984). Since peripheral branches are longer than the segments of central branches in the PNS (i.e., in the dorsal root), the loss of these signals would be greater after injury of peripheral than central branches at the same distance from the DRG. If the loss of Schwann cell-derived trophic signals triggers changes in gene expression, these changes would be greater after injury of peripheral than central branches. They would also decline as either branch is injured at increasing distances from neuron cell bodies (Richardson and Verge, 1986; Kenney and Kocsis, 1997).

The changes in gene expression induced by a CL of peripheral branches support the lengthy regeneration of central branches in a peripheral nerve graft (Richardson and Issa, 1984; Hoffman and Cleveland, 1988; Han et al., 2004). Similar changes (e.g., the elevated expression of GAP-43 and growth-associated tubulin isotypes) are also associated with the successful regeneration of optic nerve and rubrospinal axons in peripheral nerve grafts (Fournier et al., 1997; Fernandes et al., 1999). These changes, some of which occur transiently after these CNS axons are injured near neuron cell bodies, may be sustained by cytokines (e.g., oncomodulin) or trophic factors (e.g., BDNF) released by activated macrophages and proliferating Schwann cells in peripheral nerve grafts (Doster et al., 1991; Meyer et al., 1992; Fernandes et al., 1999; Yin et al., 2006). Conversely, these changes do not occur in retinal ganglion cell (RGC) or rubrospinal neurons that fail to regenerate axons in peripheral nerve grafts (Fournier et al., 1997; Fernandes et al., 1999). The specific changes necessary for the regeneration of CNS axons in a peripheral nerve graft have not been identified. However, there is ample evidence that the over-expression of individual proteins (e.g., GAP-43 or ATF-3) is insufficient to promote this regeneration (Bomze et al., 2001; Seijffers et al., 2007).

Elevated cyclic AMP overcomes CNS inhibition of elongation

Regeneration in the CNS is also limited by the presence of molecules that inhibit axon elongation, including myelin associated glycoprotein (MAG) (McKerracher et al., 1994; Mukhopadhyay et al., 1994). The ability of embryonic, but not mature axons to elongate in the presence of MAG correlates with neuronal cyclic AMP (cAMP) levels, which are much higher in embryonic than mature neurons (Cai et al., 1999, 2001). A CL transiently raises cAMP in mature DRG neurons (Qiu et al., 2002). This increase in cAMP correlates with the ability of DRG neurons harvested for culture 1 week after a CL to extend processes in the presence of MAG (Qiu et al., 2002).

Exposing neurons to dibutyryl cAMP (dbcAMP), a membrane-permeable cAMP analogue, mimics the effects of elevated cAMP by activating protein kinase A (PKA) (Cai et al., 1999; Qiu et al., 2002). Cultured DRG neurons exposed to dbcAMP are able to extend processes in the presence of MAG (Cai et al., 1999). Intraocular or intra-ganglionic injection of dbcAMP enables axons to elongate several millimeters in the optic nerve and dorsal columns of the spinal cord, respectively (Qiu et al., 2002; Neumann et al., 2002; Monsul et al., 2004; Yin et al., 2006). Although intra-ganglionic injection of dbcAMP induces some of the same changes as a CL, including the elevated expression of arginase 1 and the growth-associated tubulin isotypes (Fig. 2, right histogram), it does not promote the lengthy regeneration of dorsal column axons in a peripheral nerve graft (Cai et al., 2002; Han et al., 2004) (Fig. 2, red axon on right). This illustrates an important distinction between the effects of dbcAMP and a CL. Although dbcAMP can overcome CNS inhibition of elongation (i.e., allowing axons to elongate a short distance in the CNS), it does not increase the intrinsic growth state (Han et al., 2004).

