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The Cellular and Molecular Basis of Peripheral Nerve Regeneration

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Abstract

Functional recovery from peripheral nerve injury and repair depends on a multitude of factors, both intrinsic and extrinsic to neurons. Neuronal survival after axotomy is a prerequisite for regeneration and is facilitated by an array of trophic factors from multiple sources, including neurotrophins, neuropoietic cytokines, insulin-like growth factors (IGFs), and glial-cell-line-derived neurotrophic factors (GDNFs). Axotomized neurons must switch from a transmitting mode to a growth mode and express growth-associated proteins, such as GAP-43, tubulin, and actin, as well as an array of novel neuropeptides and cytokines, all of which have the potential to promote axonal regeneration. Axonal sprouts must reach the distal nerve stump at a time when its growth support is optimal. Schwann cells in the distal stump undergo proliferation and phenotypical changes to prepare the local environment to be favorable for axonal regeneration. Schwann cells play an indispensable role in promoting regeneration by increasing their synthesis of surface cell adhesion molecules (CAMs), such as *N*-CAM, Ng-CAM/L1, N-cadherin, and L2/HNK-1, by elaborating basement membrane that contains many extracellular matrix proteins, such as laminin, fibronectin, and tenascin, and by producing many neurotrophic factors and their receptors. However, the growth support provided by the distal nerve stump and the capacity of the axotomized neurons to regenerate axons may not be sustained indefinitely. Axonal regeneration may be facilitated by new strategies that enhance the growth potential of neurons and optimize the growth support of the distal nerve stump in combination with prompt nerve repair.

Index Entries: Nerve regeneration; axotomy; neuronal death; Schwann cells; basal lamina; macrophages; growth-associated proteins; neuropoietic cytokines; neurotrophic factors; cell adhesion molecules.

Introduction

The ability of the peripheral nerve to regenerate and reinnervate denervated targets has been recognized for more than a century. However, complete functional recovery is rarely achieved, despite the considerable advances made in microsurgical techniques and the

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understanding of nerve regeneration (Sunderland, 1978; Terzis, 1987; Lundborg, 1988; Kline and Hudson, 1995). Functional recovery is particularly poor for injuries that sever the nerve far from the target and that incur a considerable delay before target reinnervation (Gordon and Fu, 1997). The processes of nerve regeneration and target reinnervation are complex, involving a myriad of factors pertaining to the neuron, the growth environment, and the target. First, the neuron must survive the injury and mount an effective metabolic response to initiate regeneration. Second, the growth environment in the nerve stump distal to the injury site must provide sufficient support for regenerating axons. Third, the successfully regenerated axon must reinner-vate the proper target, and the target must retain the ability to accept reinnervation and recover from denervation atrophy. In this article, current knowledge on the cellular and molecular responses of axotomized neurons and the growth environment is critically examined to provide a basis for understanding why nerve regeneration often fails to restore function fully.

Survival and Response of the Axotomized Neuron

Survival

The first prerequisite for axonal regeneration is survival of the neuron following an injury. Survival depends on several factors, including neuron type, age, and the degree and proximity of the injury to the cell body. In general, spinal motoneurons are less susceptible to injury-induced cell death than cranial motoneurons and sensory neurons; mature neurons in the adult are less susceptible than immature neurons in the developing animal; neurons subjected to injuries far from cell bodies are less susceptible than injuries close to cell bodies (*see* Lieberman, 1971, 1974; Sunderland, 1978; Kuno, 1990; Snider et al., 1992; Greensmith and Vrbova, 1996 for reviews).

Spinal motoneurons rarely die after peripheral nerve section in adult mammals (Himes and Tessler, 1989; Melville et al., 1989) and remain viable even after prolonged separation from the target (Carlsson et al., 1979; Gordon et al., 1991; Vanden Noven et al., 1993). In contrast, a large proportion of cranial motoneurons die from injuries of the facial and hypoglossal nerves (Carlsson et al., 1979; Arvidsson and Aldskogius, 1982; Snider and Thanedar, 1989), since the cranial nerve lesions are generally closer to the soma than nerve lesions of the spinal motoneurons. For a given species at a given age, the proximo-distal location of the injury appears to be critical for neuronal survival. For example, even spinal motoneurons die after proximal lesions, such as avulsion of spinal roots (Wu, 1993; Koliatsos et al., 1994b). Sensory neurons are more susceptible to cell death than motoneurons. Up to 50% of the sensory neurons in a dorsal root ganglion (DRG) die after sciatic nerve transection in the adult (Schmalbruch, 1984; Melville et al., 1989). Both sensory and motoneurons, including cranial motoneurons, are more susceptible to axotomy-induced cell death in neonatal mammals (Schmalbruch, 1984; Himes and Tessler, 1989; Snider and Thanedar, 1989; Sendtner et al., 1990; Snider et al., 1992).

Cell Death

The mechanisms involved in injury-induced neuronal death are not yet fully understood. It is now clear that axotomized neurons die by apoptosis (Lo et al., 1995). The neuron displays characteristic morphological changes associated with apoptosis and undergoes DNA fragmentation (Bursch et al., 1992; Lo et al., 1995; Rossiter et al., 1996). There is compelling evidence that neuronal death is caused, at least in part, by deprivation of neurotrophic factors.

Neurotrophic factors are expressed, and released by target tissues and by glial cells, fibroblasts, and macrophages in the vicinity of both the neuronal cell body and axon (Barde, 1989; Richardson, 1991, 1994; Korsching, 1993). After axotomy, nonneuronal cells in the distal nerve stump, especially Schwann cells, synthe-

size many neurotrophic factors. These include the neurotrophins, nerve growth factor (NGF), neurotrophin 4/5 (NT-4/5), brain-derived nerve growth factor (BDNF), glial-cell-line-derived neurotrophic factor (GDNF), and insulin-like growth factors (IGFs) (for review, *see* Richardson, 1991; Bisby, 1995; and *see* Neurotrophic Factors and Receptors). These neurotrophic factors appear to be essential for the survival of axotomized neurons, since experimental procedures that deny or interfere with the access of regenerating axons to the distal nerve stump lead to an increasing magnitude of neuronal death. Capping of the proximal end of a transected peripheral nerve prevents axonal regeneration into the distal nerve stump and results in the death of 50% more DRG neurons than after surgical suture of the transected nerve (Rich et al., 1989b). The high incidence of neuronal death of the DRG neurons after nerve transection and repair has been attributed to a small number of regenerating axons entering the distal nerve stump and the target, compared to a large magnitude of neuronal survival after nerve crush (Aldskogius and Risling, 1981; Risling et al., 1983). Death of the axotomized hypoglossal motoneurons is markedly attenuated when regenerating axons gain access to the distal nerve stump regardless of the proximo-distal level of transection (Tornqvist and Aldskogius, 1994). Death of the axotomized DRG neurons is completely prevented when the sectioned proximal and distal stumps of the sciatic nerve are connected with an impermeable silicone tube to direct the trophic factors produced by the distal stump into the proximal stump (Melville et al., 1989). Trophic support of a peripheral nerve segment, grafted between the transected ends of the rat optic nerve, promotes the survival of the retinal ganglion neurons (Villegas-Perez et al., 1988).

All these observations strongly suggest that trophic support from the distal nerve stump and/or target organ plays an essential role in promoting neuronal survival after injury. However, the distal nerve stump and/or target organs may not be the only sources of neurotrophic support. For example, axotomized motoneurons survive even when they are denied access to the distal nerve stump for periods of more than 1 yr (Carlsson et al., 1979; Gordon et al., 1991; Vanden Noven et al., 1993). The trophic support for the axotomized spinal motoneurons may derive from glial cells within the spinal cord as well as from neurotrophic factors synthesized by the neurons themselves as described in Neurotrophins and Receptors.

Chromatolysis

Surviving neurons undergo morphological, physiological, and molecular changes that are characteristic of a switch from a "transmitting mode" to a "growing mode" (for reviews, *see* Watson, 1974; Grafstein and McQuarrie, 1978; Gordon, 1983; Titmus and Faber, 1990). The most conspicuous and consistent morphological changes in the neuronal cell body are dissolution of the Nissl bodies, nuclear eccentricity, nucleolar enlargement, and cell swelling, which collectively are called "chromatolytic changes" (Nissl, 1892; for reviews, *see* Lieberman, 1971, 1974; Kreutzberg, 1995). Chromatolysis is more severe and occurs earlier in neurons with axonal lesions close to the cell body (Marinesco, 1896), suggesting a correlation between the severity of chromatolysis and the amount of axonal growth that is required to establish target connections. However, chromatolysis is not necessarily a prerequisite for successful regeneration. For example, DRG neurons regenerate successfully with little chromatolysis (Hall, 1982; Perry et al., 1983; Greenberg and Lasek, 1988).

Conversion from "Transmitting Mode" to "Growth Mode"

The dissolution of the ribosomes and ordered arrays of rough endoplasmic reticulum that constitute the Nissl bodies are the morphological correlates of enhanced protein synthesis (Cragg, 1970), which is associated with increased mRNA synthesis in axotomized neurons (Watson, 1974). Expression of imme-

diate-early genes and associated changes in the production of cytoskeletal proteins, neurotransmitters, and growth factor receptors has been regarded as an indication of the switch from "transmitting mode" to "growth mode," and a prerequisite for neuronal survival and regeneration (reviewed by Watson, 1976; Gordon, 1983; Skene, 1989; Cleveland and Hoffman, 1991; Aldskogius et al., 1992; Bisby, 1995; Kreutzberg, 1995; Vrbova et al., 1995; Zigmond et al., 1997). For example, the pattern of gene expression in axotomized motoneurons is consistent with a switch from cholinergic neurotransmission to growth. Following an early increase in *jun*B and c-*jun* mRNA, the rate limiting cholinergic synthetic enzyme, choline acetyltransferase (CAT), is downregulated, whereas the neuropeptide, calcitonin gene-related peptide (CGRP), the fast transported growth-associated protein, GAP-43, and the slowly transported cytoskeletal proteins, actin and tubulin, are upregulated (Tetzlaff et al., 1988, 1991; Haas et al., 1990, 1993; Friedman et al., 1995). Among the immediate-early gene families, including *jun*, *fos*, and *krox*, only members of the *jun* family are selectively expressed in axotomized motor and sensory neurons (Jenkins and Hunt, 1991; Leah et al., 1991). However, the temporal association of these changes with regeneration does not necessarily indicate that they are essential for regeneration. For example, expression of c-*jun* and nitric oxide synthase (NOS) in axotomized neurons has been associated with both neuronal death and regeneration (Ham et al., 1995).

Growth-Associated Proteins

Correspondence between a change in gene expression after axotomy and reversal of the change after target reinnervation suggests a functional role of the gene product in nerve regeneration. An example is the induction of GAP-43, CGRP, and developmental isoforms of Tα and class IIβ tubulin after axotomy and their repression on target reinnervation (Skene and Willard, 1981; Skene et al., 1986; Hoffman and Cleveland, 1988; Hoffman, 1989; Miller et al., 1989). However the temporal correlation between the expression of a protein and growth of regenerating axons does not necessarily elucidate the function of the protein in regeneration. GAP-43, a phosphoprotein that is upregulated in growing axons, is preferentially localized to the inside of the axonal membrane, particularly near the growth cone (Meiri et al., 1988). Although in vitro experiments indicate an involvement of GAP-43 in neurite extension, recent findings from GAP-43-deficient transgenic mice indicate that GAP-43 plays a role in pathway finding of neurons rather than a prerequisite role in neurite extension (Strittmatter et al., 1995). Antisense GAP-43 oligonucleotides or intracellular anti-GAP-43 antibodies inhibit neurite extension in cultured DRG neurons, neuroblastoma, and PC-12 cells (Shea et al., 1991; Aigner and Coroni, 1993). Overexpression of GAP-43 increases the propensity of PC-12 cells to extend neurites (Yankner et al., 1990) and promotes axonal sprouting in muscles (Aigner et al., 1995). However, growth cone, neurite formation, and axonal growth rate are normal in GAP-43-deficient mice, but axons fail to grow in proper pathways (Strittmatter et al., 1995).

Cytoskeletal Proteins

There is a close correlation between the rate of slow component b transport of tubulin and actin (SCb) and the rate of axonal regeneration (Wujek and Lasek, 1983; Hoffman and Cleveland, 1988; McQuarrie and Lasek, 1989). This correlation, together with the evidence of upregulation of tubulin and actin mRNA and downregulation of neurofilament proteins after axotomy (Tetzlaff et al., 1988), strongly supports the view that regenerating axons recapitulate developing axons in transporting increased supplies of actin and tubulin to the growth cones for axonal growth (Hoffman and Cleveland, 1988). Downregulation of neurofilament proteins has been suggested to increase the fluidity of the axoplasm and thereby facilitate axonal transport (Kreutzberg, 1995; Tetzlaff, 1996). The reduced transport of neurofilaments down the axon accounts for the

decline in axon diameter and slowed conduction velocity (Hoffman et al., 1984, 1987; Gordon et al., 1991; Eyer and Peterson, 1994). However, the precise relationships among transport of actin and tubulin, reduced transport of neurofilaments, and regeneration are yet to be established (reviewed by Bisby, 1995).

