12

Regeneration and Repair

Maureen L. Condic

The central nervous system (CNS) of adult vertebrate animals is capable of considerable plasticity, both in the course of normal adult function and in response to injury. Patients suffering even severe injuries to the brain frequently achieve substantial, if not complete, recovery of function. Despite the ability of the nervous system to functionally compensate for injury, regenerative *repair* of neural injury is quite limited. Cells of the nervous system are exceptionally sensitive to ischemic insult (Goldberg and Barres, 2000; Allan and Rothwell, 2001) and cell death can be extensive following even mild injury. Neurons lost to injury or disease are rarely replaced in the adult brain (Garcia-Verdugo *et al*., 2002; Lim *et al*., 2002; Parent and Lowenstein, 2002; Turlejski and Djavadian, 2002). Even when injured neurons do not die, they are largely unable to regenerate axons and dendrites in order to reestablish functional connections with their normal synaptic partners. Thus, while significant functional recovery is often possible, the adult CNS does not appear to be capable of regenerative repair following injury. What are the possible mechanisms by which nervous system function is restored following injury?

RESTORATION OF FUNCTION VS REGENERATIVE REPAIR

Functional recovery following CNS injury in the adult can occur by at least three distinct mechanisms: restitution, substitution, and compensation (Singer, 1982). In restitutive or restorative recovery, the original system responsible for the function is repaired or its efficacy enhanced as a means of regaining normal behavior. This kind of recovery may not be complete, but the behavior observed following restitution is always qualitatively similar to the original behavior. Restitution may depend on redundancy of parallel or distributed systems that normally mediate a particular function. Alternatively, restitution of function may be based on neuronal sprouting and the generation of new synapses within the damaged system to replace those lost to injury or cell death.

Substitution, in contrast, involves the adoption of function by a related system that imperfectly replaces the failed or damaged system. Substitutive recovery is never complete, and always involves some qualitative change in the behavior. For example, patients with damage to the primary cortical visual centers ("cortical blindness") can recover significant visual behavior (avoiding obstacles, orienting toward light sources, detection of moving objects, etc.) without any conscious perception of sight, presumably by recruitment of visual processing pathways that are not normally involved in conscious experience of visual stimuli (Poppel *et al*., 1973).

Finally, compensation for CNS injury involves the recovery of function due to adaptation of the undamaged components of the normal system, so as to minimize the effects of a partial loss of function. In compensatory recovery, changes in the gain or attenuation of a system's components can result in improved functional output of the system as a whole, without strictly restoring the aspect of normal function that was lost as a consequence of damage. Gradual recovery of balance following unilateral damage to the vestibular system is an example of a compensation. Recovery of balance occurs through changes in the normal reciprocal inhibition between the two vestibular nuclei (Dieringer and Precht, 1979) that balance the firing rates of these paired groups of neurons. Neither the damaged neurons nor their connections are replaced, but the system compensates for the lost function of these neurons to restore the output of the system as a whole.

Therapeutic approaches to CNS injury attempt to exploit all three of these naturally occurring mechanisms of functional recovery. Rehabilitative medicine works to enhance the efficacy of any residual function using physical training and biofeedback techniques. Recovery mediated through physical therapy is likely to reflect both compensatory and restitutive changes in the damaged system. Pharmocologic agents that increase conduction velocity of demyelinated axons are a compensatory treatment designed to increase the gain of circuitry that remains intact following injury. The nervous system can also be trained to utilize intact, local circuitry to mediate a function normally controlled by descending cortical activity (substitution). For example, the walking function can be imperfectly recovered following complete spinal transection by a substitutive mechanism that recruits local spinal circuits normally used for maintenance of balance and foot placement to generate walking behavior (Barbeau *et al*., 1999). Entraining this "spinal walking circuit" by evoking spinal

Maureen L. Condic • Department of Neurobiology and Anatomy, University of Utah, SOM, Salt Lake City, Utah 84132.

Developmental Neurobiology, 4th ed., edited by Mahendra S. Rao and Marcus Jacobson. Kluwer Academic / Plenum Publishers, New York, 2005.

pattern generators takes considerable practice, but can ultimately result in reasonable, albeit imperfect, walking behavior in cats, rodents, and even human patients (Edgerton *et al*., 1992; Chau *et al*., 1998; Fouad *et al*., 2000; Harkema, 2001). Notably absent from the current repertoire of therapeutic approaches to CNS injury are manipulations that strictly induce or enhance the regeneration of damaged cells or axons.

FUNCTIONAL RECOVERY: RECAPITULATION OF DEVELOPMENT?

There has long been a bias in the field of regeneration research that functional recovery following CNS injury involves a recapitulation or reactivation of the processes underlying embryonic development of the brain and spinal cord. In the majority of cases, however, recovery bears little resemblance to development, appearing instead to more closely mimic the normal adaptive processes of the adult CNS that are likely to underlie learning and memory (see Chapter 10). The same flexibility that enables the mature nervous system to learn and adapt appears to provide a mechanism by which functional deficits can be circumvented, and in many cases overcome. With very few exceptions, true regenerative recovery (due to either cell or axon replacement) does not contribute to functional recovery in humans.

The minimal contribution of cell and axon regeneration to functional recovery of the CNS in humans is by no means a universal phenomenon. The failure to reactivate developmental processes following injury appears to be a limitation that is largely restricted to higher vertebrate species (avians and mammals). In reptiles and amphibians, there is extensive regeneration in the adult, in addition to the functional recovery mediated through more adaptive mechanisms (Chernoff *et al*., 2002). In most cases where the adult CNS regenerates, the process does indeed appear to mimic development. For example, the spinal cord of newts undergoes complete functional regeneration following ablation of spinal segments several millimeters in length. Spinal regeneration occurs through a process quite reminiscent of embryonic development. Following injury, specialized cells lining the ventricle (ependymal cells) dedifferentiate and migrate into the site of injury. Once there, these primitive cells proliferate to fill the ablated cavity and subsequently redifferentiate into mature spinal cord cells (Chernoff *et al*., 2002).

The failure of the adult mammalian and avian CNS to fully reinitiate a developmental program as a means of repairing injury has led to extensive investigation into the underlying reasons for this failure. Curiously, in many mammalian and avian species, the CNS exhibits robust regeneration up until roughly the last third of prenatal development (Forehand and Farel, 1982; Shimizu *et al*., 1990; Bates and Stelzner, 1993; Bandtlow and Loschinger, 1997; Sholomenko and Delaney, 1998; Wang *et al*., 1998a,b). Changes occurring in both the neurons and the environment of the CNS during development have been implicated in the shift from regeneration competency to regeneration failure. CNS regenerative failure has also been extensively compared to the relatively robust regeneration observed from adult peripheral neurons following injury. To understand regeneration failure in adult mammalian and avian CNS, it is useful to consider both what is known regarding peripheral nervous system (PNS) regeneration and what is known about the factors that prevent regenerative replacement of either cells or axons in the adult CNS.

PNS REGENERATION

The ability of peripheral nerve to regenerate following injury depends largely on the severity of the injury. Sir Sydney Sunderland (1965) defined five degrees of peripheral nerve injury from mild to severe, with the likelihood of spontaneous regeneration quite poor for all injuries above the third degree (Fig. 1). In addition, the proximity of the injury to the cell body greatly affects the likelihood of recovery, with neurons that sustain injuries close to the cell body being far less likely to regenerate than those subjected to more distal injuries. A similar correlation is seen in regenerating CNS neurons (Sunderland, 1965, 1970, 1990). The age of the individual at the time of injury also greatly affects the likelihood of peripheral regeneration, with younger individuals far more likely to regenerate peripheral nerve compared to more aged individuals.

The process of regeneration, whether it be for central or peripheral neurons, involves several distinct stages: Surviving the initial insult, initiating outgrowth (sprouting), traversing the region of injury, navigating back to the original targets, reestablishing appropriate synaptic contacts at those targets, and restoring normal myelination of regenerated axons (Fig. 2). At many of these stages, the response of peripheral nerve is distinct from that of CNS tissue (Fu and Gordon, 1997). While both CNS and peripheral nerve undergo an initial inflammatory response to injury, the damage to the CNS neurons is exacerbated by the swelling of the tissue against the rigid constraints of the vertebral column and cranium. In peripheral nerve and in the CNS, damaged neurons and glial cells undergo apoptosis and are cleared in a process known as Wallerian degeneration. Clearing of cellular debris occurs much more rapidly in peripheral nerve (within weeks) than it does following CNS damage (months). In response to cytokines released by infiltrating immune cells, quiescent peripheral nerve Schwann cell-precursors are activated, reenter the cell cycle, and actively migrate into the region of damage. A similar activation of microglia and astrocytes occurs in the CNS. However, proliferating Schwann cells are a rich source of trophic support to injured neurons, producing a wide range of beneficial factors including neurotrophins as well as other factors that enhance neuronal survival (Frostick *et al*., 1998; Yin *et al*., 1998; Terenghi, 1999). Schwann cells also produce a highly growth-promoting environment, both through their physical alignment into conduits that guide regenerating fibers as well as through the production of a specialized extracellular matrix (ECM) that stimulates regeneration. In contrast, activated CNS glia produce a highly nonpermissive environment in regions of CNS injury (see below). Finally, denervated peripheral targets participate in nerve cell regeneration by producing trophic and

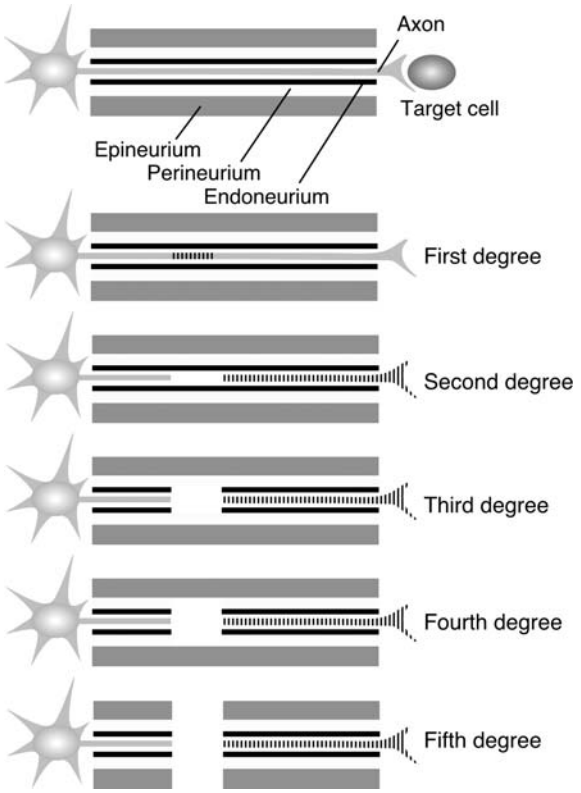

FIGURE 1. Regeneration of peripheral nerve depends on severity of the injury. Peripheral axons of mature nerves are surrounded by three layers of connective tissue: The epineurium surrounding the entire nerve, consisting of fibroblasts, fat cells, small blood vessels, and collagenous matrix; the perineurium surrounding individual nerve fascicles, consisting of a collagen matrix and specialized perineural cells (this layer also forms the blood–nerve barrier); the endoneurium surrounding individual myelinated axons, which is largely an acellular collagen layer separated from the axon itself by the Schwann cell and its basal lamina. Injuries that damage axons (by mild compression or temperature extremes) without disrupting the extracellular matrix are considered first degree injuries and are readily repaired. Second degree injuries that sever or crush the nerve result in Wallerian degeneration distal to the injury, but are also readily repaired, so long as connective tissue layers remain intact. Third through fifth degree injuries involve increasing disruption of the connective tissue of the nerve and are rarely repaired without surgical intervention to reconnect distal and proximal nerve stumps.

possibly tropic factors, a response that is generally not observed from denervated CNS targets.