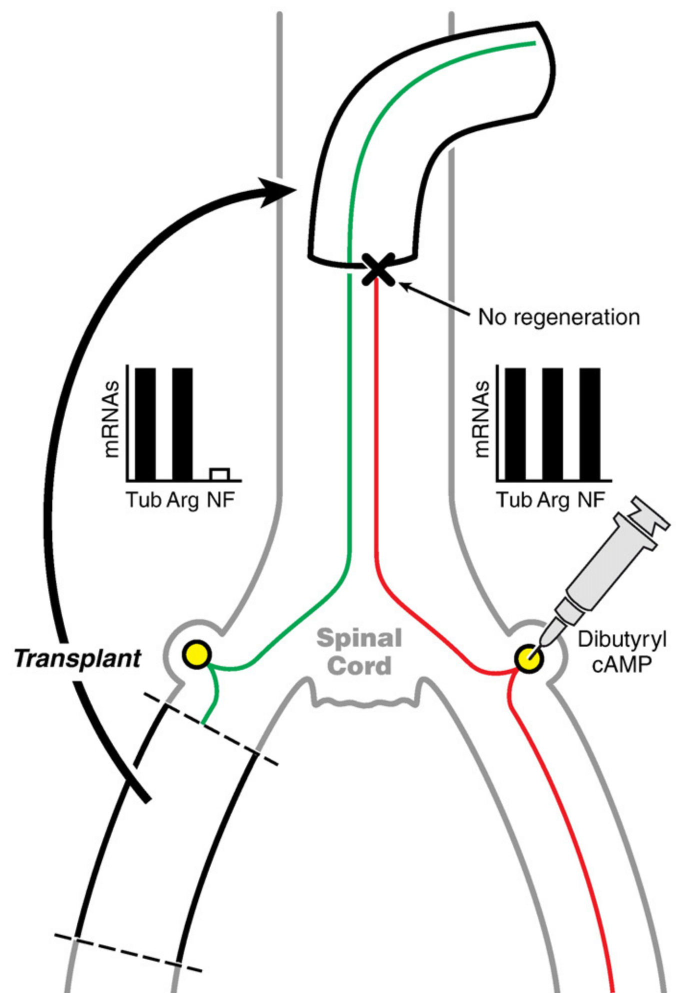

Fig. 2. Intra-ganglionic injection of dbcAMP increases the expression of arginase 1 and growth-associated tubulin isotypes (right histogram), but fails to promote the lengthy regeneration of dorsal column axons in a peripheral nerve graft (red axon on right). This indicates that dbcAMP does not increase the intrinsic growth state of axons.

The transient rise in cAMP induced by a CL overcomes MAG inhibition of elongation by triggering a transcription-dependent rise in the expression of arginase 1 (Arg), a rate limiting enzyme in the synthesis of the polyamines putrescine, spermidine, and spermine (Cai et al., 2002). Arginase 1 is also expressed at high levels in developing neurons (Cai et al., 2002). A CL also rapidly activates ornithine decarboxylase (ODC), the other rate-limiting enzyme in polyamine synthesis, through a transcription-independent mechanism (Gilad and Gilad, 1983; Tetzlaff and Kreutzberg, 1985; Boeshore et al., 2004). Together, these changes elevate neuronal polyamine synthesis after a CL (Boeshore et al., 2004). Inhibiting polyamine synthesis (using specific inhibitors of Arg or ODC) blocks dbcAMP-induced elongation in the presence of MAG (Qiu et al., 2002). Conversely, exogenous putrescine (in the absence of dbcAMP) promotes elongation in the presence of MAG through an unknown mechanism (Cai et al., 2002).

Transport along axonal microtubules

Axonal transport plays at least two important roles in regeneration. It carries signals that alter gene expression from the site of axonal injury to the cell body and it delivers molecules synthesized in the cell body to regenerating sprouts. Transport over relatively long distances involves the ATP-dependent interaction of motor proteins with axonal MTs, which are uniformly oriented with their plus ends towards the axon terminals (Heidemann et al., 1981; Burton and Paige, 1981; Schnapp et al., 1985). In contrast, movement over relatively short distances (e.g., the docking of axonal mitochondria) may involve interactions with actin filaments rather than MTs (Chada and Hollenbeck, 2004).