Neurotransmitters and Neuropeptides

Although axotomy is associated with downregulation of classical neurotransmitters and genes encoding transmitter-related proteins in motor, sympathetic, and sensory neurons (reviewed by Grafstein and McQuarrie, 1978; Gordon, 1983; Zigmond et al., 1997), several neuropeptides are upregulated. In axotomized motoneurons, CGRP is upregulated concurrent with downregulation of CAT (Haas et al., 1990). In axotomized sympathetic neurons, expression of vasoactive intestinal peptide (VIP), galanin, and substance P mRNA increases in parallel with downregulation of noradrenaline synthetic enzymes and the normally prominent neuropeptide Y (NPY), possibly in response to leukemia inhibitory factor (LIF) (Zigmond et al., 1997). In axotomized sensory ganglion neurons, the neuropeptides, substance P, CGRP, and somatostatin, which are normally expressed in the transmitting neurons, are downregulated whereas VIP, galanin, NPY, and cholecystokinin are upregulated (Verge et al., 1996; reviewed by Zigmond et al., 1997).

The switch in neurotransmitter phenotype has also been viewed in the context of the "growth mode" of axotomized neurons. The upregulation of certain peptides may contribute to both the survival of axotomized neurons and regeneration of their axons. For example, VIP prevents neurite retraction and cell death in cultured sympathetic ganglion neurons after NGF withdrawal, and VIP and NPY increase neurite outgrowth of cultured DRG neurons (Pincus et al., 1990; Tanaka and Koike, 1994; White and Mansfield, 1996). Coexpression of peptides and their receptors in axotomized neurons suggests autocrine and/or paracrine effects of the upregulated peptides (Zigmond et al., 1997). Neuropeptides released from sectioned axons may also contribute to inflammatory response to axonal injury. For example, release of VIP after upregulation in some axotomized sensory neurons may contribute to maintaining blood supply to regenerating axons by promoting vasodilatation (Said and Mutt, 1970). Release of CGRP from the cut sensory axons may enhance blood flow to the injured nerve stumps by inducing vasodilatation and promote influx of proinflammatory cells, such as macrophages, mast cells, and lymphocytes. Downregulation of CGRP in the axotomized sensory neurons presumably curtails this effect. However, the upregulation of CGRP in axotomized motoneurons (Haas et al., 1990; Kreutzberg, 1995; Sala et al., 1995) could conceivably sustain the inflammatory response to influence nerve regeneration in addition to its posited role in the formation and maintenance of neuromuscular junctions during development, sprouting, and regeneration (Fontaine et al., 1987; Matteoli et al., 1990; Lu et al., 1993; Sala et al., 1995).

There is also evidence for a neurotrophic role of neuropeptides via their mitogenic effects on glial cells. After nerve injury, VIP exerts an indirect neurotrophic effect by enhancing the proliferation of astrocytes and promoting their release of a neuronal survival factor (Brenneman et al., 1987). VIP and CGRP may also contribute to glial cell function by increasing cAMP levels, which subsequently poten tiates the effects of mitogenic growth factors on Schwann cells and endothelial cells of the blood vessels (Cheng et al., 1995). The mitogenic growth factors, which include fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and glial growth factor (GGF), are released from the cut axons as well as from the macrophages and platelets that enter the injury site, and the activated Schwann cells in the nerve stumps (Yasuda et al., 1988; Reynolds and Woolf, 1993; *see* Schwann Cell Proliferation and Fig. 1).

Cytokines and Macrophages

Axotomized neurons also synthesize and release cytokines, which appear to contribute

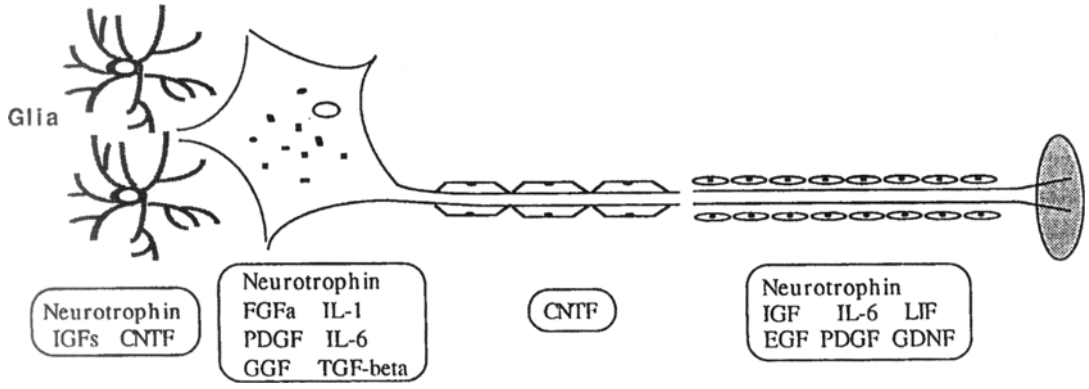

Fig. 1. Multiple sources of neurotrophic support. Neurotrophic factors are produced not only by nonneuronal cells in the distal nerve stump, but also Schwann cells in the proximal stump, neurons, and glia cells adjacent to neuronal soma. These trophic factors promote axonal regeneration via paracrine or autocrine pathways.

to the inflammatory response and synergize with cytokines released by macrophages and nonneuronal cells. These cytokines include the interleukins 1, 2, and 6 (IL-1, IL-2, IL-6), transforming growth factor (TGF-β) (Kiefer et al., 1993; Murphy et al., 1995), and interferon-γ (IFN-γ) (Rogister et al., 1993; Olsson et al., 1994). All or most of these cytokines are mitogenic for Schwann cells and influence their phenotype. For example, TGF-β released from axotomized DRG neurons is mitogenic for Schwann cells, and modulates the synthesis of NGF and deposition of extracellular matrix proteins by activated Schwann cells (Matsuoka et al., 1991; Rogister et al., 1993; Mews and Meyer, 1993).

Another important source of cytokines is the macrophages that differentiate from monocytes which invade axotomized DRG and sympathetic ganglia (elicited macrophages), and the microglia (monocyte-derived cells resident in the CNS) (Auger and Ross, 1992; Schreiber et al., 1995). After axotomy, the macrophages and microglia proliferate, surround the neuronal soma, release cytokines, such as IL-1 and IL-6, express the major histocompatibility complex (MHC) class I and II antigens (as antigen-presenting cells), and may participate directly in the cell body reaction (Perry et al., 1987, 1993; Heumann et al., 1987b; Graeber et al., 1988; Streit et al.,1989a,b; Brown et al., 1991b; Guenard et al., 1991; Auger and Ross, 1992; Schreiber et al., 1995). For example, macrophages may contribute to altered neuropeptide expression in sympathetic ganglion cells via IL-1-induced release of LIF from glial cells (Rao et al., 1993; Schreiber et al., 1995; Zigmond et al., 1996). Likewise, IL-6 and TGF-β released from neurons, astroglia, and/or microglia may contribute to the expression of neurotrophic factors in astrocytes that surround axotomized motoneurons and DRG neurons via autocrine or paracrine pathways (Kiefer et al., 1993; Murphy et al., 1995; Kreutzberg, 1995). In the process of microglial proliferation and astrocyte activation, synapses from the soma of motoneurons are "stripped" (Kreutzberg, 1995). A similar "stripping" of synapses has been described in sympathetic ganglia (Matthews and Nelson, 1975; Sumner, 1975).

Neurotrophins and Receptors

Axotomized neurons synthesize many neurotrophic factors, including NGF (Ernfors et al., 1989), BDNF and NT-3 (Ernfors and Persson, 1991; Schecterson and Bothwell, 1992; Kobayashi et al., 1996), acidic fibroblast growth factor (FGFa) (Elde et al., 1991), PDGF (Sasahara et al., 1991; Yeh et al., 1991), and GGF (Marchionni et al., 1993), all of which may have autocrine and paracrine effects on neurons in addition to their effects on nonneuronal cells

in the growth pathway (Fig. 1; Davis and Stroobant, 1990; Hardy et al., 1992; Jessen and Mirsky, 1992; Yoshida and Gage, 1992, Acheson et al., 1995; Murphy et al., 1995; Verge et al., 1996).

High-affinity receptors are upregulated in some, but not all axotomized neurons. For example, TrkB receptors are upregulated in axotomized motoneurons (Fig. 1; Koliatsos et al., 1991, 1993; Tetzlaff et al., 1992; Friedman et al., 1995), but the same receptors are downregulated in axotomized DRG neurons (Verge et al., 1989, 1990, 1992). The low-affinity 75-kDa NGF receptor p75 is transiently upregulated in axotomized motoneurons, but downregulated in axotomized DRG neurons (Raivich et al., 1985; Yan and Johnson, 1988; Ernfors et al., 1989; Verge, 1989, 1990, 1992). The transient expression of p75 on axotomized motoneurons may serve to accumulate neurotrophins released from surrounding glial cells (Raivich et al., 1985; Yan and Johnson, 1988; Ernfors et al., 1989), in addition to facilitating retrograde transport of neurotrophins from the distal nerve stump (Curtis et al., 1994).

The upregulation of high affinity receptors on axotomized neurons, for example, TrkB receptors on axotomized motoneurons, is consistent with a direct role of the neurotrophins in maintaining the viability of injured neurons. However, in this context, the downregulation of Trk receptors and p75 on axotomized DRG neurons and reduced transport of neurotrophins by regenerating axons (Raivich et al., 1991; Raivich and Kreutzberg, 1993) is surprising, particularly since exogenous application of the neurotrophins is effective in reversing many axotomy-induced changes in the neurons (Verge et al., 1995, 1996). Reversal of axotomy-induced changes in sensory neurons and motoneurons by exogenous application of neurotrophins, NGF, BDNF, and NT-4/5 is consistent with developmental evidence that neurotrophic factors promote neuronal differentiation and mature phenotype (Friedman et al., 1995; Verge et al., 1995, 1996). One possible explanation for the paradox of receptor downregulation and reduced transport in DRG neurons is that the reduced transport and Trk expression in some neurons may limit endogenous neurotrophic factors to levels that are below a threshold for switching from the "growth mode" to the mature "transmitting" neuronal phenotype, but still sufficient to promote neuronal survival and interaction of the growth cone with the growth environment.

Triggers for Altered Gene Expression

The basis for the switch of gene expression in axotomized neurons remains one of the most intriguing and still unanswered questions despite the considerable advances that have been made in the identification of neurotrophic factors and their effects since an important review of the issue by Cragg in 1970.

Reduced access of neurons to neurotrophic factors is likely to be one of the triggers for changes in gene expression from the mature transmitting to the growth mode. The ability of exogenously applied neurotrophic factors to reverse the altered gene expression in axotomized neurons (e.g., Friedman et al., 1995; Verge et al., 1995, 1996) provides support for the term "neurotrophic factors" to describe the molecules that enhance neuronal survival and differentiation (Korsching, 1993). The finding that vincristine-induced block of axonal transport is sufficient to mimic axotomy by downregulating CAT in intact motoneurons also argues that loss of target-derived neurotrophic factors is one of the signals that switch the neuron from the transmitting mode (Moix et al., 1991).

The signals for upregulation of other genes, such as p75, are less clear. The possibility that the neuron and/or the surrounding astroglia rapidly "sense" the damage to the neuron has been considered for many years (Cragg, 1970). A recent immunohistochemical demonstration of rapid induction of connexin-43 expression in the astroglia that surround an axotomized neuron suggests that the glial cells "sense" the damage within hours (Rohlmann et al., 1994). However, the issue of whether damage alone is sufficient to induce the switch in gene expression in axotomized neurons remains controversial, and it is more likely that more than

one factor is involved in triggering altered gene expression. For example, some studies have indicated that neuronal damage may be sufficient to upregulate p75 expression (Moix et al., 1990; Wood et al., 1990), whereas others obtain evidence that interruption of axonal transport by a cold block, with no apparent macrophage recruitment, is sufficient to upregulate p75 (Wu et al., 1993). It is interesting that the axotomized neuron appears to be sensitive to the extent of axon lost because the amplitude of expression of novel tubulin genes was proportional to the amount of axon lost (Mathew and Miller, 1993), consistent with early reports that chromatolytic responses are more intense for proximal as compared to distal nerve injuries (Marinesco, 1896).

Axonal Regeneration

Die-Back and Swelling

The nerve stump proximal to the lesion shows signs of traumatic degeneration usually up to the first node of Ranvier if neuronal cell bodies survive the injury (Cajal, 1928). This "die-back" degeneration is associated with calcium influx and activation of calcium-associated proteases (George et al., 1995; reviewed by Bisby, 1995). Swelling of the proximal nerve stump near the injury site is probably owing to "damming" of substances, such as cytoskeletal proteins and organelles, which are continuously transported from the cell body, via slow and fast axoplasmic transports, toward the target (Weiss and Hiscoe, 1948; Zelena et al., 1968; Heslop, 1975; Friede and Bischhausen, 1980; Smith, 1980). For example, if a nerve is crushed at two sites, swelling occurs only at the proximal crush site, which remains in continuity with the soma and from which thin nerve fibers emerge within hours after injury (Gutmann, 1958; McQuarrie, 1985). Transport of material is maintained in the isolated nerve stump and results in a minute accumulation of the material at its distal end, suggesting that axonal transport does not require continuity between the axon and soma (Lubinska and Niemierko, 1971).