The most significant challenge presented to regenerating peripheral and central neurons is the site of injury itself. If peripheral nerve injuries are relatively mild (Fig. 1; degrees 1–3), proliferating Schwann cells will successfully fill the gap between distal and proximal nerve, allowing a continuous pathway for the extension of regenerating fibers. Regenerating axons are guided to their original targets by the denervated nerve sheath that serves as a conduit for growing fibers. If injuries are more severe, however, Schwann cells form a dense mass at the injury site that regenerating axons are unable to traverse. Regeneration aborts at the injury site, with nerve fibers often forming a dense neuroma. Surgical interventions, either to ligate the severed ends of the nerve or to provide an artificial conduit that bridges the gap between distal and proximal nerve stumps, are required to promote regeneration for severe peripheral nerve injuries.

CNS REGENERATION

Following injury, CNS neurons are faced with many of the same challenges presented by injured peripheral nerve. Neurons must survive the initial insult, initiate new axons and dendrites, navigate up to and beyond the injury site, extend to appropriate targets, arrest growth, reestablish synaptic contacts, and establish normal myelination for regenerated axons (Fig. 2). In contrast to peripheral nerve, however, regeneration in the CNS is rarely, if ever, accomplished. The reasons for CNS regenerative failure have been subject to considerable debate and interpretation. For many years, it was generally accepted that, in contrast to peripheral neurons, adult neurons of the mammalian CNS were intrinsically incapable of regeneration. This pessimistic conclusion was radically altered in the early 1980s by several convincing demonstrations that adult neurons could re-extend axons over long distances if provided with the permissive environment of the adult peripheral nerve (Richardson *et al*., 1980; David and Aguayo, 1981). Over the next 20 years, the focus of regeneration research was largely the environment of the injured CNS, in an attempt to define what factors present in this environment prevent the reestablishment of contacts disrupted by injury. The dominant view was that adult CNS neurons are fully capable of regeneration, but that this intrinsic ability is somehow suppressed by the poorly supportive or actively inhibitory environment of the adult CNS.

In recent years, the pendulum of scientific opinion on the topic of adult CNS regeneration has begun to swing yet again: Away from a strict focus on the environment and toward a more nuanced and complex view of adult regeneration. It has become increasingly clear that the intrinsic ability of adult neurons to extend axons and dendrites is compromised relative to immature neurons. Even under optimal conditions, outgrowth of processes from adult neurons is weak relative to that observed from embryonic or fetal neurons. When fetal neurons are transplanted into injured adult brain, their regeneration is always superior to that of injured adult neurons in the same environment (Wictorin and Bjorklund, 1992; Nogradi and Vrbova, 1994; Lindvall, 1998; Broude *et al*., 1999; Ito *et al*., 1999), indicating that changes in cell-autonomous properties of neurons contribute to adult regenerative failure. The cell-intrinsic factors that contribute to poor adult regeneration are poorly understood, and such factors have increasingly become the topic of research.

Lastly, although restoration of function through regenerating axons and synaptic contacts has been the primary focus of research, recent work has begun to investigate the possibility of replacing damaged neurons entirely, either by supplying embryonic counterparts or by stimulating the proliferation of quiescent neuronal precursors present in the adult CNS. The use of neurons generated from either fetal or adult stem cells to replace adult neurons lost to injury or disease is an active area of research.

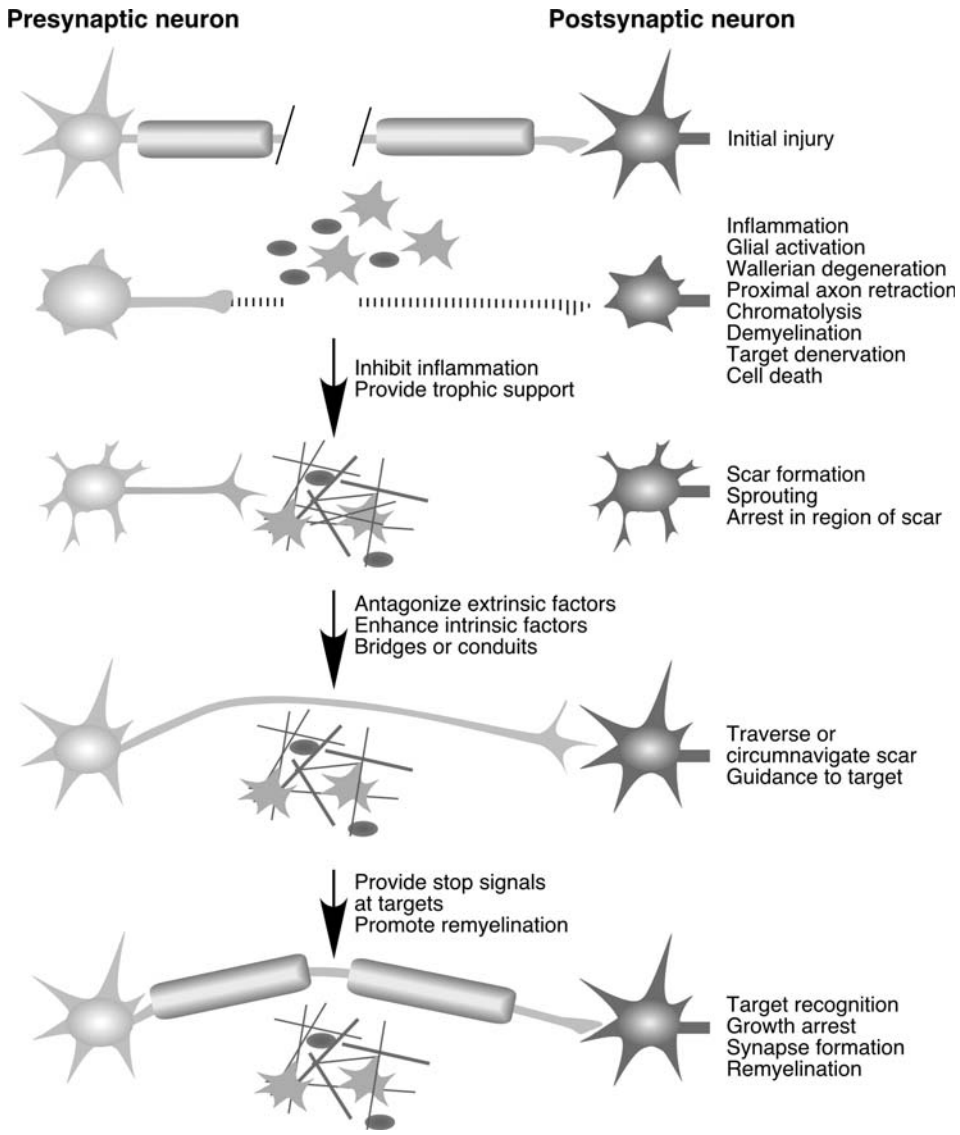

FIGURE 2. Response of the nervous system to injury involves distinct stages. Events occurring at each stage are listed at the right. Possible interventions designed to promote progression to the next stage are given adjacent to the arrows. Following initial injury, the damaged axon rapidly degenerates distal to the lesion. The proximal axon retracts from the injury site somewhat and becomes demyelinated. Cell bodies of damaged neurons swell (chromatolysis) and damaged neurons often undergo apoptosis. There is infiltration of inflammatory cells and activated glia into the injury site. Inhibiting inflammation and providing trophic support for damaged neurons at this stage can greatly enhance functional recovery by limiting cell death and scar formation. Following the acute phase of injury, surviving neurons initiate sprouts and re-extend toward the region of injury. Growth cones arrest upon encountering the glial scar associated with the injury site. At this stage, manipulations designed either to decrease scar-mediated inhibition of regeneration, to stimulate the intrinsic regenerative capability of neurons or to provide bridges or conduits that traverse the scar can improve functional recovery. Once growth cones have extended beyond the region of injury, they must be correctly guided to their original targets and must recognize those targets appropriately (i.e., arrest growth and reestablish synaptic contacts). Regenerated axons must be remyelinated to fully restore normal function. Providing stop signals at targets and introducing factors or cells that promote remyelination can improve functional recovery at this stage.

The current understanding of extrinsic environmental CNS factors, intrinsic changes in adult CNS neurons that limit their regenerative potential, and the possible mechanisms for replacing neurons lost to injury or disease will be discussed in turn below.

Extrinsic Factors: The Importance of the Glial Scar

During embryonic development, the nervous system expresses a plethora of molecules that promote the survival and

differentiation of neurons (see Chapters 5, 9, 11). Many of these molecules are further believed to play a role in guiding growth cones to their appropriate targets, enabling them to recognize those targets and to respond by ceasing outgrowth and initiating synapse formation (see Chapter 1). Generally, the expression of molecules that promote neuronal survival, outgrowth, and synapse formation declines over developmental time to low levels in the adult. Consequently, the environment of the adult CNS is believed to be relatively nonpermissive for the generation of new neurons, for the establishment of new long-distance projections and even, perhaps, for the large-scale establishment of new synapses.

The generally poor environment of the adult CNS takes a rapid turn for the worse following injury. In response to CNS injury, a large number of factors that are not normally expressed in the mature CNS are induced in regions of injury. Many of these factors are produced by astrocytes and microglia that are activated in response to injury. Activated glial cells, in particular astrocytes, migrate into the region of injury and produce a wide range of factors that influence regenerating neurons (Table 1). Ultimately, the injury site transforms into a scar composed of dense glial networks and a complex ECM (Fig. 2). Recent data strongly suggests that the glial scar is critical to regeneration failure. When labeled sensory neurons are transplanted into degenerating white matter tracts rostral to a spinal lesion, the transplanted neurons are capable of extensive and rapid regeneration (behavior that is never normally observed from sensory neurons in the injured CNS). This regeneration abruptly ceases once the growth cones encounter the glial scar (Davies *et al*., 1999). In the region of injury, regenerating growth cones arrest and assume characteristic "dystrophic" morphology (Fig. 3), originally described by Cajal over 100 years ago (Ramon y Cajal, 1991). These striking observations strongly suggest that, at least for sensory neurons, factors associated with the regions of scaring, rather than factors associated with degenerating white matter tracts are the critical components of regenerative failure.

Scar-associated molecules can have both positive and negative effects on growth cone extension *in vitro* (Table 2, Fig. 4). While it is important to understand the functions that specific molecules are *capable* of mediating under well-controlled, experimental circumstances, it is equally important to appreciate that the relationship between regeneration failure and the function(s) of scar-associated molecules is unlikely to be simple (Fig. 5). Importantly, molecules present in CNS scars that *do not function as intrinsically negative regulators* of neurite extension can contribute to regeneration failure. Factors that permit cell adhesion as well as those that prevent it can both contribute to regeneration failure by establishing an attachment state that is not conducive to growth cone motility (Fig. 5A). Mechanical barriers composed *entirely* of permissive molecules can inhibit regeneration by physically blocking the re-extension of axons. Trophic and growth factors may promote neuronal survival, and yet contribute to regenerative failure by stimulating the expression of negative growth regulators or by arresting growth cones in regions of high trophic support (Fig. 5B). Lastly, molecules with intrinsically negative functions may directly suppress growth cone migration. The complex mechanisms likely to underlie regeneration failure and the ways in which specific molecules may contribute to these mechanisms are considered below.