Cargoes linked to plus end-directed motors (e.g., kinesin-1) are transported along MTs in the anterograde direction (i.e., towards the axon terminals) (Vale et al., 1985; Brady, 1985). Conversely, cargoes attached to minus end-directed motors (e.g., dynein) are transported in the retrograde direction (i.e., towards the cell body) (Paschal and Vallee, 1987). The interaction of MTs with motor proteins can be altered by MT-associated proteins such as tau. For example, the binding of tau to MTs disrupts interactions with kinesin-1 and reverses the direction of dynein movement (Dixit et al., 2008).

Axons contain long stationary MTs, which serve as a pathway for axonal transport, and short MTs that can move bi-directionally. Existing MTs can be cut into shorter segments by MT severing proteins such as spastin and katanin (Baas et al., 2005). Unlike other cargoes, MTs appear to move by interacting with stationary motor proteins attached to immobile MTs or actin filaments. MTs move in the anterograde direction by interacting with stationary minus end-directed motors such as dynein, and in the retrograde direction by interacting with stationary plus end-directed motors such as kinesin-1 (Ahmad et al., 1998; He et al., 2005). Unlike MTs, NFs are attached to motor proteins that propel them along stationary MTs (Xia et al., 2003; He et al., 2005). The selective inhibition of NF transport by systemic intoxication with β, β'-iminodipropionitrile (IDPN) correlates with the segregation of NFs from MT-rich channels containing transported vesicles and mitochondria (Griffin et al., 1978; Griffin et al., 1983).

Adaptor proteins regulate both the activity of motor proteins and their binding to specific cargoes (Gunawardena and Goldstein, 2004). In the absence of cargo binding, the N-terminal motor region of kinesin-1 is auto-inhibited by binding to its C-terminal tail. Motor activity is restored by the simultaneous binding of FEZ1 (fasciculation and elongation factor ζ1) to the kinesin heavy chain and JIP1 (c-Jun NH_2-terminal kinase [JNK] interacting protein 1) to the kinesin light chain (Blasius et al., 2007). JIP1 also links kinesin-1 to vesicles whose membranes contain amyloid precursor protein (APP) or apolipoprotein E receptor 2 (ApoER2) (Stockinger et al., 2000; Muresan and Muresan, 2005). Other adaptor proteins link kinesin-1 to mitochondria (i.e., Milton, Miro, and syntabulin) and mRNAs (i.e., RNA-binding proteins such as HuD and zipcode binding protein 1 [ZBP1]) (Zhang et al., 1999; Krichevsky and Kosik, 2001; Eom et al., 2003; Cai et al., 2005; Guo et al., 2005; Glater et al., 2006). Dynein is linked to different cargoes by specific components of the dynactin complex (Gill et al., 1991).

The phosphorylation of adaptor proteins can alter their interaction with motor proteins. JIP3 (JNK interacting protein 3, also known as Sunday driver) is an adaptor protein that binds to JNK and its upstream activators. The binding of JIP3 to kinesin-1 is responsible for the anterograde transport of JNK in uninjured axons. After JNK has been activated by axonal injury, it phosphorylates JIP3. This phosphorylation disrupts the interaction of JIP3 with kinesin-1 and increases its binding to the $p150^{Glued}$ component of the dynactin complex, which is constitutively bound to dynein. This results in the retrograde transport of activated JNK to the cell body after axonal injury (Cavalli et al., 2005).

Slow axonal transport results from rapid, but intermittent movement punctuated by long pauses

Pulse-labeling studies reveal that cytoskeletal polymers and soluble proteins are transported two orders of magnitude slower than membrane-associated proteins, which move at a rate of 400 mm/day (Lasek, 1968). MTs and NFs are transported in slow component a (SCa), which moves at a net velocity of 1–2 mm/day in sciatic nerve axons of 10-week-old rats. Soluble proteins, including unassembled actin and tubulin, clathrin, and enzymes of glycolysis, are transported in slow component b (SCb) at a net rate of 3–6 mm/day in these axons (Lasek and Hoffman, 1976; Lasek et al., 1984; Filliatreau et al., 1988; Tashiro and Komiya, 1989). The velocity of slow axonal transport declines with age and differs in various populations of axons (Hoffman et al., 1983; McQuarrie et al., 1986). For example, it is two-fold greater in peripheral than central branches of sensory axons (Mori et al., 1979). In contrast, the velocity of membrane-associated proteins (i.e., 400 mm/day) is identical in all populations of axons and does not change with age (Ochs, 1973).