Axonal Sprouts

Growth cone formation occurs without direct support from the cell body and depends on the material locally available in the axon (McQuarrie, 1985). Preexisting cytoskeletal elements in transit are transported into daughter axons emerging from the proximal nerve stump (McQuarrie and Lasek, 1989). The microtubules provide a bridge between parent axons and daughter axons for transferring other selected materials required for axonal growth (Miller et al., 1987). Continued transport even in isolated axons is sufficient for growth cone formation in vitro (Shaw and Bray, 1977; Bray et al., 1978; Wessells et al., 1978; Kato and Ide, 1994). Materials provided by the cell body via axonal transport, however, are the major source for subsequent axonal elongation (Kato and Ide, 1994), since regenerating axons have little capacity for independent protein synthesis during growth cone formation (Davis et al., 1992).

A "latent period" has long been thought to exist before an injured nerve shows signs of axonal regeneration (Gutmann et al., 1942). Based on the assumption that the rate of regeneration is linear from the beginning, the "latent period" has been computed from extrapolation of the plot of regeneration distance (measured by means of the pinch test), as a function of time after axotomy. However, the assumption of linearity of regeneration rate is incorrect and often greatly overestimates the latent period. Axonal elongation is initially slow, but accelerates to reach a constant rate by the third day after injury (Wyrwicka, 1950; Sjoberg and Kanje, 1990).

Regeneration rate varies as a function of injury type, age, and species, especially during the early stage of regeneration (Gutmann et al., 1942; Black and Lasek, 1979; Pestronk et al., 1980). Outgrowth of axonal sprouts is more rapid after crush injury in which the continuity of the nerve sheaths and basement membrane is preserved than after nerve section (Cajal, 1928; Thomas, 1964). Regeneration is also more rapid in young mammals and varies

across species (Gutmann et al., 1942; Black and Lasek, 1979). Estimates of the rate of regeneration vary between 1 and 3 mm/d (e.g., Gutmann et al., 1942; reviewed by Seddon, 1975; Bisby, 1995).

Regenerating axonal sprouts arise from the first node of Ranvier proximal to the injury site (Cajal, 1928; Friede and Bischhausen, 1980; McQuarrie, 1985; Snyder et al., 1988). The first nerve fibers emerging from the proximal nerve stump undergo extensive branching (Perroncito 1907; Cajal, 1928; Nathaniel and Pease, 1963b). A single axon gives rise to as many as 50–100 branches (Ranson, 1912), but these new branches often form spirals or ringlets that later disappear (Perroncito, 1907). In the absence of any "guiding" structure, such as the distal nerve stump, regenerating axons form a neuroma that is a mixture of immature nerve fibers and connective tissue (Young, 1948; Sunderland, 1978), reflecting residual and yet disorganized axonal growth (Cajal, 1928; Young, 1948).

Regenerating Unit

When numerous fine nerve fibers emanating from the parent axon grow within the supportive environment of the distal nerve stump, an average of five daughter axonal sprouts per parent axon start to regenerate (Greenman, 1913; Gutmann and Sanders, 1943; Aitken et al., 1947; Bray and Aguayo, 1974; Jenq and Coggeshall, 1985a; Toft et al., 1988). The axonal sprouts that advance distally comprise a "regenerating unit" (Morris et al., 1972), which remains in the distal nerve stump unless axons make target connections. Once target connection is made, all but one daughter axon are gradually withdrawn, the process taking months and even years (Aitken et al., 1947; Mackinnon et al., 1991). More daughter axons are formed after suturing the proximal and distal stumps together in nerve transection than in crush injury in which the integrity of the basement membrane is maintained (Shawe, 1955; Jenq and Coggeshall, 1985a).

Regenerating axons in the distal nerve stump grow in diameter, but do not approach normal size unless they make functional connections (Aitken et al., 1947; Gordon and Stein, 1982). Axonal sprouts that are allowed to regenerate into the distal nerve stump, but are prevented from reinnervating the target exceed their original size of 1 μm in diameter, approach the size of the atrophic parent axons, and become myelinated. Regenerating axons can therefore mature without forming target connections and increase their diameters in proportion to those of the parent axons, the conduction velocity and the diameter of regenerating axons being usually proportional to the conduction velocity of the parent axons from the earliest time when sprouts start to elongate from the proximal stump (Devor and Govrin-Lippmann, 1979). Similar conclusions have been drawn from early experiments based on the observation that somatic nerve fibers regenerating in the narrow tubes of degenerating sympathetic fibers in the mesenteric nerve or adrenal gland attain larger diameters than nonmyelinated fibers that have previously occupied the tubes (Simpson and Young, 1945; Evans, 1947). Thus, parent axons, instead of the endoneurial tube, appear to determine the size of regenerating axons.

Remyelination of regenerating axons is initiated by contact between the axolemma and Schwann cells. The interaction induces galactocerebroside synthesis (Jessen et al., 1987a) and expression of myelin-associated proteins in Schwann cells (Lemke and Chao, 1988; LeBlanc and Poduslo, 1990), and initiates spiraling of the myelin sheath (Bunge and Bunge, 1983; Bunge et al., 1986, 1990). Although Schwann cells synthesize the myelin sheath, it is the outgrowing axons that determine the extent of myelination (Simpson and Young, 1945) with Schwann cells forming myelin lamellae that increase in number in proportion to the size of the axons (Hildebrand et al., 1994). However, recovery of conduction velocity in the regenerated axons lags the recovery of normal conduction velocity in the parent axons. This can be explained by the abnormally short internodal distances between myelin sheaths, which are formed by the more than

threefold increase in Schwann cell number in the distal nerve stumps (*see* Schwann Cell Proliferation; Vizoso and Young, 1948; Haftek and Thomas, 1968). These distances slowly increase during the course of a complex remodeling of the Schwann cell myelin sheaths (Hildebrand et al., 1986; reviewed by Hildebrand et al., 1994).

Regeneration After Prolonged Axotomy

The growth potential of regenerating axons has been suggested to be maximal 3 wk after injury, based on temporal changes of the metabolic status of axotomized neurons (Ducker et al., 1969; McCabe, 1970; Grafstein, 1975; Ducker and Kauffman, 1976). This has formed the basis of a belief that nerve repair is best performed 3 wk, instead of immediately, after injury. Additional studies have shown that a short time interval between injury and repair may benefit functional recovery (Holmes and Young, 1942; Brunetti et al., 1985). However, current evidence suggests that delayed nerve repair is detrimental to functional recovery, and the sooner the repair, the better the long-term functional recovery (Sunderland, 1978; de Medinacilli and Seaber, 1989; Mackinnon, 1989; Millesi, 1990; Barrs, 1991; Fu and Gordon, 1995a,b; Gordon and Fu, 1997). As described below, prolonged axotomy is an important factor that accounts for poor functional recovery from delayed nerve repair and from injuries that incur long delays between the time of injury and reinnervation of denervated targets.

Holmes and Young (1942) used an elegant cross-suture technique to isolate the effects of prolonged axotomy from those of prolonged denervation of both the distal nerve stumps and the target muscle. Tibial motoneurons and sensory neurons were axotomized by cutting the tibial nerve in the rat hindlimb. Regeneration was prevented for up to 1 yr prior to suture of the proximal stump of the tibial nerve to the distal stump of a freshly cut common peroneal nerve. Neither regeneration rate nor the number of axons in the distal stump, estimated by axonal count 15–25 d after cross-suture, was affected by prolonged axotomy, suggesting that prolonged axotomy does not compromise axonal regeneration (Holmes and Young, 1942). However, axonal count overestimates the number of neurons that successfully regenerate into the distal stump, because each parent axon gives rise to an average of five daughter sprouts (Aitken et al., 1947; Toft et al., 1988; Mackinnon et al., 1991). To overcome this inaccuracy, recent experiments by means of motor unit counting in our laboratory have more accurately quantified the number of motor axons that regenerate and make functional connections after prolonged axotomy (Fu and Gordon, 1995a).

Using the same cross-suture paradigm, we found that progressively longer periods of axotomy prior to nerve cross-suture result in progressive reductions in the number of motoneurons that have successfully regenerated their axons and reinnervated the denervated muscle (Fu and Gordon, 1995a). When axotomy was prolonged for 12 mo, the number of motoneurons that successfully regenerated their axons and reinnervated the target muscle decreased to one-third of the normal complement (Fu and Gordon, 1995a). Once regenerating axons reached the target muscle, they demonstrated a normal capacity to enlarge their motor units by reinnervating up to three times their normal complement of muscle fibers (Fu and Gordon, 1995a). Thereby, the increased number of muscle fibers per motoneuron has compensated for the loss of 66% of the motor innervation (Fu and Gordon, 1995a).

The molecular basis for the reduced regenerative capacity of motoneurons after prolonged axotomy has yet to be determined. Some of the regressive changes that occur after axotomy, such as astroglial stripping of synapses from the soma of axotomized motoneurons, are not completely reversed even after axonal regeneration and muscle reinnervation (Kreutzberg, 1995). Whether growth-associated changes in axotomized neurons regress during prolonged axotomy remains unknown, since most studies have been limited to the first month of axotomy. There are indications, however, that some of the changes are not sus-

tained. For example, upregulation of actin and tubulin mRNA is not maintained at the peak levels reached 7 d after nerve injury (Tetzlaff et al., 1988; Petrov et al., 1996a). However, actin and tubulin mRNAs are again upregulated when long-term axotomized motoneurons are subjected to a second injury by cutting the neuroma (Petrov et al., 1996a). It is probable that the second upregulation of cytoskeletal proteins is more transient and insufficient to support axonal regeneration of long-term axotomized neurons. Another possible cause for poor regeneration after prolonged axotomy is prolonged deprivation of neurotrophic factors from the periphery. Although this deprivation may not affect the long-term survival of adult motoneurons, it may compromise the capacity of long-term axotomized motoneurons to regenerate axons.

The Growth Environment of the Distal Nerve Stump

The success of peripheral nerve regeneration depends critically on the growth environment in the distal nerve stump following removal of axonal and myelin debris. Regenerating axons grow toward denervated targets within the endoneurial tubes formerly occupied by intact axons and their myelin sheaths. Crush injuries in which the continuity of the endoneurial tube is preserved are associated with successful regeneration and functional recovery in contrast to transection injuries, which sever the nerve sheath and require surgical repair (Seddon, 1975; Sunderland, 1978; Terzis, 1987; Lundborg, 1988). For the latter injuries, regenerating axons must cross a gap between the proximal and distal nerve stumps, which may be facilitated by microsurgical repair. Even though proliferating Schwann cells and fibroblasts from both the proximal and distal nerve stumps migrate to bridge the gap and provide a cell-matrix substratum, the number of regenerating axons that successfully cross this gap may still be less than optimal. Furthermore, the gap reduces the opportunities for regenerating axons to grow into their original endoneurial tubes. Regenerating axonal sprouts branch extensively and enter the vacant endoneurial tubes in an apparently random manner (Cajal, 1928). As a result, many regenerating axons fail to reinnervate their original targets, and this misdirection remains one of the most challenging problems in achieving optimal functional recovery (e.g., Thomas et al., 1987; reviewed by Gordon, 1994).

Profuse changes in the distal nerve stump promote a favorable growth environment. The permissive growth environment is the result of effective phagocytosis of myelin by invading macrophages and resident Schwann cells, as well as neurotrophic and substrate support provided by nonneuronal cells and extracellular matrix. The resident nonneuronal cells include Schwann cells, the fibroblasts of the endo-, peri-, and epi-neuria, and resident macrophages, all of which respond to nerve injury. Among these, Schwann cells play the most critical role in nerve regeneration (Cajal, 1928), as described in Nonmyelinating Schwann Cell Phenotype and Regeneration.

Wallerian Degeneration

Changes in the distal nerve stump are initially degenerative, and associated with removal of degrading axons and myelin debris in a process called Wallerian degeneration. Macrophages were thought not to be essential for the degradation of axon and myelin, because degradation occurs even when macrophages are denied access to the distal nerve stump (Beuche and Friede, 1984). However, the abnormally delayed Wallerian degeneration associated with impaired macrophage invasion in the C57BL/Ola mutant mouse, strongly suggests that macrophages are very important players in myelin degradation and phagocytosis (reviewed by Perry and Brown, 1992).

Both Schwann cells and macrophages contribute to phagocytosis, but the time-course and mechanisms of their action are different. During the first 2 d after injury when macrophage invasion is minimal, Schwann cells are the major cellular participants in phagocytosis;

thereafter, macrophages are the major contributors (Beuche and Friede, 1986; Bigbee et al., 1987; Stoll et al., 1989; Reichert et al., 1994; Fernandez-Valle et al., 1995; Perry et al., 1995). Within hours of injury, Schwann cells begin to express MAC-2, a galactose-specific lectin, which targets the galactolipid-rich myelin (cerebroside and sulfatide) in nonimmune opsonin-independent lectinophagocytosis and which mediates the bulk of phagocytosis within the first few hours of injury (Reichert et al., 1994). Macrophages that invade the distal nerve stump 2–3 d later express MAC-2 as well as MAC-1 (the C3b complement receptor) and the Fc receptor. The latter two mediate the immune opsonin-dependent myelin phagocytosis (Reichert et al., 1994). The multiplicity of mechanisms of phagocytosis in macrophages may contribute to the greater magnitude of phagocytosis by macrophages than by Schwann cells.