Positive Regulators: The Dual Role of Cell Adhesion

Molecules with a positive influence on growth cone extension fall into three general classes: Those that *permit* (i.e., allow) extension by interacting with the cytoskeletal machinery underlying growth cone migration; those that *promote* (i.e., encourage) extension by stimulating growth cone migration without directly mediating motility; and those that do not directly interact with growth cone receptors but *enhance* outgrowth by modulating the function of other positive factors (Table 2, Fig. 4). Many discussions of growth cone guidance and regeneration make a further distinction between molecules that are *instructive* (i.e., those capable of guiding the direction of growth cone extension) and those that are "merely" permissive, promoting, or enhancing. It is important to recognize, however, that *all* growth-influential molecules (both positive and negative factors) can be instructive if they are spatially distributed in a manner that directs growth cone extension. For example, sensory growth cones are robustly instructed to extend on low concentrations of laminin when confronted with alternating lanes containing different laminin concentrations (Fig. 6A). Under some circumstances, permissive molecules may be avoided for a more "preferred" permissive molecule. For example, growth cones turn away from borders between laminin and fibronectin (Fig. 6B), although both molecules are strongly permissive for neurite extension (Gomez and Letourneau, 1994). Thus, growth cone preference and growth cone turning indicate that specific molecules *can be* instructive under some circumstances, yet such behaviors do not necessarily provide information regarding the *intrinsic functions* of guidance molecules or how (in general) they influence growth cone behavior. Simple experimental criteria, such as whether receptors are expressed and whether those receptors mediate motility (Table 2), define molecular function far more accurately than do observations of growth cone behavior under limited experimental situations.

Many of the factors expressed in regions of CNS injury are considered permissive factors for neuronal migration and axon extension (see Chapters 8 and 9), yet regeneration invariably aborts precisely in regions of scaring where expression of growth permissive molecules is highest. Regeneration failure, despite the expression of positive growth regulators, may simply reflect the preponderance of negative regulators present in the same region (Table 1). Alternatively, permissive factors may themselves contribute to regenerative failure due to their ability to promote strong cell adhesion when present at high concentrations (Fig. 5A).

For nonneuronal cells, both theoretical calculations and empirical data indicate that cell migration only occurs over a narrow range of matrix concentrations, where cells are adhered strongly enough to generate traction, without being so strongly adhered that they are unable to change position (Palecek *et al*.,

TABLE 1. Growth-Influential Molecules That Have Altered Expression Following CNS Injury

Class	Name	Function	GAGs	Growth factors	Molecular interactions (partial list)
Proteoglycan					
Decorin/SLRP	Biglycan	Pro	CS/DS	TGFβ	
	Decorin	B	CS	TGFβ	Collagen, fibronectin, thrombospondin
Hyalectin/lectican	Aggrecan	R/B	CS/KS		
	Brevican[a]	B	CS		
	Neurocan	B/T	CS	bFGF	Cadherins, integrins, galactosyl transferase, tenascin-C, NCAM, tenascin-R, L1, TAG-1, contactin
	Versican[b]	B	CS		
Transmembrane	NG2	B	CS		
	Neuroglycan[c]	B	CS		
	RPTPβ[d]	B/T	CS/KS	bFGF	Tenascin-C, tenascin-R, NCAM, L1, TAG-1, contactin
Other	Appican[e]	Per/T	CS		Laminin
	Perlecan	Per/T	HS/CS	TGFβ FGF	Fibronectin, laminin, tenascin
	Phosphacan[d]	B/T		bFGF	Tenascin-C, tenascin-R, NCAM, L1, TAG-1, contactin
Non-proteoglycan					
	Collagen I, collagen IV	Per			SPARC, Decorin, Fibronectin, integrins
	Fibronectin	Per			Collagen, perlecan, integrins
	Laminin	Per			Entactin/nidogen, integrins
	SC1/SPARC	B		PDGF, VEGF bFGF, TGFβ	Thrombospondin, vitronectin, entactin/nidogen, collagens
	Semaphorin	C			
	Tenascin-C	B			Perlecan, phosphacan, neurocan, integrins
	Tenascin-R	B			Perlecan, phosphacan, neurocan, RPTPβ, integrins
Growth and trophic					
	Neurotrophin	Pro			
	CNTF, FGF	Pro			
	TGFβ	Pro			
	Cytokines	?			
Cell-associated					
	L1	Per			Phosphacan, neurocan, integrins, RPTPβ
	NCAM	Per			Phosphacan, neurocan, RPTPβ
	Eph-Ephrins	C/Pro			
	Trks	Pro			
Myelin-associated					
	MAG	C			
	Nogo	C			

Note: Molecules that have been molecularly cloned are considered. Functions proposed correspond to Table 2; Pro = promoting, Per = permissive, T = trapping, B = blocking, C = collapsing, R = repressing.

[a]BEHAB is a cleavage product representing approximately the N-terminal half of Brevican.

[b]GHAP is a proteolytic fragment of Versican.

[c]CALEB is likely to be the chick homolog of rat neuroglycan.

[d]Phosphacan, also known as DSD-1 and 6B4, represents the cleaved ectodomain of RPTPβ.

[e]The core protein of Appican is a splice variant of amyloid precursor protein (APP). Appican exists in transmembrane and secreted forms.

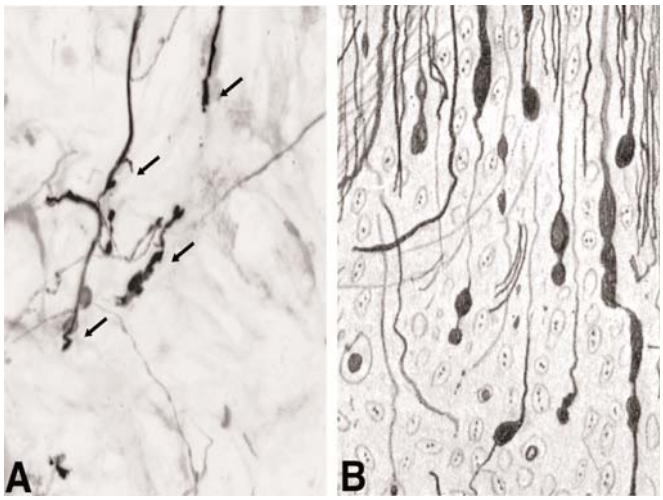

FIGURE 3. Axonal regeneration in the injured CNS is robust through degenerating white matter tracts, yet fails at the site of injury. (A) Darkly labeled, GFP-expressing sensory neurons transplanted into degenerating white matter tracts rostral to a spinal lesion regenerate robustly (1 mm/day), but form dystrophic endings (arrows) at the site of injury (S. Davies, unpublished image; Davies *et al*., 1999). Dim background staining reflects labeling for activated astrocytes (GFAP) and scar-associated CSPGs. (B) Similar dystrophic endings ("terminal end bulbs") of endogenous spinal neurons were initially described at the site of adult CNS injury by Cajal (Ramon y Cajal, 1991).

1997, 1999). At high concentrations of adhesive molecules, cells become "trapped" or "stalled" by the strong attachments they establish with the substratum (Table 2; Fig. 5A). Surprisingly, this well-established relationship between motility and adhesion has not been rigorously applied to the study of growth-cone migration.

Embryonic neurons extending on the molecule laminin may be an exception to the general rule that only intermediate levels of adhesive proteins will support motility. Growth cones of embryonic neurons efficiently migrate on laminin concentrations that vary over several orders of magnitude (McKenna and Raper, 1988; Buettner and Pittman, 1991; Condic and Letourneau, 1997). The ability of neurons to migrate over a wide range of laminin concentrations is due to an unusual regulation of neuronal receptors for laminin (Condic and Letourneau, 1997). Laminin receptors are downregulated in response to high laminin concentrations, thereby reducing adhesion and allowing an intermediate level of attachment to be maintained over a wide range of ligand concentrations.

While embryonic neurons are able to compensate for a wide range of laminin concentrations, the response of growth cones to other molecules that utilize different receptors is unknown. There is evidence that the receptor molecule L1 (also known as Ng-CAM) can be efficiently removed from the surface of embryonic growth cones (Kamiguchi *et al*., 1998; Kamiguchi and Lemmon, 2000; Long *et al*., 2001), but whether the absolute levels of L1 compensate for availability of ligand to maintain a constant level of L1-mediated attachment is unknown. Nothing is known about the regulation of other receptors that promote neuronal adhesion to components of scar matrix. Moreover, very little is known about the ability of *adult neurons* to regulate receptor levels in response to the molecular composition of the environment.

Whether the adhesive molecules expressed in regions of scaring promote or inhibit regeneration is unclear, although both functions are certainly possible. For example, appican is a chondroitin sulfate proteoglycan (CSPG) that contains an alternatively spliced version of the amyloid precursor protein as its core (Table 1). Appican expression increases following brain damage (Salinero *et al*., 1998) and is also increased in the brains of patients with Alzheimer's disease (Salinero *et al*., 1998, 2000). *In vitro* studies indicate that appican acts as a strongly adhesive matrix protein that promotes neurite extension at low concentrations (Coulson *et al*., 1997; Wu *et al*., 1997). In patients with Alzheimer's disease, appican has been proposed to trap growth cones during the formation of plaques and neural tangles (Coulson *et al*., 1997; Wu *et al*., 1997; Salinero *et al*., 1998, 2000). Whether appican plays a similar role in regeneration failure is unknown.

Mechanical Barriers: Stability and Crosslinking of the Scar Matrix

Regeneration failure at the site of injury may be strongly influenced by the physical characteristics of the scar, as well as by its molecular composition. For example, several forms of collagen are upregulated at CNS injury sites and become structurally organized into a basal lamina (BL) surrounding the wound. Most collagens are permissive molecules that mediate the extension of neurites in culture. Nonetheless, recent work suggests the collagen-BL constitutes a physical barrier to regeneration following injury. Preventing formation of the scar-associated BL reduces the expression of a number of positive (permissive, promoting, and enhancing) molecules in the region of injury and, counterintuitively, improves regeneration across the injury site (Stichel *et al*., 1999). This finding is controversial, given that other groups have reported no correlation between the formation of a collagen BL and regeneration failure (Weidner *et al*., 1999; Joosten *et al*., 2000), a discrepancy that may be due to anatomical differences between brain and spinal cord (Hermanns *et al*., 2001). These experiments strongly suggest, however, that structural aspects of the scar matrix affect the ability of neurons to regenerate through the region of injury, *independent* of the positive or negative functions mediated by the molecules composing those structures.

The ability to structurally organize the extracellular environment of the scar is not restricted to collagens. A large number of molecules expressed in regions of injury have complex interactions with other scar-associated molecules (Table 1). Structurally diverse molecules such as tenascins, perlecan, appican, neurocan, phosphacan, and decorin all exhibit high-affinity interactions with other scar components as well as with a variety of neuronal receptors that are themselves upregulated following injury (reviewed in Condic and Lemons, 2002). The role of such complex molecular interactions in regenerative failure is poorly understood. Nonetheless, it seems likely that expression of such a large number of highly interactive molecules in regions of

TABLE 2. Experimentally Distinguishing Functions for "X" and Its Receptor "R_x"

Function	Definition	Example	Requires that	Criteria			Mechanisms[c]
				R_x	A/S[a]	2°[b]	
Positive							
Permissive	X supports extension	Laminin	• X is substratum-bound • R_x interacts with cytoskeleton to generate force and motility	Yes	Yes	No	R_x interacts with cytoskeleton or recruits receptors that do
Promoting	Extension is improved by X; X is not permissive	NGF	• R_x enhances extension without directly mediating migration or attachment	Yes	No	Yes	R_x enhances actin polymerization or enhances function of permissive pathway
Enhancing	Extension is improved by X; R_x is not expressed	Nidogen/ entactin	• X neither promotes nor permits extension	No	No	Yes	X changes function of permissive or promoting factors or their receptors
Negative							
Collapsing	X induces growth cone collapse	Semaphorin	• X neither promotes nor permits extension • R_x induces de-adhesion and collapse	Yes	No	No	R_x depolymerizes actin or antagonizes permissive receptor function
Repressing/ silencing	X blocks extension without inducing collapse	Aggrecan (?)	• X neither promotes nor permits extension	Yes	No	Yes	R_x inhibits the signaling pathway downstream from a permissive or promoting receptor
Blocking	X blocks extension or induces collapse	CSPGs (?)	• X neither promotes nor permits extension	No	No	Yes	X changes the function of permissive or promoting factors
Trapping/ stalling	High [X] blocks extension without collapse	Appican (?)	• X is permissive at low concentrations	Yes	Yes	No	R_x mediates strong adhesion and does not desensitize to high [X]
Neutral							
Neutral	No response to X	Silicon	• R_x does not exist or is not expressed	No	No	n/a	Neurons do not express R_x
Nonpermissive	No neurites form on X	NGF	• R_x does not support neurite extension	Yes	No	No	R_x does not interact with cytoskeleton
Nonpromoting	X does not enhance extension	Substance P	• R_x does not alter neurite extension	Yes	No	No	R_x does not affect motility pathways

Notes:

[a]Promotes adhesion and spreading.