The movement of individual NFs and MTs has been visualized in real-time imaging studies using fluorescent markers to label these polymers in the axons of cultured neurons (Wang et al., 2000; Wang and Brown, 2002). These studies reveal that individual polymers undergo rapid, but intermittent bi-directional movement punctuated by long pauses (Wang et al., 2000). The instantaneous velocity of this movement is comparable to that of vesicle transport (Wang et al., 2000; Wang and Brown, 2002). The soluble proteins transported in SCb appear to be associated with multi-protein complexes that also move intermittently along axonal MTs. The faster velocity of SCb reflects shorter pauses for SCb than SCa (Roy et al., 2007). Thus, factors that regulate the duration of transport pauses are likely to account for differences in the net rate of slow transport in various populations of axons and for reductions that occur with age.

The duration of transport pauses and the net velocity of NF transport may be influenced by the interaction of axons with myelin-forming cells (i.e., Schwann cells in the PNS and oligodendrocytes in the CNS) (de Waegh et al., 1992). The radial growth of myelinated axons during postnatal development correlates with elevated NF expression and a dramatic increase in the number of axonal NFs, which outnumber MTs by more than 10:1 in large-caliber axons (Friede and Samorajski, 1970; Hoffman and Cleveland, 1988). Since NFs move by interacting with MTs, increasing the ratio of NFs to MTs may raise the average duration of transport pauses by reducing the probability that an individual NF will interact with a MT. This is consistent with an age-dependent decline in the net velocity of NF transport as myelinated axons grow in caliber (Hoffman et al., 1983; Hoffman et al., 1985).

The axonal transport of mRNAs

The abundance of mRNAs encoding growth-associated proteins increases in neuron cell bodies after a CL (Hoffman and Cleveland, 1988; Hoffman, 1989; Smith and Skene, 1997). In addition to being translated in cell bodies, specific mRNAs may be transported into axons and undergo local translation (Tobias and Koenig, 1975). Granules containing mRNAs, RNA-binding proteins, motor proteins, and ribosomes are rapidly transported along axonal MTs (Bassell et al., 1998; Krichevsky and Kosik, 2001). These RNA-binding proteins (e.g., HuD and ZBP1) interact with sequences in the 3′ untranslated regions (3′UTRs) of specific mRNAs (Aronov et al., 2002; Smith et al., 2004). For example, ZBP1 specifically binds to a sequence in the 3′UTR of β-actin mRNAs known as zipcode (Bassell et al., 1998; Zhang et al., 2001). The axonal transport of β-actin mRNAs in embryonic neurons is regulated by neurotrophins through a cAMP-dependent mechanism (Zhang et al., 1999).

Mature axons contain several mRNAs that are translated in response to axonal injury. The local translation of vimentin and importin-β at the site of injury triggers the retrograde transport of activated (i.e., phosphorylated) MAP kinases Erk1 and Erk2 to the neuron cell body (Hanz et al., 2003; Perlson et al., 2005). Vimentin, an insoluble intermediate filament protein, is cleaved by calpain (which is activated by the influx of calcium at the site of injury) to form a soluble fragment that links importin-β to phosphorylated MAP kinases Erk1 and Erk2. Importin-β also binds to importin-α, an adaptor protein constitutively present in axons and bound to dynein. This results in the formation of a multi-protein complex in which these activated MAP kinases are linked to dynein and undergo retrograde transport (Hanz et al., 2003; Perlson et al., 2005).