Delayed Wallerian degeneration in Ola mouse is owing to defects in Schwann cells, axonal membranes, and macrophages. A defective axonal membrane in Ola nerves fails to recruit macrophages as demonstrated by delayed Wallerian degeneration in Ola nerve grafts in both macrophage-competent and macrophage-incompetent mice: Wallerian degeneration was delayed when the Ola nerve graft was placed between normal nerves in a normal mouse host (Glass et al., 1993) or when the normal macrophage levels were produced in Ola mice by a bone marrow transplant from a normal donor mouse (Perry et al., 1990). The finding that Ola nerves undergo more rapid Wallerian degeneration in vitro where Schwann cells are responsible for myelin phagocytosis (Reichert et al., 1994) suggests that Ola nerves release an inhibitory factor in vivo, which prevents Schwann cells from participating in Wallerian degeneration.

The phagocytic activity of macrophages contributes directly to nerve regeneration by removing inhibitory substances associated with myelin and indirectly by release of a plethora of factors that appear to be important for successful cellular repair. Vacating the endoneurial sheath of the distal nerve stump by removal of myelin and axons has been thought to be crucial for regeneration, since axonal growth fails to occur when regenerating axons are directed into nondegenerated nerve (Langley and Anderson, 1904; Brown et al., 1991a). Adult sensory neurons do not extend neurites along a sectioned peripheral nerve in vitro, unless it is predegenerated in vivo (Bedi et al., 1992). There is evidence that inhibitors to regeneration are present in the myelin of nondegenerating nerve. Growth-inhibitory effects have been demonstrated in myelin extracts from peripheral nerve devoid of laminin (David et al., 1995). One possible regeneration inhibitor is myelin-associated glycoprotein (MAG), which has been shown to effectively inhibit neurite outgrowth (Mukhopadhyay et al., 1994; Schafer et al., 1996).

Factors released by macrophages include mitogens for Schwann cells and fibroblasts (reviewed by Reynolds and Woolf, 1993), and cytokines that stimulate the synthesis of growth factors and adhesion molecules by nonneuronal cells of the nerve sheath and endothelial cells of the blood vessels *(see* Regeneration-Associated Molecules*)*. The growth factors and adhesion molecules, in turn, play an essential role in growth of blood vessels (angiogenesis) *in situ*, activating Schwann cells and guiding regenerating axons through vacated distal nerve stumps.

Schwann Cell Proliferation

Normally, Schwann cells outnumber other nonneuronal cells in the peripheral nerve (Reichert et al., 1994). Fibroblasts are associated primarily with nerve sheaths, and there is a small component of macrophages. The latter comprise <5% of the total nonneural cells (Perry and Brown, 1992; Griffen et al., 1993). Both Schwann cells and fibroblasts proliferate after nerve injury, but Schwann cells remain the predominant cell type in vacated endoneurial tubes in the distal nerve stump (Salonen et al., 1988). Previously, mitotically

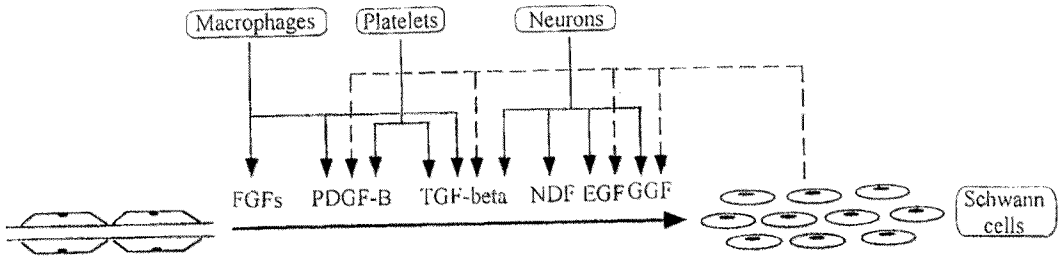

Fig. 2. Regulation of Schwann cell mitosis. An array of molecules released by neurons, macrophages, and platelets promote the proliferation of Schwann cells. These molecules include FGFs, PDGF-B, TGF-β, NDF, EGF, and GGF. Proliferating Schwann cells themselves also produce some of these molecules, such as PDGF-B, TGF-β, EGF, and GGF, which may exert autocrine effects on Schwann cells.

quiescent Schwann cells, deprived of axonal contact, began to proliferate and form linear arrays (bands of Bungner) along the empty basement membrane of the endoneurial tube (Cajal, 1928; Nathaniel and Pease, 1963a; Thomas, 1964; Bunge, 1987). The nonmyelinating Schwann cells reach a peak of proliferation 2 d after injury, about 1 d earlier than myelinating Schwann cells (Clemence et al., 1989).

Proliferation of Schwann cells is regulated by a multitude of factors (Fig. 2). Loss of axonal contact alone is not a sufficient signal for the proliferation of Schwann cells, because axonal degradation occurs within hours of injury in contrast to Schwann cell mitosis, which begins later and peaks on the second or third day (Pellegrino et al., 1986; Clemence et al., 1989). Schwann cell mitosis is temporally correlated with macrophage invasion into the distal nerve stump, suggesting that the presence of macrophages may be required for Schwann cell proliferation. Macrophages that have digested myelin membrane release Schwann cell mitogens (Baichwal et al., 1988), which include cAMP-dependent mitogens, such as PDGF-B, and acidic and basic fibroblast factors (FGFs) (Davis and Stroobant, 1990; Eccleston et al., 1990; Weinmaster and Lemke, 1990; reviewed by Reynolds and Woolf, 1993). Macrophages and platelets also release TGF-β, which also induces Schwann cell mitosis (Ridley et al., 1989; Rogister et al., 1993 but *see* Chandross et al., 1995). As TGF-β mRNA is detected in Schwann cells, TGF-β may be released and induce Schwann cell mitosis via an autocrine pathway (Ridley et al., 1989; Scherer et al., 1993). TGF-β constitutes a family of molecules involved in wound repair and includes at least five distinct isoforms (Mustoe et al., 1987; Roberts et al., 1990). In general, TGF-β stimulates the proliferation of cells of mesenchymal origin, but inhibits division of other cell types, particularly those of epithelial origin (Rogister et al., 1993). TGF-β resembles cAMP-dependent mitogens in inducing the premyelinating status of Schwann cells, including downregulation of p75 and upregulation of myelin-related proteins and type IV collagen (Raff et al., 1978; Carey et al., 1986; Jessen et al., 1991; Morgan et al., 1991; Matsuoka et al., 1991; Mew and Meyer, 1993; Rogister et al., 1993).

There is normally a second stage of Schwann cell proliferation when regenerating axons grow into the denervated nerve stump (Pelligrino and Spencer, 1985), which may contribute to the more than threefold increase in Schwann cell number to form the shorter internodes of regenerating axons (Hildebrand et al., 1986). In vitro, axolemma and its fragments are mitogenic (Ratner et al., 1988; Dent et al., 1992). Nevertheless, the Schwann cell proliferation associated with regeneration may not always be detected in vivo (Salonen et al., 1988; Siironen et al., 1994). Possibly, the proliferation is maximally stimulated by other mitogens in the distal nerve stump by the time regenerating axons arrive at the stump.

GGF belongs to a novel growth factor group, the Neu differentiation factors (NDF), which have been cloned from rat (NDF; Ben-Baruch and Yarden, 1994), human (heregulin; Holmes et al., 1992), bovine (GGF; Marchionni et al., 1993), and chick (acetylcholine receptor-inducing activity [ARIA]; Falls et al., 1993) tissues. They are encoded by a single gene, produced by embryonic sensory and motoneurons, and are strongly mitogenic for Schwann cells in vitro (Marchionni et al., 1993). The mitogenic effects of the factors, when combined with agents that elevate cAMP, are particularly notable and mediated via $p185^{erbB2/neu}$ receptor tyrosine kinase, but may also require formation of heterodimers with erB3 or erbB4 (Holmes et al., 1992; Levi et al., 1995). In vivo and in vitro experiment demonstrate that NDF and GGF regulate the survival, proliferation and maturation of immature Schwann cells (Dong et al., 1995; Trachtenberg and Thompson, 1996). However, the role of the factors in Schwann cell function after nerve injury in adults is not yet known. Recent in vitro experiments demonstrate that GGF may indirectly promote neurite outgrowth by increasing the motility of Schwann cells and their secretion of neural growth-promoting activities (Mahanthappa et al., 1996).

Binding of epithelial growth factor (EGF) to its receptor on fibroblasts leads to phosphorylation of $p185^{erb2/neu}$ receptor tyrosine kinase, and may be linked to proliferation of fibroblasts and Schwann cells (Wada et al., 1990; Toma et al., 1992). Proliferation of fibroblasts is most prominent at or near the injury site (Nieke and Schachner, 1985; Daniloff et al., 1986; Martini and Schachner, 1988).

In summary, although several molecules derived from macrophages and regenerating neurons have been identified as mitogens for Schwann cells in culture, their roles in damaged axons in vivo are yet to be determined. Presently, mitogens derived from macrophages and possibly the regenerating axons appear to be involved in the proliferation of Schwann cells and fibroblasts after nerve injury.

Conversion of Schwann Cell Phenotype from Myelinating to Nonmyelinating Mode

By analogy with a switch of a mature neuron from transmitting to growth mode after nerve injury, Schwann cells in the distal nerve stump appear to switch their function from myelination of electrically active axons to growth support for regenerating axons. Within 3 d of nerve injury, DNA and RNA syntheses in Schwann cells increase (Oderfold-Nowak and Niemierko, 1969). Genes that code myelin-associated proteins, such as P_0, myelin basic protein (MBP), MAG, and PMP22, are downregulated (Trapp et al., 1988; LeBlanc and Poduslo, 1990; DeLeon et al., 1991), whereas proteins that are normally expressed only by nonmyelinating axons, such as p75 and GAP-43, are upregulated (Taniuchi et al., 1986; Curtis et al., 1992). Other upregulated proteins include:

1. Transcription factors, including c-*fos* (Liu et al., 1995) and c-*jun*, which is selectively expressed in nonmyelinating Schwann cells in the normal peripheral nerve (Vaudano et al., 1992; Shy et al., 1996);
2. Neurotrophic factors, including three members of the neurotrophin family, NGF, NT-4/5, and BDNF, and their low-affinity receptor (p75), as well as EGF and its receptor, insulin-like growth factors I and II (IGF-I; IGF-II), and GDNF (Richardson and Ebendal, 1982; Hansson et al., 1986; Heumann et al., 1987a,b; Raivich and Kreutzberg, 1987a; Taniuchi et al., 1988; Acheson et al., 1991; Meyer et al., 1992; Toma et al., 1992; Funakoshi et al., 1993; Trupp et al., 1995; You et al., 1997);
3. Cell adhesion molecules (CAMs), such as L1, N-CAM, and N-cadherin (Nieke and Schachner, 1985; Martini and Schachner, 1988; Cifuentes-Diaz et al., 1994); and
4. Basement membrane components, including laminin, fibronectin, thrombospondin, various proteoglycans, collagens, and tenascin/entactin/J1 (Bunge and Bunge, 1983; Daniloff et al., 1986; Bixby et al., 1988; Brodkey et al., 1993; Martini, 1994).

It is important to note, however, that <1% of all DNA sequences is either induced or

repressed in the distal nerve stump after injury (DeLeon et al., 1991). The most abundant mRNA sequence is that of vimentin, an intermediate filament protein expressed by Schwann cells, consistent with immunohistochemical evidence for its abundance in the denervated distal nerve stump (Neuberger and Cornbrooks, 1989).

Since many alterations in gene expression occur in vivo once Schwann cells have lost contact with axons and are reversed by regenerating axons, the trigger for this switch is, at least in part, owing to the loss of axonal contact (Taniuchi et al., 1988; LeBlanc and Poduslo, 1990; Bolin and Shooter, 1993). However, many changes in gene expression in Schwann cells may be more directly associated with the interaction between Schwann cells and invading macrophages. For example, the prolonged second phase of the biphasic upregulation of NGF in Schwann cells and fibroblasts correlates with the invasion of macrophages, and is triggered directly by IL-1 released from macrophages (Heumann et al., 1987a,b; Lindholm et al., 1987, 1988). Upregulation of p75 mRNA also correlates with macrophage invasion and declines within 4 mo even when axonal regeneration is prevented (Petrov et al., 1996b; Rehovet et al., 1996b; You et al., 1997). The decline in p75 mRNA follows the same time-course as clearance of macrophages from the denervated distal nerve stump (You et al., 1997), suggesting that interaction between invading macrophages and Schwann cells is important in maintaining the "growth mode" of Schwann cells. This, in turn, implies that axons regenerate in less than optimal growth conditions in the more distal region of the distal nerve stump when injury occurs far from the target and may account for the dramatically reduced regenerative success after delayed nerve repair (Fu and Gordon, 1995b). Some changes in Schwann cell gene expression, on the other hand, are short-lived. For example, induction of IL-6 in an immortalized Schwann cell line by tumor necrosis factor-α (TNF-α) in vitro or in crushed rat nerves in vivo is not maintained (Bolin et al., 1995), although IL-6 release may continue for up to 3 wk in degenerating nerves in vivo (Reichert et al., 1996).