[b]Does the effect on neurite extension require a specific secondary factor?

[c]Possible mechanisms are not intended to provide an exhaustive list, but rather to illustrate how functional terms *limit* the possible mechanisms and inform the direction of future experiments.

injury will contribute to the stability and crosslinking of the scar matrix in a manner that may impede the advance of axons.

Adding to the dense structural environment provided by scar-associated extracellular molecules is the dense accumulation of glial cells in regions of injury. Glia are the major source of the scar matrix and are found in great numbers associated with injury sites. Evidence suggests that both astrocytes and microglia actively migrate into regions of injury in response to cytokines and chemokines released during the inflammatory response (Goldberg and Barres, 2000; Allan and Rothwell, 2001). Glial densities remain high in the region of scarring for long periods following injury, perhaps indefinitely. Whether or not high glial densities themselves constitute a mechanical barrier to regeneration is difficult to determine, although some evidence suggests that high densities of astrocytes do not, in and of themselves, constitute a barrier to the advance of regenerating axons (Davies *et al*., 1999).

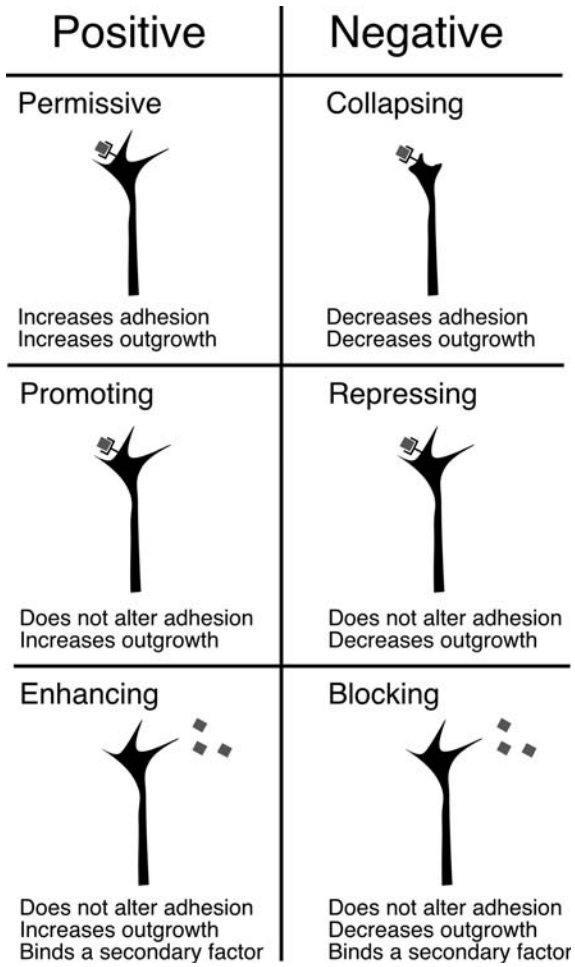

FIGURE 4. Positive and negative functions of growth influential molecules (see also Table 2). Molecules with permissive functions work through neuronal receptors to "permit" or allow growth cone migration by mediating attachment and interacting with cytoskeletal components required for cell motility. Collapsing molecules function through cellular receptors to oppose the activity of permissive factors. Molecules with collapsing function decrease cell attachment and suppress motility. Promoting molecules do not mediate cell adhesion directly, but activation of their receptors stimulates the rate of neurite extension. Analogously, repressing factors do not alter cell adhesion, but decrease motility without inducing collapse. Finally, enhancing and blocking molecules do not directly interact with neuronal receptors, but bind to secondary factors to increase (i.e., enhance) or decrease (i.e., block) neurite extension.

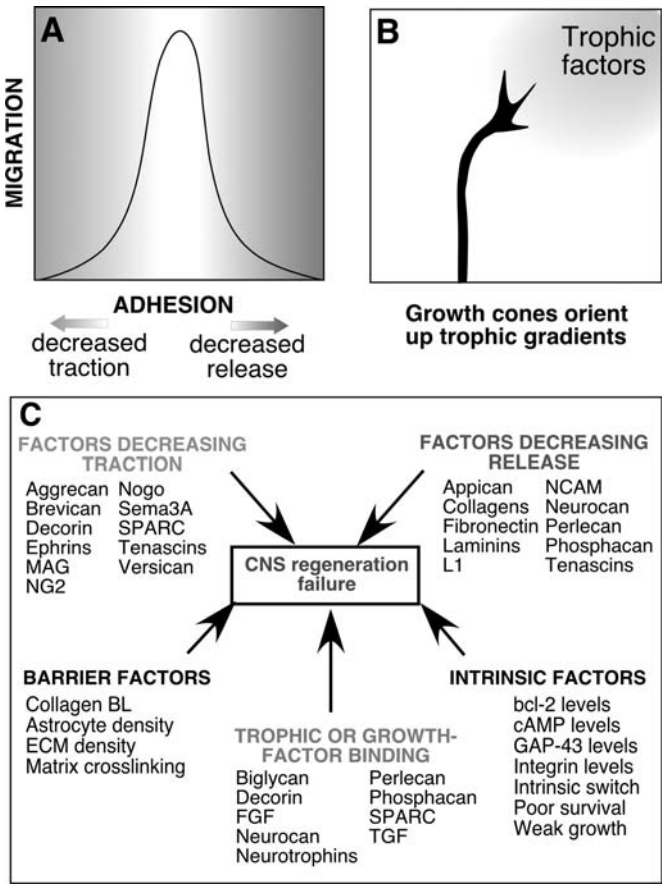

FIGURE 5. Molecules with both positive and negative functions may prevent migration of growth cones in regions of injury. (A) For nonneuronal cells, adhesion to the substratum increases linearly with the amount of bound matrix protein, yet peak motility is only supported by a narrow range of adhesion states (Palecek *et al*., 1997, 1999). Low levels of attachment/matrix inhibit motility by reducing traction while high levels of attachment/matrix inhibit motility by reducing the ability of cells to release from the substratum. (B) Trophic and growth factors can function as attractive signals for growth cones (McFarlane, 2000). High concentration of such factors in regions of injury may attract and subsequently retain regenerating axons at the scar. (C) Growth cone motility may be reduced by more than a single mechanism following CNS injury. Cellular mechanisms and specific molecules that may contribute to those mechanisms are listed. Factors providing mechanical barriers to migration may physically block growth cone advance. Factors that antagonize the function of cell-matrix receptors or directly inducing growth cone collapse will prevent migration by reducing traction. Extracellular matrix molecules and receptors that increase growth cone adhesion will prevent migration by preventing release from the substratum. High levels of neurotrophic molecules or molecules that recruit growth factors may retain growth cones in regions of injury due to high trophic support. Finally, cell autonomous limitations to regeneration may prevent CNS neurons from regenerating efficiently, independent of environmental factors.

Trophic Molecules: Effects on Neurons and Glia

The role of trophic molecules in regeneration and regeneration failure is far from simple. Adult CNS neurons are highly sensitive to insult and die in large numbers following even mild injury (Allan and Rothwell, 2001). Extensive evidence suggests that providing trophic support can improve the survival of neurons following CNS injury, and in some cases, stimulate regeneration as well (Goldberg and Barres, 2000). Both neurotrophins and other growth factors, such as TGFβ and FGF, can promote neuronal survival and regeneration. However, in many cases, regeneration observed following exogenous application of trophic factors is disorganized and perhaps even maladaptive. Animals expressing NT3 in the spinal cord exhibit extensive invasion of sensory afferents into the dorsal horn following dorsal root crush. Yet many of these animals become excessively sensitive to painful stimuli (Romero *et al*., 2000), suggesting

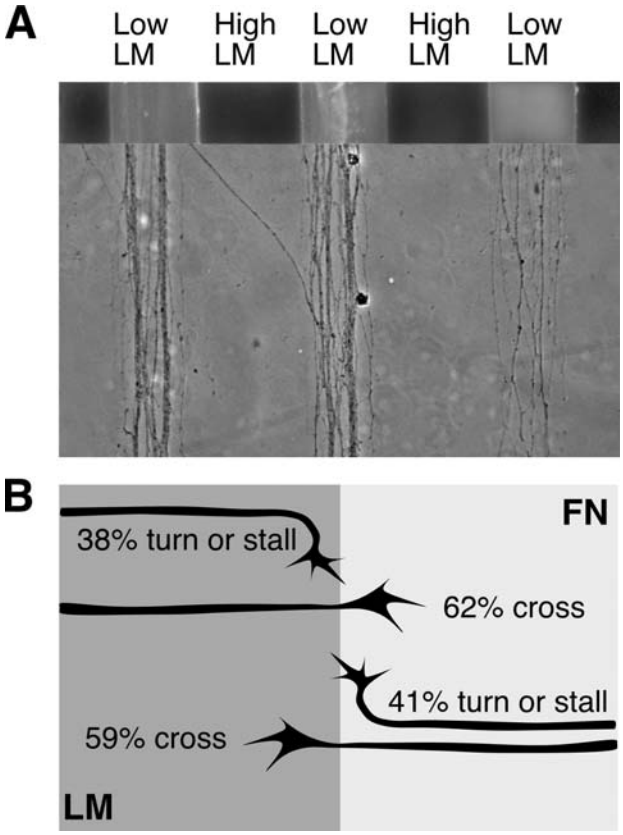

FIGURE 6. Growth-influential molecules are "instructive" for growth cone guidance if they are presented in discontinuous or graded distributions. (A) Sensory neurons preferentially elect to extend on lanes containing low levels of laminin and avoid lanes containing high levels of the same molecule (M. Condic, unpublished). (B) Some sensory growth cones will turn away from a border of two permissive molecules (Laminin and Fibronectin), although neither molecule is a negative regulator of sensory neurite extension (data taken from Gomez and Letourneau, 1994).

that the strong but diffuse response to exogenously supplied neurotrophins may induce the formation of inappropriate or inordinate synaptic contacts.

There is evidence for endogenous upregulation of trophic factors and growth factors in regions of CNS injury (Goldberg and Barres, 2000; Allan and Rothwell, 2001). The contribution of these factors to regeneration is likely to be complex. Neurotrophins and other growth factors can clearly promote both neuronal survival and neuronal regeneration, yet the neuroprotective effect of growth factors is often transient or specific for only subclasses of neurons. In some cases, neurotrophins may actually potentiate neural injury (Behrens *et al*., 1999). The same factors that promote neuronal survival and regeneration also contribute to the activation of glia and the subsequent formation of the glial scar. Whether the positive effects of growth factors on neurons can be experimentally separated from the deleterious effects these same factors have in promoting scar formation is not known.