Twiss and coworkers have examined the axonal transport of mRNAs in regenerating axons of cultured DRG neurons. These neurons were grown on membranes containing pores large enough to accommodate elongating axons, but not migrating cell bodies (Zheng et al., 2001). By harvesting axons that have grown through pores and along the under-surface of these membranes, they found that mRNAs encoding a large number of proteins, including β-actin (but not γ-actin, which is confined to cell bodies) and the growth-associated tubulin isotypes, are transported in regenerating axons that have attained a length of several micrometers (Willis et al., 2005). It remains to be determined whether these mRNAs are also transported over distances three orders of magnitude greater (>100 mm) in the regenerating axons of rat sciatic nerve. Although local translation plays important roles in growth cone formation, growth cone guidance, and neurotrophic signaling, it does not appear to be necessary for axon elongation (Campbell and Holt, 2001; Brittis et al., 2002; Verma et al., 2005; Cox et al., 2008). In fact, the elongation of embryonic axons can be blocked by inhibiting translation in cell bodies, but not in axons (Blackmore and Letourneau, 2007).

Changes in axonal transport induced by axonal injury

Axonal injury reduces NF expression and decreases the amount of NF protein transported in SCa (Hoffman and Lasek, 1980; Hoffman et al., 1987). This results in a fall in axonal caliber and NF content that start proximally and spread away from the cell body (somatofugally) along nerve fibers at a rate equal to the net velocity of NF transport in SCa, which is two-fold greater in peripheral than central branches of sensory axons (Hoffman et al., 1984, 1987). This process, referred to as somatofugal axonal atrophy, results in reduced conduction velocity (Cragg and Thomas, 1961).

Significant amounts of actin and tubulin are constitutively transported in mature axons in the absence of a prior CL. Thus, SCb provides a continuous stream of unassembled actin and tubulin emanating from the cell body that can be interrupted at any point along its length, leading to the accumulation of these proteins at the site of axonal injury (Lasek and Hoffman, 1976; Hoffman and Lasek, 1980). Actin filaments are directly involved in the motility of growth cones at the tips of elongating axons (Yamada et al., 1971). Tubulin is preferentially added to the plus ends of MTs, which are uniformly oriented towards the growing tip (Heidemann et al., 1981; Burton and Paige, 1981). The local assembly of MTs at the axon tip is a prerequisite for successful outgrowth (Bamburg et al., 1986). Tubulin transported in parent axons in SCb slows to the velocity of SCa after entering regenerating sprouts (McQuarrie and Lasek, 1989). This is consistent with a model in which unassembled tubulin transported in SCb is added to MTs moving in regenerating sprouts in SCa.

The rate of regeneration is identical to the velocity of SCb (Lasek and Hoffman, 1976; Wujek and Lasek, 1983). The two-fold greater velocity of SCb in peripheral than central braches of sensory axons correlates with identical differences in the rates of regeneration of these axons in the sciatic nerve and dorsal root, respectively (Komiya, 1981; Wujek and Lasek, 1983). An age-dependent decline in the velocity of SCb in both branches of sensory axons correlates with identical reductions in the rate of regeneration (Black and Lasek, 1979; Komiya, 1980; Hoffman et al., 1983). Thus, the intrinsic growth state of axons is reflected in the rate of regeneration, which is identical to the velocity at which unassembled tubulin is transported in SCb. Although other proteins are undoubtedly required for axon elongation, the relatively slow velocity of actin and tubulin in SCb (e.g., as compared to 400 mm/day for GAP-43) may be rate-limiting for regeneration (Lasek and Hoffman, 1976).

The inability of RGC axons to regenerate in the optic nerve correlates with the absence of tubulin in SCb and with the near-cessation of tubulin transport after axonal injury. Tubulin is transported exclusively in SCa in RGC axons (McQuarrie et al., 1986). The velocity of SCa, which is normally 10-fold slower in optic nerve than sciatic nerve axons (i.e., 0.1–0.2 mm/day), declines further after axonal injury (i.e., to 0.01–0.02 mm/day) (McQuarrie et al., 1986; McKerracher et al., 1990b). The successful regeneration of RGC axons in a peripheral nerve graft correlates with the elevated expression of growth-associated tubulin isotypes and the transport of tubulin in SCb (McKerracher et al., 1990a; Fournier et al., 1997). This is consistent with the hypothesis that the intrinsic growth state of axons reflects the velocity of tubulin transport in SCb (i.e., the transport of tubulin in SCb is necessary for effective regeneration) (Lasek and Hoffman, 1976).