Nonmyelinating Schwann-Cell Phenotype and Regeneration

The altered phenotype of Schwann cells after nerve injury has been directly associated with nerve regeneration. In the frequently used experimental paradigm of sciatic nerve crush, upregulation of neurotrophic factors, CAMs and basal lamina proteins, and their downregulation following regeneration implicates their roles in nerve regeneration. Many of these molecules, expressed by nonmyelinating Schwann cells even in the normal nerve, support regenerating axons. In the C57Bl/Ola mouse, nonmyelinating Schwann cells may support axonal regeneration into the distal nerve stump prior to axonal degeneration and conversion of myelinating Schwann cells to nonmyelinating phenotype (Lunn et al., 1989; Brown et al., 1992). Although delayed Wallerian degeneration reduces the growth rate of sensory axons in particular and impairs functional recovery (Bisby and Chen, 1990; Brown et al., 1990, 1991b; Chen and Bisby, 1993), it has little effect on the regeneration of motor axons (Lunn et al., 1989; Bisby and Chen, 1990; Brown et al., 1992). If, however, nonmyelinating Schwann cells are sparse, as they are in the phrenic nerve, regeneration of motor axons does not occur in the Ola mouse until Wallerian degeneration has progressed (Brown et al., 1994).

Cellular and Acellular Nerve Grafts

The presence of viable nonmyelinating Schwann cells is essential for axonal growth in the distal nerve stump, even though nerve fibers can grow on artificial surfaces coated with one or more extracellular matrix molecules of the Schwann cell basal lamina in vitro and in vivo, such as laminin, fibronectin, and/or collagen (Ard et al., 1987; Bailey et al., 1993; for review, *see* Letourneau, 1988; Sephel et al., 1989; Bixby and Harris, 1991; Carbonetto, 1991; Reichardt and Tomaselli, 1991; Bixby, 1992). The success of axonal regeneration through the

acellular nerve grafts (Zalewski and Gulati, 1982; Anderson et al., 1983, 1991; Ide et al., 1983; Hall, 1986a,b; Osawa et al., 1986; Bresjanac and Sketelj, 1989; Sketelj et al., 1989) or through the acellular basal lamina sheaths of the denervated muscle (Fawcett and Keynes, 1986, 1990; Glasby et al., 1986; Enver and Hall, 1994) depends critically on the migration of Schwann cells from both the proximal and distal nerve stumps into the grafts (Thomas, 1966; Hall, 1986a,b; Nadim et al., 1990; Enver and Hall, 1994). Acellular grafts fail to support regeneration when Schwann cell migration from the nerve stumps is prevented by inhibiting Schwann cell proliferation in the nerve stumps using mitomycin-C (Hall, 1986b; Enver and Hall, 1994). The inability of axons to regenerate over acellular grafts longer than 40 mm has been attributed to the finite migratory ability of Schwann cells (Hall, 1986a; Nadim et al., 1990; Anderson et al., 1991; Chong et al., 1994; Enver and Hall, 1994).

The finding that Schwann cell migration precedes axonal regeneration into acellular grafts (Sjoberg et al., 1988; Nadim et al., 1990) is further supported by experiments that use shorter (<15 mm) artificial silicone or biodegradable tubes to examine the spatio-temporal participation of cellular and chemical factors in axonal regeneration (Danielsen, 1990; Karlsson et al., 1993). Plasma-like fluid accumulates in the tube followed by formation of a fibrin/fibronectin cable, which bridges the nerve stumps (Noback et al., 1958; Longo et al., 1983; Williams et al., 1983, 1987; Liu, 1992). The fibrin/fibronectin cable derives from the plasma that is extravasated into the wound as a result of the increased vascular permeability associated with the trauma. The fluid extracted from the tube within a day of injury promotes Schwann cell proliferation and adhesion to substrate in vitro; the fluid extracted later supports Schwann cell migration (Le Beau et al., 1988). Schwann cells, endothelial cells, macrophages, and fibroblasts first migrate into the fibrin/fibronectin cable followed by axons that regenerate along Schwann cells (Williams et al., 1983; Williams and Varon, 1985; Liu, 1992; Karlsson et al., 1993; Dahlin et al., 1995). These findings are consistent with Cajal's (1928) original proposal that proliferating Schwann cells that comprise the bands of Bungner within the endoneurial sheaths form the substrate for axonal regeneration. Fibroblasts that migrate along the cable form a perineurial ensheathment of regenerating axons in the silastic chamber giving rise to multiple "minifascicles" (Azzam et al., 1991; Schroder et al., 1993).

The Schwann cell migration demonstrated on the fibrin-fibronectin cable in the silicone chamber also explains how Schwann cells migrate out from the proximal and distal nerve stumps to form a bridge across the gap between the nerve stumps. The fibrin-fibronectin cable forms the substrate for both the migration of endothelial cells for neovascularization and the Schwann cells that, in turn, form the substrate for regenerating axons.

The fibrin-fibronectin cable is only effective in bridging short, but not long gaps in contrast to acellular grafts that support regeneration across 40-mm gaps (Lundborg et al., 1982; Rich et al., 1989a,b; reviewed by Fields et al., 1989). This comparison shows that the inner surface of the basal lamina tubes derived from degenerated peripheral nerve or skeletal muscle is an important substrate for Schwann cell migration. Superior regeneration through cellular compared to acellular nerve grafts with respect to both the rate of regeneration and the number of regenerating axons shows that the number of Schwann cells migrating into acellular grafts is not sufficient for full support of axonal regeneration (Anderson et al., 1983; Gulati, 1988; Sjoberg et al., 1988). The abnormally large number of unmyelinated axons in an acellular nerve graft (Jenq and Coggeshall, 1985b) supports the idea that the number of Schwann cells migrating into the acellular graft is less than the number of Schwann cells in cellular grafts.

Regeneration-Associated Molecules

A large number of molecules can potentially regulate axonal regeneration either directly or

indirectly via their effects on nonneuronal cells. The molecules can be roughly divided into three groups: neurotrophic factors, cells adhesion molecules, and extracellular matrix proteins. Alteration in gene expression of many of these molecules after nerve injury and their roles in peripheral nerve regeneration are briefly outlined below.

Neurotrophic Factors and Receptors

The upregulation of neurotrophic factors in the distal nerve stumps of injured nerves and their return to normal levels after axonal regeneration and target reinnervation have led to the natural and direct association of the factors with regeneration (Richardson and Ebendal, 1982; Hansson et al., 1986; Heumann et al., 1987a,b; Raivich and Kreutzberg, 1987a; Acheson et al., 1991; Meyer et al., 1992; Funakoshi et al., 1993). Although a direct effect of these molecules on axonal regeneration has often been sought, increasing evidence indicates that these molecules act directly to promote survival and indirectly on regenerating axons via nonneuronal cells.

Neurotrophins and Their Receptors

The neurotrophin family includes NGF, BDNF, neurotrophin 3 (NT-3), and NT-4/5, which bind to high-affinity tyrosine kinase receptors called Trks and a low affinity NGF receptor, p75 (for review, *see* Lindsay et al., 1994; Chao and Hempstead, 1995; Mendell, 1995). All the neurotrophins, except NT-3, are upregulated in the distal nerve stump after axotomy (Fig. 1; Heumann et al., 1987a; Meyer et al., 1992; Funakoshi et al., 1993), and are taken up and transported to the cell body (DiStefano et al., 1992), although some at a lower rate than normal (Raivich et al., 1991, 1993). The low-affinity NGF receptor, p75, which is upregulated in axotomized motoneurons (Ernsfors et al., 1989), may assist in retrograde transport of NT-4/5 and BDNF from the distal nerve stumps (Curtis et al., 1995). Expression of p75 on the axotomized motoneurons and Schwann cell membranes in the distal nerve stump may also act to accumulate neurotrophins for presentation to the high-affinity Trk receptors on the soma and regenerating axons, respectively (e.g., Taniuchi et al., 1988). In addition, neurotrophins released from the neurons themselves are an additional source of neurotrophic support *(see* Neurotrophins and Receptors*)*.

Neuronal Survival

Compelling evidence for a role of the neurotrophins in promoting survival of axotomized neurons comes from both in vivo and in vitro studies. NGF, the classic target derived neurotrophic factor that binds to Trk A, is able to prevent the death of axotomized sensory neurons completely following exogenous administration (Yip et al., 1984; Otto et al., 1987; Rich et al., 1987; Melville et al., 1989). Knockout of NGF or Trk A in mice results in a dramatic loss of DRG and sympathetic neurons, but has little effect on motoneurons (Crowley et al., 1994; Smeyne et al., 1994). NGF is ineffective in rescuing immature motoneurons from axotomy-induced cell death, but other members of the neurotrophin family, BDNF, NT-3, and NT-4/5, have been shown to promote the survival of motoneurons in vitro (Henderson et al., 1993). Local or systemic administration of BDNF or NT-3 in vivo, acting primarily via Trk B and Trk C receptors, respectively, is more effective than NT-4/5 in promoting the survival of embryonic and neonatal motoneurons (Oppenheim et al., 1992; Sendtner et al., 1992a; Yan et al., 1992; Clatterbuck et al., 1994; Koliatsos et al., 1994a; Li et al., 1994; Vejsada et al., 1995; Kobayashi et al., 1996). In the adult, exogenous application of NGF, BDNF, and NT-4/5 can reverse axotomy-induced changes in sensory ganglion neurons and motoneurons (Friedman et al., 1995; Verge et al., 1995, 1996), consistent with a role of these neurotrophins in maintaining the viability of injured neurons and their mature phenotypes as described in Neurotrophins and Receptors.

Despite preferential binding of NGF to Trk A, BDNF and NT-4/5 to Trk B, and NT-3 to Trk C, there is overlap of ligand specificity (reviewed by Chao, 1992; Meakin and Shooter,

1992). This can account for the relatively small effects of deletion of either BDNF, NT-3, or NT-4/5 compared to the dramatic effects of deletion of the Trk B or Trk C receptor on motoneuronal survival in knockout mice (Klein et al., 1993, 1994; Conover et al., 1995). There is, however, a neuron-specific sensitivity for different neurotrophins. For example, survival of vestibular and trigeminal sensory neurons depends on BDNF, but not on NT-4/5, whereas survival of nodose-petrosal sensory neurons depends on both (Conover et al., 1995; for review, *see* Snider, 1994).

REGENERATION

The evidence for a direct effect of neurotrophins on axonal regeneration is less compelling. Rather, the available evidence points to the neurotrophins mediating effects indirectly via the nonneural cells in the growth environment.

Exogenous NGF or NGF antibodies have no apparent effect on the regeneration of sensory (Diamond et al., 1987, 1992; Saunders, 1992) and motor axons (Finkelstein et al., 1992), in contrast to the capacity of exogenous NGF to promote collateral sprouting of sensory neurons (Diamond et al., 1987, 1992). However, NGF applied to silastic nerve bridges between the ends of transected sciatic and facial nerves increases regeneration rate (Chen et al., 1989; Rich et al., 1989a; Derby et al., 1993; Spector et al., 1993). This enhanced regeneration rate is associated with increased outgrowth of nonneuronal cells into the chambers (Derby et al., 1993). Since the migration of Schwann cells is an early prerequisite for regeneration across these chambers (as described above), NGF-induced enhancement in regeneration rate appears to be owing to its indirect effect of promoting migration of nonneuronal cells (Anton et al., 1994) rather than a direct growth-promoting effect on regenerating axons. Another indirect effect of NGF on regeneration across the silicone bridge is to enhance angiogenesis and thereby promote blood flow to the regenerating axons (Santos et al., 1991). The lack of effect of NGF on sensory nerve regeneration in a crush model (Diamond et al., 1987, 1992) indicates that migration of nonneural cells and blood flow are not likely to be limiting factors for axonal growth of crushed peripheral nerves.

Interestingly, NGF antibodies reduce the extent of myelination of regenerating axons (Hulsebosch et al., 1987), consistent with a role of NGF in Schwann cell function. The demonstration that NGF increases the expression of the cell adhesion molecule L1 in cultured Schwann cells (Seilheimer and Schachner, 1987) is also consistent with an autocrine effect of NGF on Schwann cells. Since NGF also induces L1 expression on neurites (Friedlander et al., 1986), the net effect of this NGF-induced L1 expression is likely to promote adhesion between regenerating axons and Schwann cells in the growth pathway. Thus, NGF could promote the outgrowth of neurons by promoting both Schwann cell migration and adhesion between regenerating axons and Schwann cells in the growth pathway. However, an autocrine effect of neurotrophins on Schwann cells has generally been ruled out because Trk receptors are not expressed either in myelinating or nonmyelinating Schwann cells. However, the putative second messenger, ceramide, derived from the sphingomyelin cycle (Dobrowsky et al., 1995; Hannun and Obeid, 1995), could possibly mediate the paracrine and autocrine effects of neurotrophins on Schwann cell function via the p75 receptor. The function of p75 expression in the Schwann cells after injury is unclear. The most influential suggestion to date is that p75 accumulates neurotrophins for presentation to regenerating axons, which express high affinity Trk receptors *(see* Neurotrophins and Receptors*)*.

There is now evidence that p75 may be involved in apoptosis, and that NGF binding prevents the p75-mediated apoptosis. P75 has striking homology with TNF-R, CD40, and FAS (APO-1) receptors that contain a "death domain" in their intracellular region, and is required for apoptosis in serum-deprived cerebellar neurons and antimitotic agent-treated neuroblastoma cells in vitro (Ibanez et al., 1992; Rabizadeh et al., 1993; Chapman and Kuntz, 1995; Cortazza et al., 1996). NGF binding res-

cues neurons from apoptosis in these experimental systems. Expression of p75 alone is sufficient to induce apoptosis in these cell lines. However, Trk receptors are required in others, such as P12 pheochromocytoma cells and sympathetic neurons (Ibanez et al., 1992), where there is a cooperative relationship between p75-bound NGF and Trk A to enhance NGF binding to Trk A and Trk A autophosphorylation (Hempstead et al., 1991; Barker and Shooter, 1994; Verdi et al., 1994). Whether an injury-induced p75 upregulation in Schwann cells in the distal nerve stump affects their ability to survive has not been explored. There is evidence that Schwann cell apoptosis occurs in embryonic nerves as part of the adjustment of the final axon–glia ratio in myelinated nerves (Ciutat et al., 1996) and in intramuscular nerves after neonatal nerve section (Trachtenberg and Thompson, 1996). Apoptotic death of Schwann cells has also been described in vivo and in vitro in injured sciatic nerves from immature mice, particularly at the injury site (Ekstrom, 1995). The possibility that NGF and/or other neurotrophins prevent Schwann cell apoptosis in the distal nerve stump has not been explored.