A number of molecules expressed at injury sites are able to bind growth factors with high affinity (Table 1). Expression of growth-factor binding proteins is likely to increase the localization of these factors to regions of injury. However, it is not clear whether increased growth factor function will have a positive or a negative effect on regeneration. Moreover, growth factor expression does not necessarily translate into increased activity. For example, the scar-associated proteoglycan decorin binds and inactivates TGFβ (Ruoslahti *et al*., 1992; Hildebrand *et al*., 1994). Exogenous application of decorin (presumably, inactivating TGFβ) attenuates scar formation (TGFβ induces many scar-associated molecules) and enhances regeneration (Logan *et al*., 1999), suggesting that TGFβ has a net negative effect on regeneration.

A final role for scar-associated growth factors may be chemoattraction (Fig. 5B). Numerous studies have indicated that growth cones in culture will orient up growth factor gradients (McFarlane, 2000). Localization of such factors to the region of injury may induce arrest of growth cones at the high point of such chemoattractive gradients. Indeed, neurotrophic factors are believed to contribute to the arrest and terminal differentiation of growth cones in their appropriate target tissues during development (Chapter 10). Whether high expression of growth factors and trophic factors contribute to growth cone arrest is currently unknown.

Negative Regulators: Collapse, Repression, and Blocking

There are numerous proteins expressed in the CNS following injury that are believed to negatively regulate axonal regeneration (Tables 1, 2). Most of these molecules can be assigned to one of three categories: CSPGs, non-proteoglycan molecules, and factors associated with myelin. CSPGs are of particular interest due to their highly localized expression in regions of CNS scarring, their roles during development, and their influence on neuronal growth in culture (Bovolenta and Fernaud-Espinosa, 2000; Asher *et al*., 2001; Condic and Lemons, 2002). Myelin-associated factors are expressed generally in the CNS and do not appear to play a critical role in regeneration; regenerating axons extend up to 1 mm/day in CNS regions expressing high concentrations of myelin-associated factors (Davies *et al*., 1999).

Molecules that have a negative influence on growth cones can function in more than one manner (Table 2; Fig. 5C). As noted above, molecules that permit neurite extension at low concentrations can potentially suppress extension at high concentrations, due to trapping or stalling (Fig. 5A). Whether this form of negative regulation applies to neurons has not been tested, but appears likely given the precedent from non-neural cells.

Molecules can also have a negative impact on growth cone extension by inducing growth cone *collapse* (Table 2, Fig. 4). The morphology of dystrophic endings *in vivo* (Fig. 3) is similar to that of growth cones undergoing collapse and retraction *in vitro* (see Chapter 9), suggesting that factors present in regions of injury may induce growth cone collapse. Collapsing molecules directly promote cytoskeletal depolymerization and release of growth cones from the substratum. Collapsing molecules are not dependent on the context or on the presence of specific positive factors to mediate their effects on growth cones.

For example, the Eph-family tyrosine kinase receptors are a large class of cell surface molecules that interact with both cell-surface and matrix-associated ephrin ligands (see Chapter 9). In development, Eph–ephrin interactions most commonly mediate inhibitory or repulsive growth cone responses, independent of the substratum on which neurites are extending (reviewed in Wilkinson, 2001). Recent work has shown that several Eph receptors, including EphB3 (Miranda *et al*., 1999), EphA4, EphA5, and EphB2 (Moreno-Flores and Wandosell, 1999) are upregulated following either traumatic or excitotoxic injury to the CNS, suggesting that these collapsing factors may contribute to regenerative failure.

Similar to ephrins, the collapsing factor semaphorin 3A is upregulated following CNS injury in regions of scarring (Pasterkamp *et al*., 1998, 1999). Semaphorins are a large family of cell surface and secreted proteins that are believed to act as repulsive and/or stop signals in neural development (Nakamura *et al*., 2000; Pasterkamp and Verhaagen, 2001). Thus far, there is no direct evidence for a role of semaphorins in CNS regenerative failure, yet by analogy to the role of semaphorins in development (Nakamura *et al*., 2000) and based on the ability of semaphorins to inhibit the regeneration of adult sensory neurons in culture (Tanelian *et al*., 1997), semaphorins could readily contribute to adult regenerative failure by collapsing growth cones in regions of injury.

Negative regulators can also act *via* receptors to *repress* neurite outgrowth without inducing collapse (Table 2, Fig. 4). Repressing factors do not directly antagonize the function of receptors that mediate adhesion, but rather inhibit the signaling pathway downstream of such receptors such that growth cone motility is suppressed. For example, growth cones extending on laminin arrest but do not collapse when they encounter the CSPG aggrecan (Snow *et al*., 1994; Challacombe *et al*., 1996, 1997). Aggrecan induces a rapid and sustained increase in growth cone calcium, suggesting the effects of this molecule are mediated by an uncharacterized neuronal receptor (Snow *et al*., 1994). Thus, aggrecan does not disrupt growth cone attachment to laminin (although it can reduce whole cell attachment; Condic *et al*., 1999), but suppresses growth cone motility that normally results from such attachment.

In contrast to factors that have an intrinsic negative effect on growth cones, *blocking* molecules act predominantly by suppressing the positive effects of growth-promoting or growth-permissive molecules (Table 2; Fig. 4). Molecules with blocking functions have often been described as "inhibitory," because such factors inhibit the function of something else. Yet, the term "inhibitory" is inherently imprecise as a descriptor of molecular function, due to the fact that "inhibition" can describe *either* the effect of a molecule on the rate of neurite extension *or* the impact of one molecule on the function of another. *All* molecules that slow or abolish neurite extension (i.e., all negative regulators) inhibit outgrowth, yet *only* molecules that antagonize the function of other factors have inhibitory (i.e., blocking) molecular function.

Molecules that alter neurite outgrowth *via* a blocking mechanism would be predicted to have *no direct effect on neurons when presented alone* (Table 2). Some of the CSPGs found in regions of injury are believed to inhibit growth cone extension by blocking the effects of growth-permissive molecules present in the CNS. Importantly, molecules that block neurite extension are entirely dependent on the context in which they are encountered and will only inhibit the positive functions of specific promoting or permissive molecules.

It is interesting to note that under this definition, blocking factors need not exclusively mediate negative effects on growth cones. While thus far the growth cone equivalent of a "derepressor" (i.e., an antagonist of a molecule that normally mediates a negative function) has not yet been described, it is possible that both positive and negative blocking molecules exist.

Molecules with More than One Function

A further complication in the study of regeneration failure is that a large number of growth-influential factors can have more than a single effect on neurite extension (Fig. 7). Growth-influential molecules can work through more than one receptor to mediate opposing effects on neurite extension (Fig. 7A). For example, netrin-1 can act through the receptor DCC to mediate attraction (Vielmetter *et al*., 1994; Keino-Masu *et al*., 1996; Kolodziej *et al*., 1996) and through Unc-5 to mediate repulsion (Hedgecock *et al*., 1990; Leonardo *et al*., 1997; Colavita and Culotti, 1998). Differential expression of these receptors in particular populations of neurons or in the same neurons at different times results in netrin-1 having widely varying effects on growth cones. For example, commissural neurons of the spinal cord (that normally extend toward a source of netrin-1 during development) are attracted to netrin-1 *in vitro* (Kennedy *et al*., 1994) while trochlear motor axons (that normally extend down a netrin-1 gradient) are repelled (Colamarino and Tessier-Lavigne, 1995). These findings indicate that subpopulations of CNS neurons are likely to have widely differing responses to the same scar-associated molecule, depending on which receptors are expressed.

In addition, the response of a neuron to the same factor can be modified by the internal state of the growth cone (see Chapter 9), most notably the levels of cyclic nucleotides (Fig. 7B; reviewed in McFarlane, 2000). The fact that the state of the neuron can critically alter its response suggests that the recent history of the growth cone can influence behavior. Indeed, while commissural axons of the spinal cord are initially attracted to netrin-1, they lose responsiveness to this molecule after having crossed the ventral midline (Shirasaki *et al*., 1998), indicating that recent encounters can alter growth cone response. Similarly, sensory neurites extending on fibronectin will accelerate and change morphology in response to a single encounter with a laminin-coated bead (Kuhn *et al*., 1995), yet a second encounter with laminin within a narrow time window following the first causes the growth cone to completely arrest (Diefenbach *et al*., 2000). The differing response of growth cones to the *same molecule* depending on the recent history of the growth cone argues against a simple view of any specific molecule as having a strictly positive or negative function.

The effects of specific molecules on regeneration can be further complicated by the ability of one factor to modify or even eliminate the response of a growth cone to a second molecule

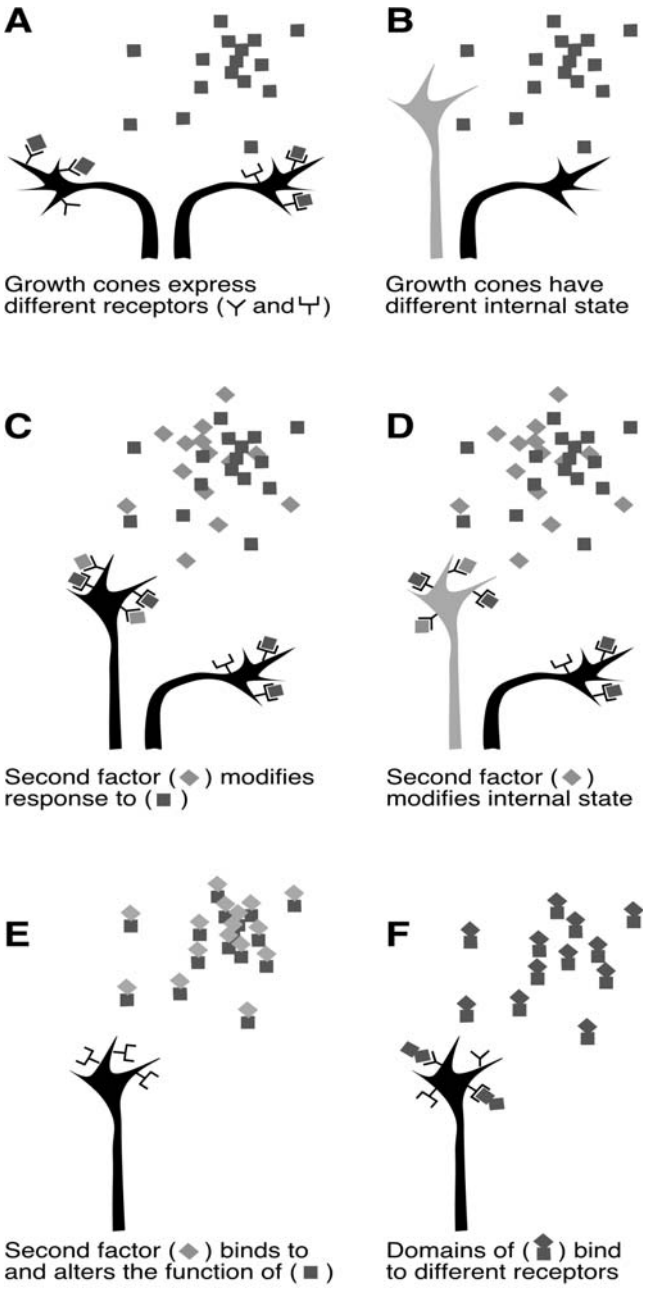

FIGURE 7. Molecules that influence regeneration can have more than a single function. (A) Different classes of neurons can express receptors that mediate different responses to the same factor. (B) The internal state of the growth cone at the time the factor is encountered can alter growth cone response. (C) Secondary factors can modify the response of the growth cone through interaction of distinct receptors or their downstream signaling pathways. Examples include "silencing" of growth cone response to netrin-1 by Robo-slit signaling or inactivation of integrin signaling as a consequence of Eph–ephrin signaling. (D) Secondary factors can modify the internal state of the growth cone, thereby altering growth cone response, without a direct interaction between the receptors. Promoting and repressing factors work in this manner. (E) Secondary factors can modify the function of other growth influential factors. Enhancers, such as nidogen/entactin and blockers (possibly scar-associated CSPGs), work in this manner. (F) Single molecules can possess different functional domains that interact with distinct receptors.