GAP-43 is transported rapidly enough (i.e., 400 mm/day) for elevated expression to influence the initial elongation of axons injured relatively far from the cell body in the absence of a prior CL. This is not true for the actin and tubulin transported in SCb (Lasek and Hoffman, 1976; Hoffman and Lasek, 1980) (Fig. 3). There is a 1-

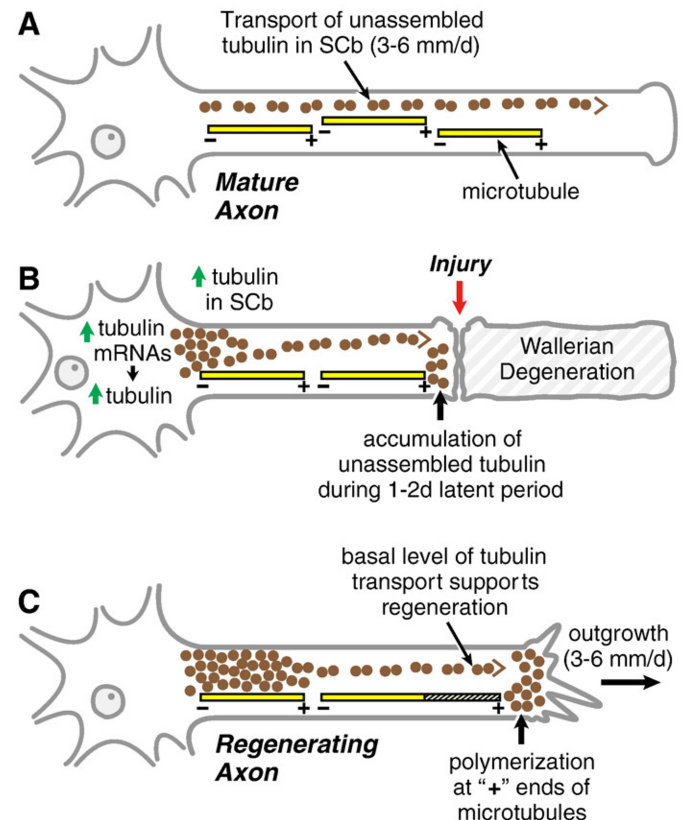

Fig. 3. (A) In the absence of a CL, unassembled tubulin is transported in SCb at a velocity equal to the rate of regeneration (3–6 mm/day in rat sciatic nerve). (B) Although the tubulin content of SCb increases after axonal injury, this tubulin does not move fast enough to reach the tips of axons injured far from the cell body before elongation begins. (C) In this model, tubulin already en route in SCb at the time of injury supports MT assembly at the growing tip.

to 2-day latent interval between the time of injury and the onset of elongation (Forman et al., 1980). In sciatic nerve axons injured 60 mm from neuron cell bodies, tubulin moving in SCb at a maximum velocity less than 10 mm/day would not reach the site of injury until at least 6 days after leaving neuron cell bodies. By that time regenerating axons would extend 16 mm beyond the site of injury, assuming that elongation begins 2 days after injury and proceeds at a rate of 4 mm/day. This suggests that the quantities of actin and tubulin constitutively transported in SCb are sufficient to support the elongation of axons injured far from neuron cell bodies (Fig. 3). This hypothesis is consistent with the observation that pulse-labeled tubulin already en route at the time of injury is transported into regenerating sprouts (McQuarrie and Lasek, 1989). Local translation is another potential source of tubulin for assembly at the growing axon tip (Fig. 4). At present, it is unclear whether significant amounts of actin or tubulin mRNAs are rapidly transported to the tips of regenerating axons *in vivo*.