Neuropoietic Cytokines

Neurotrophic factors of the cytokine family include ciliary neurotrophic factor (CNTF), IL-6, oncostatin M (ONC), granulocyte colony-stimulating factor (GCSF), and LIF. They share common structural motifs, bind to common receptor components, and use the JAK/STAT signaling pathways (Gearing et al., 1992; Ip et al., 1992; Stahl et al., 1994; reviewed by Patterson, 1992). The expression of these molecules after nerve injury (Fig. 1) suggests an involvement in promoting the survival of injured neurons in both central and peripheral nervous systems, at least within the first month after injury (Bolin et al., 1995; Curtis et al., 1995; Murphy et al., 1995; Reichert et al., 1996).

Survival

CNTF, originally identified as a survival-promoting factor for embryonic cholinergic neurons of the chick ciliary ganglion (Adler et al., 1979; Manthorpe et al., 1980), facilitates the survival of the dorsal root and autonomic ganglion neurons, motoneurons, and hippocampal neurons (Arakawa et al., 1990; Ip et al., 1991; Sendtner et al., 1991; Clatterbuck et al., 1993). In vivo, exogenous CNTF rescues chick embryonic motoneurons from naturally occurring cell death (Oppenheim et al., 1991) and neonatal facial motoneurons from axotomy-induced cell death (Sendtner et al., 1990). CNTF also prevents early death of motoneurons in murine motoneuron diseases (Sendtner et al., 1992b). As shown for neurotrophins, deficiency of CNTF receptors is much more deleterious than deficiency of CNTF itself. Motoneuron death is extensive in the CNTF receptor knockout mouse (DeChiara et al., 1995) in contrast to a modest (22%) loss of spinal motoneurons after deletion of the CNTF gene (Masu et al., 1993). Thus, molecules, in addition to CNTF, must bind to the CNTF receptor and mediate neuronal survival.

CNTF has been regarded as a postnatal survival promoting factor because of its abundance in Schwann cells of the intact nerve and the observation that its deficiency in transgenic mice does not affect neuronal survival until neurons attain maturity (Masu et al., 1993; DeChiara et al., 1995). Without a secretory signal sequence, CNTF is not likely to be released under physiological conditions (Stockli et al., 1989), unless there is an alternative secretory pathway (Muesch et al., 1990). Damage of Schwann cells owing to nerve injury may trigger the release of CNTF to the extracellular matrix where it could serve as a "lesion factor" (Sendtner et al., 1992b; Unsicker et al., 1992). However, the availability of CNTF is limited because of its downregulation in the distal nerve stump within the first day of injury (Friedman et al., 1992; Sendtner et al., 1992b; Seniuk et al., 1992). In addition, CNTF is less effective in preventing axotomy-induced motoneuron death in neonates than neurotrophins and IGF-I (Li et al., 1994; Vejsada et al., 1995).

LIF is retrogradely transported and promotes the survival of embryonic motor and sensory neurons in vitro (Martinou et al., 1992; Curtis et al., 1994). In contrast to CNTF, IL-6

and LIF are upregulated in the distal nerve stump (Seniuk et al., 1992; Curtis et al., 1994; Reichert et al., 1996). LIF is also expressed in glial cells surrounding the soma of neurons in the cervical sympathetic ganglion (Banner and Patterson, 1994). IL-6 and LIF could both contribute to the survival of injured motoneurons, at least for the short duration of their production, in view of their survival-promoting effects on embryonic acetylcholinesterase-positive spinal cord neurons in vitro (Kushima and Hatanaka, 1992).

IL-6 is a stress-associated protein that is strongly induced after tissue injury to counteract tissue damage. Synthesis of IL-6 in fibroblasts, macrophages, mast cells, and Schwann cells is stimulated by IL-1, TNF-α (Ng et al., 1994), LIF (Villiger et al., 1993), oncostatin M (Brown et al., 1991), and IL-6 itself (Bolin et al., 1995). IL-6 is induced within hours of nerve injury (Bolin et al., 1995; Reichert et al., 1996). It remains elevated for 3 wk in degenerating mouse nerves (Reichert et al., 1996), but not in crushed rat nerves (Bolin et al., 1995). IL-6 release from degenerating nerve stumps continues over a 20-d period (Reichert et al., 1996) during which macrophage numbers are high (Avellino et al., 1995). This finding indicates that macrophages and fibroblasts are the major contributors to IL-6 production, particularly in view of the findings that IL-6 release was considerably less in C57Bl/Ola mice, where macrophage recruitment is defective, and in freeze-damaged nerves (Reichert et al., 1996). Moreover, there was little IL-6 release from Schwann cell cultures established from degenerating nerves, in apparent contrast to the IL-6 produced by an immortal Schwann cell clone in response to IL-1 or TNF-α (Bolin et al., 1995). In vivo, IL-6 may also be induced by IL-1, which is released from injured nerves within hours of injury (Rotshenker et al., 1992). Although TNF-α is also a potent inducer of IL-6 expression (Aloisi et al., 1992; Ng et al., 1994), TNF-α is detected too late in the degenerating nerve, 14 d after the injury (Stoll et al., 1993), to be a likely factor that induces IL-6 expression.

Regeneration

There is some evidence for the involvement of CNTF in axonal regeneration after crush and transection injuries. Systemic or local infusion of CNTF increased rate of axonal elongation, number of reinnervated muscle end plates, muscle mass, and functional recovery (Sahenk et al., 1994; Ulenkate et al., 1994; Newman et al., 1996). Whether this effect is related to CNTF's capacity to induce neuronal sprouting in muscles is not clear (Gurney et al., 1992). A posited trophic support of axotomized neurons by the high levels of CNTF in the myelinating Schwann cells of the proximal nerve stump has yet to be demonstrated directly (Unsicker et al., 1992).

The precise role of IL-6 in axotomized neurons and in the distal stump is presently unknown. As described in Neurotransmitters and Neuropeptides, LIF and IL-6 are intermediates in the upregulation of some neuropeptides by axotomized sensory neurons, and thus, may play a role in the neuronal response to injury in addition to a possible role in promoting survival of axotomized neurons. The early upregulation of IL-6 in the distal nerve stump, probably in the fibroblasts, suggests a potentially important role in promoting the infiltration of blood-borne cells into the degenerating nerve stump. These include the macrophages, which in turn release cytokines, including IL-1 and IL-6, which serve as triggers for altered gene expression in axotomized neurons and Schwann cells. Infiltrating lymphocytes and platelets, particularly at the injury site, play an important role in Schwann cell proliferation (*see* Schwann Cell Proliferation; Fig. 1). In addition, the lymphocytes supply interleukins, which include interferon-α (IFN-α) and TNF-α, and which provide positive feedback for IL-6 production.

Cytokines induce the expression of MHC class I and II molecules on nonmyelinating Schwann cells (Wekerle et al., 1986; Kingston et al., 1989), which together with the capacity of Schwann cells to synthesize and release IL-1 (Rotshenker et al., 1992) allow Schwann cells to function as accessory cells to T-cell-mediated immune responses by presentation of antigen

to T-lymphocytes (*see also* Bergsteinsdottir et al., 1991). However, the role of this immune competence in nerve regeneration has not been explored. The immune competence is a major factor in rejection of allografts from the same species (Zalewski and Gulati, 1982; reviewed by Evans et al., 1994).

Fibroblast Growth Factors

The demonstrated effects of both FGFa and basic FGF (FGFb) on neurite outgrowth of PC12 cells and mammalian retinal ganglion neurons in vitro suggest a direct effect of these trophic factors on nerve growth (Togari et al., 1985; Neufeld et al., 1987; Lipton et al., 1988). However, the decline in FGF expression in the distal nerve stump after nerve injury suggests that this support may be limited to developmental growth rather than regeneration after injury in the adult (Eckenstein et al., 1991; Ishikawa et al., 1992). The FGFs resemble CNTF in their lack of a signal peptide sequence necessary for both secretion (Burgess and Maciag, 1989) and their receptor mediated uptake and retrograde transport (Ferguson and Johnson, 1991; Grothe and Unsicker, 1992). The resemblance suggests that FGFs play a role similar to CNTF in providing neurotrophic support for mature neurons.

FGFs enhance regeneration of axons across collagen-filled nerve or synthetic bridges between proximal and distal nerve stumps (Aebischer et al., 1989; Cordeiro et al., 1989; Walter et al., 1993; Laquerriere et al., 1994). Their involvement in regeneration is likely owing to the well-described mitogenic effect on Schwann cells and promotion of angiogenesis (Danielsen et al., 1988; Stewart et al., 1991; Walter et al., 1993; Gimenez-Gallego and Cuevas, 1994). In addition, FGFs alone or in combination with PDGF induce the synthesis of DNA in Schwann cells (Jessen and Mirsky, 1992), which includes the gene for NGF (Yoshida and Gage, 1992).

Insulin-Like Growth Factors (IGFs)

IGF-I and IGF-II, members of the insulin gene family, are upregulated in the distal nerve stump after axotomy and downregulated once axons regenerate into the distal nerve stump (Fig. 1; Ishii et al., 1994).

Survival

Low concentrations of IGFs enhance the survival of embryonic sensory, sympathetic, and motoneurons in vitro (Recio-Pinto et al., 1986; Arakawa et al., 1990; Ang et al., 1992). In vivo, IGF-I treatment rescues lumber motoneurons from both naturally occurring and injury-induced neuronal death in chicken embryos (Neff et al., 1993). In neonatal mice, IGF-I completely prevents motoneurons from axotomy-induced neuronal death (Li et al., 1994). IGF-II also has survival-promoting effects on lumber motoneurons of the chick embryo during naturally occurring neuronal death, though to a lesser extent than IGF-I (Neff et al., 1993).

Regeneration

Both IGF-I and IGF-II promote neurite outgrowth of embryonic sensory, motor, and sympathetic neurons in vitro (Recio-Pinto et al., 1986; Caroni and Grandes, 1990; Near et al., 1992; Fernyhough et al., 1993; Edbladh et al., 1994) and increase regeneration rate in vivo when delivered locally after crush, freezing, and transection injuries of the sciatic nerve (Sjoberg et al., 1988; Kanje et al., 1989; Sjoberg and Kanje, 1989; Glazner et al., 1993). Conversely, antibodies against IGF-I and II, when delivered locally to the injury site, markedly inhibit regeneration (Kanje et al., 1989; Near et al., 1992).

IGFs have both direct and indirect effects on axonal regeneration. Neurite outgrowth in vitro is a direct effect of the IGFs and does not require Schwann cells (Recio-Pinto et al., 1986), although the extent of neuronal transport of IGFs in vivo is controversial (Hansson et al., 1987; Caroni and Grandes, 1990; Caroni and Becker, 1992). In vivo, IGF-I promotes proliferation of Schwann cells and their migration into acellular nerve grafts (Sjoberg and Kanje, 1989).

Glial-Cell-Line-Derived Neurotrophic Factor (GDNF)

GDNF, a distant member of the TGF-β superfamily, is expressed in the rat limb bud, embry-

onic myotubes, and Schwann cells (Henderson et al., 1994). RNA transcripts for GDNF have been found in Schwann cell lines in vitro and in the distal nerve stump of injured nerves in vivo (Fig. 1; Springer et al., 1994; Trupp et al., 1995). GDNF is retrogradely transported in a receptor-mediated fashion in axotomized motoneurons (Yan et al., 1995).

Survival

Originally described as a survival-promoting factor for central dopaminergic neurons (Lin et al., 1993; Stromberg et al., 1993), GDNF has been shown to be the most potent survival-promoting factor for embryonic spinal motoneurons in vitro and in vivo (Henderson et al., 1994; Oppenheim et al., 1995; Zurn et al., 1996) and adult motoneurons in vivo (Li et al., 1995). Exogenous GDNF is transported retrogradely to the cell body of neonatal facial and spinal motoneurons, and completely prevents axotomy-induced neuronal death and atrophy (Oppenheim et al., 1995; Yan et al., 1995). Exteroceptive and cutaneous sensory neurons respond to GDNF at later developmental stages than motoneurons (Buj-Bello et al., 1995). The upregulation of GDNF in the distal nerve stump after injury (Trupp et al., 1995) and the evidence for its potent survival-promoting effect on motoneurons indicate its potential role as a survival-promoting factor for axotomized motoneurons.

Regeneration

Recombinant GDNF has marked stimulatory effects on neurite outgrowth of sympathetic and ciliary ganglion neurons in vitro, but lesser effects on sensory neurons (Ebendal et al., 1995). The medium conditioned from immortalized Schwann cells that contain GDNF, NGF, BDNF, NT-3, and CNTF is mitogenic for themselves and long-term cultured Schwann cells (Watabe et al., 1995). The relative contribution of GDNF to the mitogenic effect, however, is not known. In addition, GDNF, like NGF, has been found to increase the expression of L1 on cultured Schwann cells (Schachner et al., 1990).