(Figs. 7C–E). Such "context-dependent" effects can be either transient (i.e., dependent on the presence or absence of the modifying factor) or long lasting, thereby contributing to the "history-dependent" effects described above. In addition to factors that act as classical agonists or antagonists (i.e., activating or inactivating ligands for the same receptor) there are at least three ways in which secondary factors can alter a growth cone's response. First, direct interactions between the receptors or signaling pathways downstream from different molecules can modify the response a growth cone shows to either molecule in isolation (Fig. 7C). For example, a positive turning response to netrin-1 can be "silenced" via a physical interaction of the netrin-receptor DCC and an unrelated receptor robo, when robo is bound by its ligand, the extracellular protein slit (Stein and Tessier-Lavigne, 2001). In this situation, presence or absence of slit (itself a negative guidance cue) can radically alter the effects of netrin-1 on growth cone behavior.

In addition to mechanisms that depend directly or indirectly on receptor–receptor interactions, growth cone response can be altered by secondary factors that alter the internal state of the growth cone (Fig. 7D). For example, retinal growth cones are attracted to a soluble gradient of netrin-1 when they are extending on a fibronectin substratum, but repelled by the same gradient when extending on laminin (Hopker *et al*., 1999), despite the absence of evidence for a physical interaction between receptors for netrin and receptors for either laminin or fibronectin. Similarly, the scar-associated molecule versican strongly inhibits the extension of sensory neurites on laminin (Schmalfeldt *et al*., 2000), but does not inhibit regeneration on fibronectin (Braunewell *et al*., 1995), despite the lack of evidence for a direct interaction between versican and either laminin or fibronectin receptors. In these cases, it is likely that independent receptor-ligand pathways alter the internal state of the growth cone, which in turn alters growth cone response (Fig. 7D).

Independent of cellular receptors, factors themselves can interact to modify function in both positive (Table 2, Fig. 4; enhancing) and negative (Table 2, Fig. 4; blocking) ways. The effects of enhancing and blocking factors do not require specific receptors or downstream signaling pathways, but rather occur via direct action on other growth influential molecules (Fig. 7E). The presence or absence of blocking or enhancing factors can alter or reverse the function of specific molecules that in isolation have only a single effect on neurons.

Finally, the effects of some growth-influential molecules are complicated by the multidomain structure of the molecules themselves (Fig. 7F). For example, members of the tenascin gene family have complex molecular structure (Faissner, 1997; Jones and Jones, 2000; Meiners *et al*., 2000) and diverse effects on neurite extension. Tenascins can possess adhesive, counteradhesive, growth-promoting, and inhibitory activities all on the same molecule (Jones and Jones, 2000). Different tenascin domains interact with a wide range of cellular receptors expressed by neurons as well as with a large number of scar-associated molecules (Jones and Jones, 2000). How a particular neuron responds to tenascin will reflect both the receptors expressed by that neuron (Fig. 7F) and the crosslinking of tenascin to other molecules that

may modify (or eliminate) the function of specific tenascin domains (Fig. 7E). Given the diversity of high-affinity interactions tenascin is able to maintain, it is difficult to predict what the functional properties of tenascin would be in a complex molecular environment and what net affect this molecule would have on a neuron expressing multiple tenascin receptors.

Dissecting the Contributions of Specific Molecules to Regeneration Failure

Understanding how specific molecules contribute to regeneration failure clearly goes well beyond a simple matter of understanding the functions those molecules are capable of mediating *in vitro* (Table 2, Fig. 4). While molecular function is clearly relevant to regeneration failure, in complex environments, the net effect of a specific factor can be hard to predict. In light of the multiple ways that molecules can function to prevent regeneration (Table 2; Figs. 4 and 5) and the multiple functions any given molecule can mediate (Fig. 7), sorting out the contribution of individual factors is extraordinarily complicated. While it is clear that the net effect of the scar matrix is to inhibit regeneration, the precise manner in which a specific molecule participates in this net function is hard to discern. Is scar-associated laminin a positive factor whose beneficial effect is masked by the numerous inhibitory molecules present in regions of injury or is it present in sufficiently high concentrations that it acts as a stalling factor, thereby itself contributing to regenerative failure? How can the net effect of a specific molecule in such a complex environment be determined?

One approach has been to generate animals deficient for a molecule believed to contribute to regenerative failure and then challenge those animals with CNS injury. This approach cannot be applied to the majority of molecules believed to play a role in regeneration failure, due to the essential functions these molecules play in development and normal physiology. However, in a small number of cases, genetic knockouts have proven informative. For example, myelin-associated glycoprotein (MAG) is a prominent component of CNS myelin that can induce growth cone collapse when applied to neurons *in vitro* (Li *et al*., 1996). Animals deficient for MAG are viable and show subtle defects in myelin ultrastructure and in axonal conduction velocity (Bartsch, 1996). Surprisingly, animals deficient for MAG show no significant improvement in regeneration following CNS injury (Bartsch *et al*., 1995), suggesting that MAG is not critical to regeneration failure. This conclusion has recently been supported by experiments demonstrating robust regeneration in intact (Davies and Silver, 1998) and degenerating white matter tracts (Davies *et al*., 1999), sites where MAG is present in high concentrations. Thus, while MAG is clearly able to collapse axons *in vitro*, it appears either to play a minor role in regeneration failure or (more likely) to be merely one of a large number of players *in vivo*.

As for all genetic approaches, the interpretation of the MAG knockout experiment is compromised by the fact that it is difficult to know how removal of a major component of myelin may have altered the normal development of the CNS or its response to injury. While proteins such as MAG could play an important role in regenerative failure in wild-type animals, this role may be compensated for by other molecules in animals deficient in MAG. Thus, while genetic ablation indicates that MAG does not make a *critical* contribution to regenerative failure, the actual role of MAG in regeneration failure in wild-type animals is difficult to access from such an experiment.

An alternative approach has been to acutely antagonize the function of specific molecules or classes of molecules either *in vivo* or in an *in vitro* model. This approach has the advantage of studying regeneration failure in a genetically wild-type individual and asking what role does a specific molecule or combination of molecules normally play. For example, the contribution of CSPGs to regeneration failure has been examined using chondroitinase ABC, a glycolytic enzyme that removes chondroitin sulfate side chains from proteoglycan cores and greatly reduces the inhibition mediated by this class of proteins. Both in the cortex (Yick *et al*., 2000; Moon *et al*., 2001) and in the spinal cord (Bradbury *et al*., 2002), chondroitinase treatment significantly improves regeneration. In an *in vitro* model system, McKeon *et al*. (1995) have similarly shown that CSPG associated with CNS scars inhibits axon outgrowth; as CSPG accumulates in the region of injury, axon outgrowth is progressively inhibited. Treatment of the scar-associated matrix with chondroitinase results in a significant increase in neurite extension over scar tissue *in vitro*. Interestingly, co-treatment with both chondroitinase and function-blocking anti-laminin antibodies reverses the growth-promoting effect of chondroitinase alone, suggesting that scar-associated laminin plays a positive role in regeneration that is antagonized by CSPGs co-expressed in regions of injury (McKeon *et al*., 1995).

Acute manipulations allow for the contribution of particular molecules to be accessed, but are often limited by the specificity and reliability of the available reagents. In the example given above, the positive effects of chondroitinase treatment suggest that this class of molecules plays an important role in regeneration failure, yet it is not possible to determine the contribution of specific CSPGs from these experiments. Moreover, it is difficult to interpret negative findings of acute manipulations. Does a failure to improve regeneration indicate that a particular factor is not involved or merely that the manipulation did not sufficiently reduce the function of that factor? As noted above for manipulations of collagen deposition in injury models (Stichel *et al*., 1999; Weidner *et al*., 1999; Joosten *et al*., 2000; Hermanns *et al*., 2001), subtle differences in technique or even anatomical differences between CNS regions can potentially alter the experimental outcome.

Intrinsic Factors

In addition to the critical role of scar-associated factors in regeneration failure, the intrinsic state of neurons can play an important role in regeneration (Figs. 7B, D). The intrinsic ability of neurons to regenerate changes over developmental time and in response to injury itself. Although the cell-autonomous factors controlling (and limiting) adult regeneration are poorly understood, such factors present attractive targets for therapeutic

intervention. To promote the maximum level of regeneration with the lowest level of side effects on undamaged regions of the nervous system, one would ideally like to control both the temporal and spatial extent of the manipulation. In most cases, it is quite difficult to control the effective temporal and spatial extent of manipulations designed to alter the extracellular environment of the CNS. Even with genetic or molecular manipulations, once molecules are secreted into the extracellular space, the time course over which they persist and the distances over which they diffuse are difficult to regulate.

Targeting cell intrinsic factors as a means of promoting adult regeneration presents several technical advantages. Inducible promoters can be used to regulate the temporal expression of intrinsic factors: Turning genes on to promote regeneration and turning them off once regeneration is accomplished. The ability to return adult gene expression to normal adult levels once connections have been reestablished is a strong advantage of approaching CNS regeneration from the perspective of cell-intrinsic factors. In addition, gene expression can be *locally* altered in the region of injury using microinjection techniques. Viral gene-delivery systems can be selected for high neuronal affinity that results in a very minimal spread of the viral agent away from the site of injection. Spatially restricting the manipulation to the region of injury minimizes any unintended effects on undamaged neurons distant from the site of injury. Damaged neurons take up factors from the environment (including viral vectors) more readily than do undamaged cells, an effect that serves to further enhance the "targeting" of gene manipulation to the cells actually affected by the injury. Thus, while relatively little is currently known regarding the contribution of cell-autonomous factors to regeneration failure, such factors are an active area of investigation and attractive targets for therapeutic intervention.

Changes in the Intrinsic Properties of CNS Neurons with Maturation

It is abundantly clear that there are developmental changes in the ability of neurons to regenerate axons. Both retinal (Cohen *et al*., 1986, 1989; Neugebauer and Reichardt, 1991; Bates and Meyer, 1997) and sensory (Sango *et al*., 1993; Golding *et al*., 1999) neurons in culture show decreased rates of axon extension at progressively older stages. Several studies suggest that young neurons transplanted into injured adult CNS tissue show more extensive regeneration than do the adult neurons of the host (Wictorin and Bjorklund, 1992; Nogradi and Vrbova, 1994; Lindvall, 1998; Broude *et al*., 1999; Ito *et al*., 1999). The basis for this age-dependent decline in intrinsic regenerative potential is unknown.

A large number of intrinsic factors that may contribute to regeneration show altered expression over developmental time (reviewed in Caroni, 1997; Rossi *et al*., 1997), but in most cases, the contribution of these factors to adult regeneration failure is unclear. For example, while GAP43 expression is associated with regeneration in some adult neurons (Vaudano *et al*., 1995), many regenerating axons do not express GAP43 (Schreyer and Skene, 1991; Andersen and Schreyer, 1999). Overexpression of GAP43 in transgenic animals does not stimulate adult neuronal regeneration (Buffo *et al*., 1997; Mason *et al*., 2000), while overexpression of GAP-43 in combination with a related protein, CAP-23, does improve adult performance (Bomze *et al*., 2001). Similarly, increased expression of other genes expressed at high levels in embryonic stages but at low levels in adults (e.g., the anti-apoptotic gene *bcl2* [Chen *et al*., 1997] or receptors of the integrin class [Condic, 2001]) can improve the regeneration of adult neurons *in vitro*. Potentially relevant cell-intrinsic factors are not limited to molecules classically associated with axon extension and guidance. For example, as neurons mature, cAMP levels decline, and pharmacologically increasing cAMP can stimulate regeneration both *in vitro* (Cai *et al*., 2001) and *in vivo* (Neumann *et al*., 2002; Qiu *et al*., 2002).