The latent interval is reduced in regenerating axons injured 1–2 weeks after a CL (Forman et al., 1980). Since significant amounts of actin and tubulin are normally transported in SCb in uninjured axons, the increased expression and transport of these proteins after a CL may influence the intrinsic growth state much less than the elevated transport of proteins like GAP-43, which are normally absent in mature axons. If these proteins must be rapidly transported to the tips of injured axons before elongation can begin, then the latent interval would include the delay between the time of injury and the arrival of these molecules at the axon tip. A CL can eliminate this delay by ensuring that these molecules are already en route in axons at the time of the test lesion.

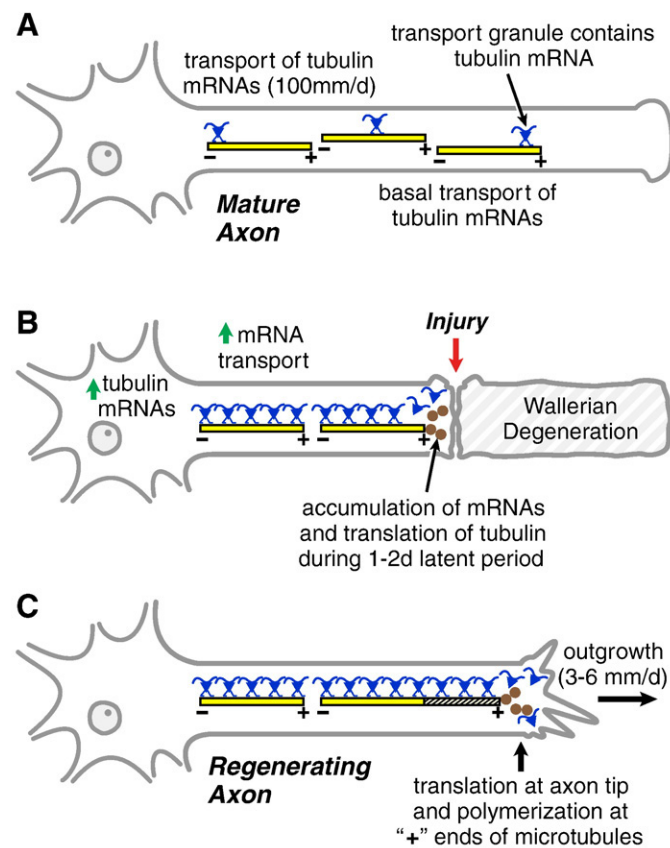

Fig. 4. (A) Few, if any tubulin mRNAs are transported in axons prior to injury. (B) It remains to be determined whether elevated expression and transport after injury results in the accumulation of these mRNAs at axon tips before elongation begins. (C) In this model, local translation of tubulin supports MT assembly at the axon tip.

The rate of elongation and the number of regenerating sprouts increase in axons re-injured 1–2 weeks after a CL (McQuarrie et al., 1977; McQuarrie, 1985; Richardson and Verge, 1987; Jenq et al., 1988). Elevated tubulin expression after a CL results in an increase in the amount of unassembled tubulin transported in SCb (Hoffman and Lasek, 1980; Hoffman et al., 1992). The increased delivery of unassembled tubulin to the axon tip may support the assembly of more MTs at a faster rate. Additional MTs can be generated locally through the action of MT severing proteins such as katanin and spastin, which cut existing MTs into shorter segments (Yu et al., 2008). Even in the absence of a prior CL, there is a 10-fold increase in the number of MTs in regenerating sprouts 3 days after injury (Friede and Bischhausen, 1980). If the rate of regeneration is limited by the speed of MT assembly, increasing the rate of assembly might accelerate regeneration (Lasek and Hoffman, 1976). Furthermore, if each sprout contains a fixed number of MTs, the assembly of more MTs might support the formation of additional sprouts.

Conclusion

Axonal regeneration in the CNS is limited by the death of injured neurons, the presence of molecules that inhibit elongation, and a failure to increase the intrinsic growth state of axons (Han et al., 2004). Once the changes in gene expression and axonal transport necessary to increase the intrinsic growth state have been identified, it may be possible to induce them in CNS neurons as part of a strategy to promote robust regeneration.

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