Other Growth Factors

Other molecules that affect axonal regeneration include glial maturation factor-β (GMF-β) and PDGFs. GMF-β is synthesized in Schwann cells in the distal nerve stump after nerve injury (Bosch et al., 1989) and increases the number of regenerating axons in the distal nerve stump when delivered to the injury site (Harman et al., 1991). PDGF-B promotes neurite outgrowth (Smits et al., 1991) and stimulates Schwann cell proliferation (Davis and Stroobant, 1990; Eccleston et al., 1990; Weinmaster and Lemke, 1990).

PDGF is only one of several growth factors that have both mitogenic effects on Schwann cells and potent angiogenic effects via their mitogenic effects on endothelial cells. Other factors with mitogenic effects include TGF-β, FGF, and EGF. TGF-β and PDGF released from the damaged axons and infiltrating macrophages, much like helper cells of the immune system, help to recruit macrophages to the injury site and to stimulate the synthesis of other growth factors, such as FGF, EGF, and IL-1 by macrophages (Wahl et al., 1987; Brodkey et al., 1993). Thus, TGF-β and PDGF amplify and prolong the duration of macrophage invasion, activation and synthesis, and release of themselves and other growth factors. Consequently, a supportive growth environment is induced by promoting Schwann cell proliferation and expression of their "growth support mode," and by promoting neovascularization. Owing to the delay in macrophage invasion of the distal nerve stump and their release of growth factors, which influence blood vessel growth in the injured nerve, neovascularization occurs slowly (Lundborg, 1987).

The very rapid induction of specific PDGF binding to the endoneurial connective tissue and the subperineurial and subendothelial mesenchyme indicates that PDGF has direct effects on fibroblasts in the distal nerve stump in addition to its more indirect effects on macrophages (Raivich and Kreutzberg, 1987b).

Synopsis

In summary, there is good evidence, particularly from studies of injured immature neu-

rons, that several of the neurotrophic factors that are expressed in the distal nerve stump promote the survival of injured neurons. In contrast, there is relatively little in vivo evidence for a direct effect of growth factors in promoting axonal outgrowth during regeneration. There is, however, extensive evidence for their effects on neurite outgrowth in vitro. Since the evidence in vitro primarily concerns neurite outgrowth from immature neurons and the lack of effect in vivo is demonstrated primarily in crush models where axonal regeneration is already optimal, it is still not clear whether neurotrophic factors may promote axonal regeneration directly. Presently, the available evidence suggests that the positive effects of growth factors in enhancing regeneration across artificial bridges between the cut nerve ends in vivo may be owing to paracrine and autocrine effects on nonneuronal cells in the distal nerve stump rather than direct effects on regenerating axons.

The indirect effects of neurotrophic factors include their mitogenic stimulation of Schwann cells and other endoneurial cells, which contribute to providing a permissive and well-perfused growth environment for axonal regeneration. For example, NGF's enhancement of early, but not late axonal regeneration across silicone chambers (Rich et al., 1989a; Derby et al., 1993) may be owing to its indirect effects on Schwann cell migration and expression of CAMs rather than direct effects on the regenerating axons themselves.

Most in vitro and in vivo experimental paradigms have assayed the effects of growth factors independently of each other. However, their relative effects are likely to depend on the time of expression and on which growth factors are expressed simultaneously. The effects of TGF-β, for example, depend critically on its coexpression with other growth factors, concentrations, and status of differentiation of target cells (reviewed by Brodkey et al., 1993). For instance, TGF-β facilitates fibroblast proliferation in the presence of PDGF, but inhibits fibroblast proliferation in the presence of EGF (Sporn and Roberts, 1986). In addition, the neurotrophin dependence may differ as a function of stage of development or nerve repair (Davis, 1994, 1996).

Cell Adhesion Molecules (CAMs)

Also representing a switch from myelinating to nonmyelinating phenotype is enhanced expression of CAMs of the immunoglobulin superfamily, L1/Ng-CAM/NILE, *N*-CAM, and *N*-cadherin (Daniloff et al., 1986; Mirsky et al., 1986; Martini and Schachner, 1988; Cifuentes-Diaz et al., 1994) and extracellular matrix molecules, such as laminin, collagen, fibronectin, and tenascin-C (Erickson, 1993; Martini, 1994; Bisby, 1995). Many of these molecules are involved in adhesion between axon and axon, axon and Schwann cells, and axon and basement membrane, and thereby regulate axonal growth in the distal nerve stump (Fig. 3).

L1 and N-CAM

L1 and *N*-CAM are members of the immunoglobulin superfamily that also include CAMs, MAG, and Thy-1. L1 and *N*-CAM are expressed only by nonmyelin-forming Schwann cells, whereas MAG is normally only expressed by myelinating Schwann cells (Martini and Schachner, 1986; Mirsky et al., 1986). During and after axonal degeneration in vivo, myelinating Schwann cells downregulate MAG and form slender processes that are L1- and *N*-CAM-positive (Martini and Schachner, 1988). L1 and *N*-CAM are downregulated concurrent with re-expression of MAG when Schwann cell processes begin to encircle regenerating axons and have turned approximately one and a half loops around regenerating axons (Kruse et al., 1984; Kunemund et al., 1988; Martini and Schachner, 1988). MAG disappears from the turning loop once myelin compaction occurs, coinciding with the expression of myelin basic protein (reviewed by Schachner et al., 1990). In contrast to L1, *N*-CAM is expressed at low level in myelinated axons, and is mainly located periaxonally and more weakly in compact myelin. In vitro, Schwann cells express L1 and *N*-CAM on their

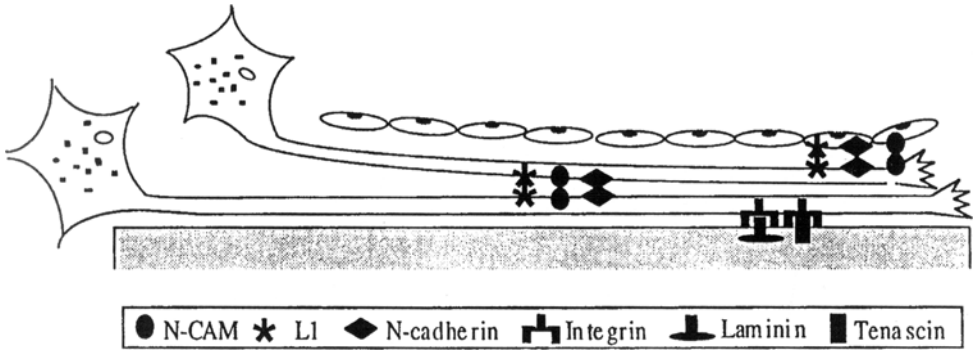

Figure 3. Schematic illustration of molecules that mediate adhesion between axons, between axons and Schwann cells, and between axons and basal lamina. Adhesions between axons and between axons and Schwann cell are primarily mediated by homophilic binding of L1, N-CAM, and N-cadherin, although heterophilic binding of N-CAM to L1, N-CAM to integrins, and L1 to integrins are also observed. Adhesion between axons and the basal lamina is primarily mediated by binding of extracellular matrix molecules, such as laminin and tenascin, to integrin.

entire surface in the absence of contact with other cells. Cultured Schwann cells only express L1 and *N*-CAM in regions of contact with either cocultured neurons or other Schwann cells (Martini et al., 1994a).

The presence of L1 and *N*-CAM on the nonmyelinating Schwann cells and on the growth cone of growing axons (Tomascelli et al., 1986; Jessen et al., 1987b; Bixby et al., 1988; Kleitman et al., 1988; Martini and Schachner, 1988; Seilheimer and Schachner, 1988) suggests their role in mediating interactions between regenerating axons and the longitudinal array of Schwann cells in the endoneurial tube of the distal nerve stump. In vitro experiments that use antibodies against L1 and *N*-CAM provide strong evidence for this role. L1 antibodies inhibit at least 80% of neurite outgrowth on the Schwann cell surface in cocultures of Schwann cells and the DRG, embryonic chick ciliary ganglion, and retinal ganglion neurons; *N*-CAM antibodies are generally less effective and are completely ineffective in the chick ciliary ganglion–Schwann cell cocultures (Bixby et al., 1988; Kleitman et al., 1988; Seilheimer and Schachner, 1988). A soluble recombinant L1-Fc chimera promotes neurite outgrowth of cerebellar neurons on the surface of 3T3 fibroblasts, and this growth-promoting effect is inhibited by L1 antibodies (Doherty et al., 1995).

L1 and *N*-CAM mediate cellular interactions mainly by binding to the same molecules (homophilic binding) in a calcium-independent manner. L1 and *N*-CAM can also mediate cell adhesion by binding to other CAMs of the immunoglobulin family and to integrins (Bixby, 1992). The integrins belong to a family of α-β heterodimeric receptors, which are transmembrane proteins that interact internally with cytoskeleton and externally with extracellular matrix molecules of the basal lamina and receptors on adjacent cells (reviewed by Clark and Brugge, 1995). The integrin-cytoskeletal assembly plays an important role in adhesion and growth by its direct action on the organization of cytoskeleton and by forming the foundation for construction of signaling complexes, including the focal adhesion kinase (FAK), and a cascade of tyrosine and serine-threonine kinase-induced phosphorylations (Clark and Brugge, 1995). Since integrins affect cell survival by regulating cell death (Schwartz and Ingber, 1994), L1 and *N*-CAM expression on Schwann cells may also synergize with neurotrophic factors as survival-promoting molecules for axotomized neurons, particularly as integrins and neuro-

trophins converge on the same components of the signaling pathway. An example is the synergy in enhancement of Ras-MAP kinase activation (Clark and Brugge, 1995). Moreover, upregulation of L1 expression on Schwann cells by neurotrophic factors, such as NGF and GDNF (Schachner et al., 1990), may enhance L1–integrin interactions between Schwann cells and regenerating axons.

The functional capacities of L1 and *N*-CAM can be modulated by binding to carbohydrate moities such as L2/HNK-1 and polysialic acid (PSA). L2/HNK-1 is shared by L1, *N*-CAM, MAG, and an extracellular matrix molecule, tenascin, whereas PSA modification is restricted primarily to *N*-CAM (reviewed by Schachner et al., 1990). The preferential expression of L2/HNK-1 on Schwann cells in the motor pathway after nerve injury has been linked to the significant preference of regenerating motor axons for motor rather than sensory nerve branches in young animals in vivo (Brushart, 1988, 1990, 1993; Martini et al., 1988, 1994a,b). In vitro, embryonic chick motoneurons elongate neurites farther on cryosections of L2-positive nerves than on those of L2-negative nerves (Martini et al., 1992). Polysialization of *N*-CAM reduces the adhesiveness of *N*-CAMs and likely promotes axonal branching in the developing muscle by reducing axonal fasciculation mediated by *N*-CAM (Landmesser et al., 1990; *see* Rutishauser and Jessell, 1988; Rutishauser and Landmesser, 1991; Martini, 1994 for reviews). The roles of PSA and *N*-CAM in regeneration are not well understood, although PSA has been found in lesioned nerves (Daniloff et al., 1986), and antibodies to the highly polysialized, embryonic *N*-CAM in silicone bridging chambers delays the recovery of the evoked muscle action potential (Remsen et al., 1990).

N-Cadherin

N-cadherin is a member of a multigene family that mediates homophilic Ca^{2+}-dependent cell adhesion. Normally, *N*-cadherin is expressed by membranes of all Schwann cells where they contact other Schwann cells and the membrane of nonmyelinated axons (Shibuya et al., 1995). After injury, proliferating Schwann cells continue to express *N*-cadherin in the distal nerve stump (Cifuentes-Diaz et al., 1994). When axons regenerate into the distal nerve stump, *N*-cadherin immunoreactivity is localized to the axon and Schwann cell membrane where axon–axon or axon–Schwann cell contact is made, suggesting that *N*-cadherin is involved in axonal growth by mediating adhesion between axons and Schwann cells (Shibuya et al., 1995). *N*-cadherin either as a substrate or transfected into heterologous nonneuronal cells has potent effects in stimulating neurite outgrowth in vitro (Matsunaga et al., 1988; Bixby and Zhang, 1990; Doherty et al., 1991; Harper et al., 1994). Neurite outgrowth on glia cells in vitro is at least partially blocked by antibodies against *N*-cadherin (Bixby et al., 1987, 1988; Neugebauer et al., 1988; Tomaselli et al., 1988; Letourneau et al., 1991; for review, *see* Doherty and Walsh, 1991).

Extracellular Matrix Proteins

Molecules in the basal lamina and endoneurium constitute a distinct class of neurite-promoting molecules that are synthesized and secreted by Schwann cells and fibroblasts of the peripheral nerve. These include laminin, fibronectin, entactin/J1/tenascin, heparan sulfate proteoglycan (HSPG), types IV and V collagens and variable amounts of fibronectin (reviewed by Bunge et al., 1989, 1990). Axonal contact is required for elaboration of the basal lamina by Schwann cells (reviewed by Bunge et al., 1986, 1989, 1990). Nevertheless, some components of the basal lamina, such as laminin, continue to be synthesized under denervated condition (Bunge and Bunge, 1983; Cornbrooks et al., 1983). In addition, visualized by laminin and HSPG antibodies, the basal lamina in the distal nerve stumps remains intact for 3–4 wk following injury (Neuberger and Cornbrooks, 1989), corroborating early morphological studies (Holmes and Young, 1942; Thomas, 1964). Over longer durations, however, the Schwann cell basal laminae

become fragmented, and laminin mRNA and immunoreactivity decrease if regeneration does not occur (Bignami et al., 1984; Roytta and Salonen, 1988; Giannini and Dyck, 1990; Doyu et al., 1993). These changes are likely to contribute to the poor axonal regeneration and target reinnervation after delayed nerve repair (Fu and Gordon, 1995b).