The mechanism underlying the age-dependent decline in regenerative potential is not known. One possibility is that molecules required for efficient regeneration are incompatible with normal adult CNS function and are therefore downregulated at adult stages in order to promote stabile adult CNS function. For example, receptors of the integrin class are the primary receptors for growth-promoting matrix proteins and are expressed at high levels in embryonic neurons during periods of axon extension (Reichardt and Tomaselli, 1991; Letourneau *et al*., 1994). In contrast, adult CNS neurons express low levels of integrin message (Jones and Grooms, 1997; Pinkstaff *et al*., 1999) and the majority of integrin protein expressed in the adult brain is found at synapses (Grotewiel *et al*., 1998; Nishimura *et al*., 1998; Chavis and Westbrook, 2001). Consistent with these observations, the major phenotype associated with loss of integrin function in the adult CNS is a learning defect (Grotewiel *et al*., 1998). These results suggest that integrin expression is constitutively suppressed in the adult CNS and that integrins have distinct functions at adult and embryonic stages. Low levels of integrin protein appear to be required for the maintenance or formation of synaptic connections in the adult. Quite possibly, low levels of integrin expression also serve to explicitly *prevent* the large-scale formation of new neuronal connections in the adult, "crippling" adult neurons, to promote a stable pattern of circuitry in the brain.

An alternative (albeit, not mutually exclusive) possibility is that age-dependent changes in regeneration potential reflect a stable developmental switch from production of axons to production of dendrites (Fig. 8). In this view, molecules required for efficient long-distance regeneration of axons are downregulated once long-distance axonal projections have been established. This switch reflects a normal developmental program that promotes dendrite formation at the expense of axons. Due to the stability of this developmental switch, adult neurons are unable to revert to production of axons following injury, although they are able to produce short, dendrite-like processes. In retina, factors associated with amacrine cell membranes are able to induce a stable shift from production of axons to production of dendrites from retinal ganglion cell neurons (Goldberg *et al*., 2002). Whether such a developmental switch could be reversed or inactivated to promote the regeneration of axons will depend on the precise nature of the switch.

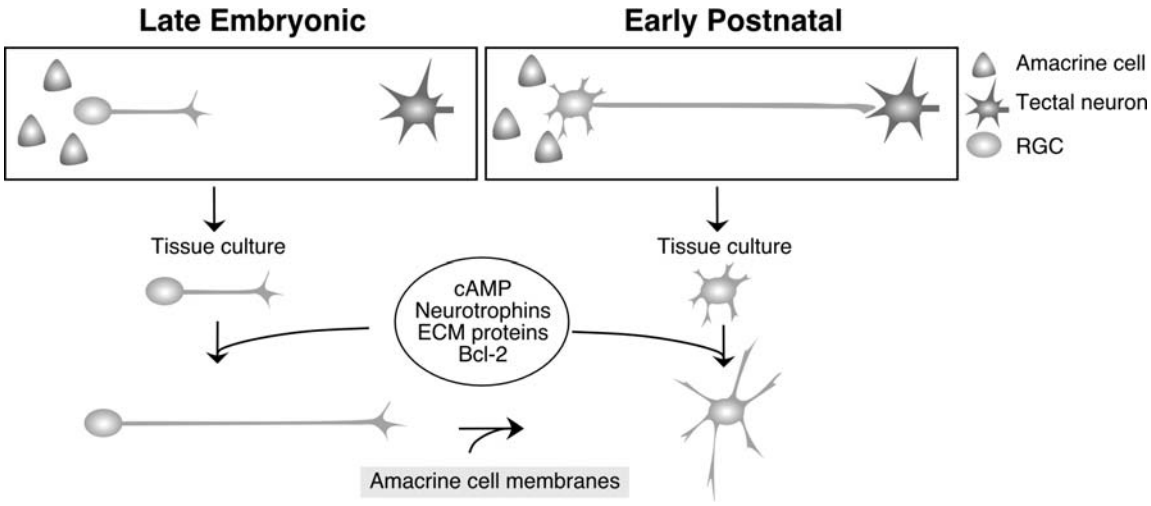

FIGURE 8. Maturing neurons may undergo a cell autonomous switch from production of axons to production of dendrites. Retinal ganglion cells (RGCs) *in vivo* (boxes) extend axons to innervate targets in the brain during late embryonic stages, and extend dendrites during postnatal stages. RGCs placed in tissue culture at embryonic or postnatal stages regenerate processes that are similar to the ones they generate *in vivo*; young neurons re-extend a single axon while older neurons extend multiple short dendrites. Factors that stimulate neurite extension (oval) can increase the length of the regenerated processes, but do alter the axonal vs dendritic nature of the process, suggesting that RGCs have undergone a stable, cell-intrinsic switch from production of axons to production of dendrites. Contact with cell membranes derived from postnatal amacrine cells is sufficient to switch embryonic RGCs to a postnatal pattern of growth in culture, suggesting that amacrine-associated factors may mediate this maturational switch in retina (Goldberg *et al*., 2002). Figure adapted from Condic (2002).

Changes in Intrinsic Properties of CNS Neurons in Response to Injury

Independent of maturational changes in neuronal gene expression, the intrinsic state of adult neurons can be a key factor in CNS regeneration. For example, adult sensory neurons that have sustained a "conditioning" peripheral lesion regenerate more readily into the CNS following dorsal root injury (Neumann and Woolf, 1999). How such conditioning lesions enhance the ability of neurons to regenerate into the CNS is unknown, but it is possible that peripheral injuries indirectly promote expression of genes that are not upregulated in response to CNS injuries (Frostick *et al*., 1998; Terenghi, 1999; Kury *et al*., 2001). For example, activated Schwann cells may supply trophic factors to sensory neurons that are not supplied by activated central glia. Consequently, neurons that have been appropriately "conditioned" may have a distinct state of gene activation that enhances their ability to regenerate.

In the absence of a beneficial conditioning lesion, injured adult CNS neurons exhibit altered patterns of gene expression that can both improve and detract from their ability to regenerate. Following injury, CNS neurons express higher levels of cell adhesion molecules, such as NCAM (Becker *et al*., 2001; Tzeng *et al*., 2001) and L1 (Jung *et al*., 1997), both of which interact with components of the scar matrix as well as with the surfaces of other neurons. The net effect of increased cell-adhesion molecule expression is hard to predict. Enhanced axon–axon interactions may promote regeneration along axon scaffolds. However, increased adhesion to the scar ECM may contribute to regenerative failure by stalling growth cones in the region of injury (Fig. 5A). Adult neurons also upregulate receptors for collapsing factors, including members of the Eph-family (Miranda *et al*., 1999; Moreno-Flores and Wandosell, 1999). Lastly, neurotrophin receptor expression is upregulated following injury, suggesting that the response of neurons to growth factors may be enhanced (Goldberg and Barres, 2000). The effect of such enhanced responsiveness on regeneration is unclear, with some evidence suggesting that neurotrophins may potentiate rather than reduce neuronal injury (Behrens *et al*., 1999).

Adult CNS neurons are as much characterized by their *failure* to respond to injury as by their response. In the PNS, for example, numerous beneficial genes are upregulated in response to injury, including growth-associated molecules, neurotrophin receptors, and matrix receptors (Frostick *et al*., 1998; Yin *et al*., 1998; Terenghi, 1999). In many cases, these genes fail to increase in expression following CNS injury. Whether the failure to adaptively regulate gene expression reflects some suppressing property of the CNS environment or an intrinsic limitation of CNS neurons appears to vary depending on the cell type. For example, injured adult Purkinje neurons *in vivo* fail to upregulate the growth-associated molecule GAP-43 and do not express this gene even when provided with a permissive environment for regeneration (Gianola and Rossi, 2002). In contrast, adult retinal neurons only weakly upregulate GAP-43 *in vivo*, yet respond to permissive environments *in vitro* with a strong upregulation (Meyer *et al*., 1994). While there may not be general rules that apply to *all* CNS neurons, it appears that failure to respond adaptively to injury can contribute to the limited intrinsic regenerative capability of some CNS neurons.

Cell Replacement: Endogenous or Transplanted Neuronal Stem Cells

Following CNS injury, there is extensive death of injured neurons. Replacing neurons lost to injury has long been considered an attractive option for the repair of CNS injury, particularly in light of the superior ability of young transplanted neurons to extend axons in the damaged adult CNS. Attempts to restore CNS function by replacing damaged or dead neurons have taken two general approaches; stimulating the division and differentiation of endogenous neuronal stem cells and transplanting stem cells or their derivatives into the injured CNS.

In most areas of the CNS, new neurons are not born in adult animals. Until quite recently, it was believed that all neurogenesis was completed during development and that new neurons were never added to the adult CNS. Recent work has modified this view somewhat. It is clear that in limited areas of the brain, there is ongoing neurogenesis during adult life (Garcia-Verdugo *et al*., 2002; Turlejski and Djavadian, 2002). It is likely that new neurons are generated throughout the CNS, albeit in very small numbers for most regions. The source of new neurons in the adult brain and spinal cord appears to be a resident population of adult neural stem cells. The existence of an adult stem cell population is in many ways quite surprising. What function do these cells normally serve, and why do they fail to repair the CNS following injury? The factors that stimulate and suppress the generation of mature neurons from endogenous stem cells are clearly of great scientific and therapeutic interest, yet remain poorly understood (Lim *et al*., 2002). It is also unclear whether stem cells derived from adult CNS tissue are capable of forming all, or only some of the neurons found in the mature nervous system. A significant advantage of stimulating endogenous cell replacement mechanisms or utilizing stem cells derived from patients is that autologous stem cell transplants would not be subject to immune rejection (Subramanian, 2001).

In contrast to adult CNS tissue, neural stem cells are abundant in fetal and embryonic CNS. Transplantation of fetal-derived stem cells and/or neurons into adult injury models has thus far had mixed results (Temple, 2001; Cao *et al*., 2002; Rossi and Cattaneo, 2002). In some cases, fetal tissue improves recovery following CNS injury. Typically this improvement is not due to fetal stem cells generating neurons, but rather due to fetal-derived astrocytes or other nonneuronal cells providing unknown factors that enhance the survival and regenerative performance of injured adult neurons. It is possible that the environment of the adult CNS promotes the differentiation of bipotential stem cells along a glial pathway. Alternatively, it is possible that newly generated fetal neurons are unable to survive or to integrate into existing adult CNS tissues. One beneficial aspect of the propensity of transplanted neural stem cells to form glia has been the generation of oligodendrocytes that are capable of myelinating axons. Much of the functional deficit experienced following CNS injury is attributable to reduced conduction velocities as a consequence of demyelination. Oligodendrocytes derived from transplanted stem cells readily migrate into areas of injury and can participate in myelination of existing axon tracts (Lundberg *et al*., 1997).

A significant concern for the use of cell-replacement strategies is the long-term survival and fate of such transplanted cells. Very few experiments have been done testing the function of stem cells or their derivatives over the long survival times (Temple, 2001; Cao *et al*., 2002; Rossi and Cattaneo, 2002). Little is known regarding the functional properties of replacement cells *in vivo* and the stability of those properties over time. It is critical to determine whether tissue differentiated in culture from stem cells remains stable and functional once transplanted into the CNS. The stability and normalcy of transplanted cells is of particular concern for derivatives of embryonic stem cells (ESCs). ESCs form teratomas in adult tissue with high frequency (Kirschstein and Skirboll, 2001). Whether ESCs can be safely differentiated into stable cell types that do not form teratomas is largely unknown. Lastly, immune rejection of allografts is also a concern for potential cell replacement therapies (Subramanian, 2001). Although the CNS enjoys a certain degree of "immune privilege," replacement cells would nonetheless be rejected by the immune system over the long term if immunosupression is not employed.