In contrast to many in vitro studies that have demonstrated neurite outgrowth on basement membrane alone or even components of the basement membrane (Ard et al., 1987; for reviews, *see* Letourneau, 1988; Bixby and Harris, 1991; Carbonetto, 1991; Reichardt and Tomaselli, 1991; Bixby, 1992), few studies have examined the role of the basal lamina in nerve regeneration in vivo (*see* Laminin). These studies have demonstrated an essential role for laminin and possibly tenascin in providing an essential substrate for supporting axonal regeneration. In addition to their roles in providing a substrate for growth, extracellular matrix molecules bind to and regulate the activity and stability of several growth factors, most notably FGF and TGF-β (McCaffrey et al., 1989; Rifkin and Moscatelli, 1989).

Laminin

Laminin is present in the nervous systems that are able to regenerate and absent in those that are not, indicating that laminin is a prerequisite for successful axonalregeneration (Hopkins et al., 1985; Liesi, 1985). For example, the presence of laminin in the basal lamina of the peripheral nerve is correlated with its ability to regenerate and may account for growth of CNS axons, which normally do not regenerate, through peripheral nerve grafts (David and Aguayo, 1981; Liesi, 1985; David et al., 1995).

A critical role of laminin in peripheral nerve regeneration has been demonstrated in experiments in which sectioned peripheral nerves are bridged by artificial or natural grafts in which the content of basal lamina proteins is experimentally controlled. Several experiments have demonstrated that regenerating axons fail to cross silicone or collagen grafts unless laminin is present (Madison et al., 1985; Williams et al., 1987; Yoshii et al., 1987; Bailey et al., 1993; Kauppila et al., 1993). Regeneration across silicone chambers or acellular nerve grafts is drastically impaired by antibodies to laminin or integrin receptors (Toyota et al., 1990; Wang et al., 1992). In vitro, neurite growth cones prefer laminin to other growth-promoting substrates, such as fibronectin (reviewed by Bixby and Harris, 1991). Consistent with this finding, neither exclusion of fibronectin from nerve grafts in vivo nor antifibronectin antibodies greatly affects axonal regeneration across the graft (Wang et al., 1992; Bailey et al., 1993). Fibronectin contrasts with laminin in its more diffuse distribution in the endoneurial tube (Bunge et al., 1986).

The B2 chain of laminin is as effective as laminin in promoting the growth of regenerating axons (Kauppila et al., 1993). It is notable that axonal regeneration fails to occur across grafts containing collagen or collagen coated with the cell attachment domain of laminin, despite the fact that Schwann cells have migrated into these grafts (Kauppila et al., 1993). These findings demonstrate that the migration of Schwann cells alone could not explain the growth-promoting effects of laminin in artificial or acellular nerve grafts. Thus, the presence of both laminin and Schwann cells is necessary for successful regeneration. This conclusion is consistent with the morphological finding that regenerating axons navigate between Schwann cells and the basement membrane in the distal nerve stump (Nathaniel and Pease, 1963b; Haftek and Thomas, 1968; Scherer and Easter, 1984; Schwab and Thoenen, 1985; Martini and Schachner, 1988).

Tenascin C

Tenascin C is normally confined to the extracellular matrix around the node of Ranvier and the perineurium (Daniloff et al., 1989; Martini et al., 1990). After injury, tenascin-C is expressed along the entire length of the distal nerve stump, the distal end of the proximal nerve stump, and the bridge between the proximal and distal nerve stumps (Martini et

al., 1990). Its immunoreactivity is associated with the basal lamina of Schwann cells (Martini et al., 1990). In vitro, tenascin promotes neurite outgrowth (Wehrle and Chiquet, 1990; Husmann et al., 1992; Lochter et al., 1991). In vivo, immunohistochemistry reveals a close association of regenerating axons with tenascin-positive regions in the distal nerve stump (Martini et al., 1990). The spatial and temporal expression of tenascin together with its growth-promoting effects in vitro suggests its possible involvement in axonal regeneration.

Expression of tenascin is closely related to Wallerian degeneration and Schwann cell proliferation. Nonmyelinating Schwann cells that reach their peak of proliferation a day earlier than myelinating Schwann cells (Clemence et al., 1989) are the first Schwann cells to express tenascin-C, followed by the myelinating Schwann cells (Fruttiger et al., 1995). In the latter cells, tenascin-C upregulation may be causally linked to Wallerian degeneration, because tenascin-C immunoreactivity parallels the delayed time-course of degeneration in C57Bl/Ola mice. It is probable that IL-1 and TGF-β released from invading macrophages play a role in the upregulation of tenascin in Schwann cells, since both cytokines enhance tenascin-C expression in other cell types (Pearson et al., 1988; McCachren and Lightner, 1992). Nevertheless, the sustained expression of tenascin in chronically denervated nerve stumps and its downregulation by regenerating axons suggest that loss of axonal contact is a sufficient signal to induce tenascin expression (Martini et al., 1990; Martini 1994) as part of the repertoire of changes in gene expression associated with conversion of Schwann cells to the nonmyelinating phenotype.

Synopsis

In summary, the dramatic molecular changes that occur in the distal nerve stump after injury promote the formation of a conducive environment for axonal regeneration. Loss of contact between Schwann cells and axons together with chemical signals from invading macrophages induce Schwann cells to change their phenotype to that associated with axonal regeneration. In vivo experiments, particularly those that utilize artificial and neural grafts, have demonstrated theessential role of Schwann cells and their basal lamina in supporting regenerating axons.

Regeneration After Prolonged Denervation of the Distal Nerve Stump

Prolonged denervation of the distal nerve stumps is one of the three factors that account for poor functional recovery after delayed nerve repair as well as after injuries that incur long delays between the injury and the reinnervation of denervated targets by regenerating axons. The other two factors are prolonged axotomy and prolonged target denervation.

Freshly sectioned axons that are forced to regenerate in a long-term denervated nerve stump regenerate very slowly (Holmes and Young, 1942; Vuorinen et al., 1995) and largely fail to traverse through the distal nerve stump and reinnervate the target muscles successfully (Fu and Gordon, 1995b; Vuorinen et al., 1995). When denervation is prolonged for 1 yr, <15% axons regenerate and make functional connections with the denervated muscle (Fu and Gordon, 1995b). Although the few successfully regenerated motor axons branch extensively to reinnervate three times as many muscle fibers as normal, the increase in muscle fibers per motoneuron is insufficient to compensate for the small number of regenerated axons (Fu and Gordon, 1995b). As a result, far fewer muscle fibers are reinnervated after long-term denervation (Fu and Gordon, 1995b). In addition, reinnervated muscle fibers do not fully recover from denervation atrophy (Fu and Gordon, 1995b). This incomplete muscle fiber recovery exacerbates the effects of poor regeneration with the end result that functional recovery is poor following long-term denervation of the target muscle and the distal nerve stump (Gutmann, 1948; Irintchev et al., 1990; Fu and Gordon, 1995b).

These findings indicate that the growth environment of the distal nerve sheaths deterio-

rates with time when regeneration is prevented or when the distances over which axons regenerate prolong the period of sheath denervation. However, the nature of deterioration of the distal nerve sheaths is poorly understood. Most studies have been directed to observe the immediate changes in gene expression in the denervated nerve stumps and their reversal after axonal regeneration. The few studies that have examined the condition of the distal nerve stump when regeneration is prevented have shown that the deterioration is associated with Schwann cellatrophy, disruption of the basal laminae, progressive fibrosis, and collagenization (Sunderland and Bradley, 1950; Sunderland, 1978; Weinberg and Spencer, 1978; Pellegrino and Spencer, 1985; Salonen et al., 1985, 1987; Giannini and Dyck, 1990; Siironen et al., 1995). The molecular basis for the deterioration of the basal lamina is not clear. Laminin immunoreactivity has been shown to decline concurrently with atrophy of Schwann cell columns. When axonal regeneration in the long-term denervated distal nerve stump is permitted, axons regenerate only inside the laminin-positive columns (Neuberger and Cornbrooks, 1989; Salonen et al., 1987). The integrin's β1-subunit, a receptor for laminin and CAMs, is undetectable in the distal nerve stump when regeneration into the distal nerve stump is delayed (Siironen et al., 1995). Upregulation of p75 mRNA in the distal nerve stump is not maintained beyond the first month even when axonal regeneration does not occur (You et al., 1996). The protein and mRNA of p75 are undetectable in long-term denervated distal nerve stump 6 mo after injury, indicating that an active signal is required to maintain the synthesis of the low-affinity neurotrophin receptors. These few studies indicate that unless regeneration occurs, the permissive growth environment of the distal nerve stump emerges after injury and deteriorates within months. This deterioration may also account for the poor reinnervation of denervated muscle, since regenerating axons fail to traverse the deteriorating intramuscular nerve sheaths (Gutmann and Young, 1944; Fu and Gordon, 1995b). The small number of regenerating axons and the failure of these axons to reinnervate all the muscle fibers may account for the loss of muscle fibers and severe atrophy of long-term denervated muscles, which cannot be fully reversed by reinnervation (Gutmann, 1948; Anzil and Wernig, 1989; Fu and Gordon, 1995b). An additional factor that exacerbates the poor muscle recovery is the incomplete recovery of muscle fiber diameter after reinnervation of long-term denervated muscles (Anzil et al., 1989; Fu and Gordon, 1995b).

Conclusions

Although the capacity of peripheral nerves to regenerate their axons after injury has been recognized for more than a century and the cellular and molecular basis for this capacity has been progressively unraveled, poor functional recovery after peripheral nerve injuries remains a frustrating clinical problem (e.g., Sunderland, 1978; Kline and Hudson, 1995). The techniques of microsurgical repair of severed nerves have improved considerably, and yet, regenerative success, especially for proximal nerve lesions that sever large nerve trunks, may be minimal (Gordon, 1994). The traditional views that attribute poor recovery to poor alignment of severed ends of the injured nerves and/or the irreversible atrophy of denervated targets are now being revised to include the perspectives gained from the extensive work on the survival and response of the injured neurons, the nonneural cells that surround the soma of the injured neurons, and the nonneural cells of the distal nerve stump through which regenerating axons grow.

The evidence shows that injured neurons switch from the normal adult phenotype of the transmitting mode to a growth mode in which they synthesize many growth-associated molecules. The injured neurons and their surrounding glial cells appear to "sense" the damage and/or the cessation of access to target-derived neurotrophic support, and to alter

their gene expression according to the extent of axonal loss. The susceptibility of neurons to apoptotic cell death when lesions are close to the cell body or if the neuron is immature suggests that the adaptive growth response may be overwhelmed if the "task" of growth is too extensive and if the neuron is insufficiently mature. Even if the neurons survive the injury, their capacity to regenerate may deteriorate with prolonged neurotrophic factor deprivation. As a result, many of axotomized neurons that attempt to regenerate their axons after delayed nerve repair may fail. Even when regenerating axons gain access to the distal nerve stump after immediate microsurgical delay, many axons must regenerate over long distances to reach their denervated targets and, at a regeneration rate of 1–3 mm/d, they remain without target connections for periods of up to 6 mo. The scant evidence that is available suggests that axotomized neurons may not sustain the growth program sufficiently long to support the regeneration over these prolonged periods of time. The failure of the growth program may result, in turn, from loss of positive signals, which trigger the program, and/or the encountering of negative signals, which prevent the neuron from sustaining the growth program.

Wallerian degeneration of the isolated axons and myelin sheaths of the injured peripheral nerve is a prerequisite to establishing a permissive growth environment for regenerating axons. The capacity of blood-borne cells, particularly macrophages, to enter into the damaged peripheral nerve is pivotal to Wallerian degeneration, which removes inhibitory factors to axonal growth, and the conversion of the myelinating Schwann cells in the distal nerve stump to the nonmyelinating phenotype, which supports axonal regeneration. Complex interplay among regenerating axons, macrophages, Schwann cells, fibroblasts, and extracellular matrix molecules in the basement membranes underlies the growth of regenerating axons between Schwann cells and the basement membrane. However, the acute response of the neurons and the nonneural cells may not be sustained such that axons that regenerate over long distances are likely to grow under progressively poorer growth conditions.

Although the acute responses of the neurons and nonneural cells may be appropriate for regeneration of axons over relatively short distances, they appear to be insufficient to support regeneration over long distances, which incur considerable delays between the onset of regeneration and the reinnervation of denervated targets.

The complexity of nerve regeneration has been increasingly recognized with the advent of genetic and molecular biological techniques. As we acquire more understanding of the triggers for the altered gene expression in injured neurons and the associated nonneural cells, the interplay between the cells, and the complex interactions of the many molecular species involved in axonal regeneration, it is likely that molecular interventions will be devised that can sustain the growth program for sufficient time and intensity to allow all neurons to survive, to regenerate their axons successfully, and make functional target connections. Finally, the problem of misdirection of regenerating axons to inappropriate targets has to be solved by ensuring that regenerating axons find their original and appropriate targets.

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