SUMMARY

1. In mammals and in avians, restoration of function is unlikely to be due to recapitulation of developmental mechanisms, but rather appears to come about through recruitment of the normal mechanisms underlying adult plasticity and learning. Restitution, substitution, and compensation can all contribute to recovery of function.

2. In lower vertebrates and during the embryonic life of most mammals, the CNS is capable of extensive regenerative repair that occurs largely through the dedifferentiation and redifferentiation of damaged CNS tissue.

3. In both the CNS and the PNS of adult mammals, regeneration involves distinct, sequential challenges: Surviving the initial insult, initiating new axons and dendrites, circumnavigating the region of injury, guidance back to original targets, recognition of appropriate synaptic partners, reestablishment of synaptic contacts, and reestablishment of myelination.

4. In the PNS, the effects of inflammation, the response of glia, and the ability of the nerve to serve as a permissive conduit for regeneration and guidance all contribute to superior performance.

5. In the CNS, regeneration is limited by both the intrinsic properties of CNS neurons and the extracellular environment of the CNS that suppresses regeneration.

6. CNS regeneration failure is largely due to factors present at the site of CNS injury. While factors that inhibit axon extension are expressed throughout CNS white matter, regeneration can be nonetheless robustly accomplished in degenerating white matter tracts. Regeneration abruptly fails once growth cones encounter the glial scar at the region of injury.

7. Numerous factors with both positive and negative effects on axon extension in culture are associated with CNS scar tissue. Regeneration is likely to be inhibited by a number of

distinct mechanisms, including mechanical barriers, growth cone collapse, inhibition of outgrowth, and growth cone trapping.

8. Specific molecules expressed in regions of CNS scarring have complex and changing effects on regeneration, depending on the type on neuron encountering the factor, the internal state of the growth cone at the time the factor is encountered, and the molecular context in which the factor is encountered. Dissecting the role of individual molecules in regeneration failure is a task of exceptional difficulty.

9. Adult CNS regeneration failure reflects maturational changes in the intrinsic properties of CNS neurons and the maladaptive response of these neurons to injury.

10. Cell replacement therapy may prove to be a means of restoring function lost due to death of CNS neurons, either by stimulating the division of endogenous neural stem cells or by transplanting fetal or ESCs into the CNS. Very little is known regarding the long-term survival and function of transplanted stem cell or their derivatives, due in part to the immune rejection of these cells and the tendency of ESCs to form teratomas in adult tissue.

ACKNOWLEDGMENT

This work was supported by grant R01 NS382138.

REFERENCES

Allan, S.M. and Rothwell, N.J., 2001, Cytokines and acute neurodegeneration, *Nat. Rev. Neurosci.* 2:734–744.

Andersen, L.B. and Schreyer, D.J., 1999, Constitutive expression of GAP-43 correlates with rapid, but not slow regrowth of injured dorsal root axons in the adult rat, *Exp. Neurol.* 155:157–164.

Asher, R.A., Morgenstern, D.A., Moon, L.D., and Fawcett, J.W., 2001, Chondroitin sulphate proteoglycans: Inhibitory components of the glial scar, *Prog. Brain. Res.* 132:611–619.

Bandtlow, C.E. and Loschinger, J., 1997, Developmental changes in neuronal responsiveness to the CNS myelin-associated neurite growth inhibitor NI-35/250, *Eur. J. Neurosci.* 9:2743–2752.

Barbeau, H., McCrea, D.A., O'Donovan, M.J., Rossignol, S., Grill, W.M., and Lemay, M.A., 1999, Tapping into spinal circuits to restore motor function, *Brain Res. Brain Res. Rev.* 30:27–51.

Bartsch, U., 1996, Myelination and axonal regeneration in the central nervous system of mice deficient in the myelin-associated glycoprotein, *J. Neurocytol.* 25:303–313.

Bartsch, U., Bandtlow, C.E., Schnell, L., Bartsch, S., Spillmann, A.A., Rubin, B.P. *et al*., 1995, Lack of evidence that myelin-associated glycoprotein is a major inhibitor of axonal regeneration in the CNS, *Neuron* 15:1375–1381.

Bates, C.A. and Meyer, R.L., 1997, The neurite-promoting effect of laminin is mediated by different mechanisms in embryonic and adult regenerating mouse optic axons in vitro, *Dev. Biol.* 181:91–101.

Bates, C.A. and Stelzner, D.J., 1993, Extension and regeneration of corticospinal axons after early spinal injury and the maintenance of corticospinal topography, *Exp. Neurol.* 123:106–117.

Becker, C.G., Becker, T., and Meyer, R.L., 2001, Increased NCAM-180 immunoreactivity and maintenance of L1 immunoreactivity in injured optic fibers of adult mice, *Exp. Neurol.* 169:438–448.

Behrens, M.M., Strasser, U., Lobner, D., and Dugan, L.L., 1999, Neurotrophin-mediated potentiation of neuronal injury, *Microsc. Res. Tech.* 45:276–284.

Bomze, H.M., Bulsara, K.R., Iskandar, B.J., Caroni, P., and Pate Skene, J.H., 2001, Spinal axon regeneration evoked by replacing two growth cone proteins in adult neurons, *Nat. Neurosci.* 4:38–43.

Bovolenta, P. and Fernaud-Espinosa, I., 2000, Nervous system proteoglycans as modulators of neurite outgrowth, *Prog. Neurobiol.* 61:113–132.

Bradbury, E.J., Moon, L.D., Popat, R.J., King, V.R., Bennett, G.S., Patel, P.N. *et al*., 2002, Chondroitinase ABC promotes functional recovery after spinal cord injury, *Nature* 416:636–640.

Braunewell, K.H., Pesheva, P., McCarthy, J.B., Furcht, L.T., Schmitz, B., and Schachner, M., 1995, Functional involvement of sciatic nerve-derived versican- and decorin-like molecules and other chondroitin sulphate proteoglycans in ECM-mediated cell adhesion and neurite outgrowth, *Eur. J. Neurosci.* 7:805–814.

Broude, E., McAtee, M., Kelley, M.S., and Bregman, B.S., 1999, Fetal spinal cord transplants and exogenous neurotrophic support enhance c-Jun expression in mature axotomized neurons after spinal cord injury, *Exp. Neurol.* 155:65–78.

Buettner, H.M. and Pittman, R.N., 1991, Quantitative effects of laminin concentration on neurite outgrowth in vitro, *Dev. Biol.* 145:266–276.

Buffo, A., Holtmaat, A.J., Savio, T., Verbeek, J.S., Oberdick, J., Oestreicher, A.B. *et al*., 1997, Targeted overexpression of the neurite growth-associated protein B-50/GAP-43 in cerebellar Purkinje cells induces sprouting after axotomy but not axon regeneration into growth-permissive transplants, *J. Neurosci.* 17:8778–8791.

Cai, D., Qiu, J., Cao, Z., McAtee, M., Bregman, B.S., and Filbin, M.T., 2001, Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate, *J. Neurosci.* 21:4731–4739.

Cao, Q., Benton, R.L., and Whittemore, S.R., 2002, Stem cell repair of central nervous system injury, *J. Neurosci. Res.* 68:501–510.

Caroni, P., 1997, Intrinsic neuronal determinants that promote axonal sprouting and elongation, *Bioessays* 19:767–775.

Challacombe, J.F., Snow, D.M., and Letourneau, P.C., 1996, Actin filament bundles are required for microtubule reorientation during growth cone turning to avoid an inhibitory guidance cue, *J. Cell Sci.* 109: 2031–2040.

Challacombe, J.F., Snow, D.M., and Letourneau, P.C., 1997, Dynamic microtubule ends are required for growth cone turning to avoid an inhibitory guidance cue, *J. Neurosci.* 17:3085–3095.

Chau, C., Barbeau, H., and Rossignol, S., 1998, Effects of intrathecal alpha1- and alpha2-noradrenergic agonists and norepinephrine on locomotion in chronic spinal cats, *J. Neurophysiol.* 79:2941–2963.

Chavis, P. and Westbrook, G., 2001, Integrins mediate functional pre- and postsynaptic maturation at a hippocampal synapse, *Nature* 411:317–321.

Chen, D.F., Schneider, G.E., Martinou, J.C., and Tonegawa, S., 1997, Bcl-2 promotes regeneration of severed axons in mammalian CNS, *Nature* 385:434–439.

Chernoff, E., Sato, K., Corn, A., and Karcavich, R., 2002, Spinal cord regeneration: Intrinsic properties and emerging mechanisms, *Semin. Cell. Dev. Biol.* 13:361.

Cohen, J., Burne, J.F., Winter, J., and Bartlett, P., 1986, Retinal ganglion cells lose response to laminin with maturation, *Nature* 322:465–467.

Cohen, J., Nurcombe, V., Jeffrey, P., and Edgar, D., 1989, Developmental loss of functional laminin receptors on retinal ganglion cells is regulated by their target tissue, the optic tectum, *Development* 107:381–387.

Colamarino, S.A. and Tessier-Lavigne, M., 1995, The axonal chemoattractant netrin-1 is also a chemorepellent for trochlear motor axons, *Cell* 81:621–629.

Colavita, A. and Culotti, J.G., 1998, Suppressors of ectopic UNC-5 growth cone steering identify eight genes involved in axon guidance in Caenorhabditis elegans, *Dev. Biol.* 194:72–85.

Condic, M.L., 2001, Adult neuronal regeneration induced by transgenic integrin expression, *J. Neurosci.* 21:4782–4788.

Condic, M.L., 2002, Neural development: Axon regeneration derailed by dendrites, *Curr. Biol.* 12:R455–R457.

Condic, M.L. and Lemons, M.L., 2002, Extracellular matrix in spinal cord regeneration: Getting beyond attraction and inhibition, *NeuroReport* 13:A37–48.

Condic, M.L. and Letourneau, P.C., 1997, Ligand induced changes in integrin expression regulate neuronal adhesion and neurite outgrowth, *Nature* 389:852–856.

Condic, M.L., Snow, D.M., and Letourneau, P.C., 1999, Embryonic neurons adapt to the inhibitory proteoglycan aggrecan by increasing integrin expression, *J. Neurosci.* 19:10036–10043.

Coulson, E.J., Barrett, G.L., Storey, E., Bartlett, P.F., Beyreuther, K., and Masters, C.L., 1997, Down-regulation of the amyloid protein precursor of Alzheimer's disease by antisense oligonucleotides reduces neuronal adhesion to specific substrata, *Brain Res.* 770: 72–80.

David, S. and Aguayo, A.J., 1981, Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats, *Science* 214:931–933.

Davies, S.J., Goucher, D.R., Doller, C., and Silver, J., 1999, Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord, *J. Neurosci.* 19:5810–5822.

Davies, S.J. and Silver, J., 1998, Adult axon regeneration in adult CNS white matter, *Trends Neurosci.* 21:515.

Diefenbach, T.J., Guthrie, P.B., and Kater, S.B., 2000, Stimulus history alters behavioral responses of neuronal growth cones, *J. Neurosci.* 20:1484–1494.

Dieringer, M. and Precht, W., 1979, Synaptic mechanisms involved in compensation of vestibular function following hemilabrinthectomy, *Brain Res.* 50:607–615.

Edgerton, V.R., Roy, R.R., Hodgson, J.A., Prober, R.J., de Guzman, C.P., and de Leon, R., 1992, Potential of adult mammalian lumbosacral spinal cord to execute and acquire improved locomotion in the absence of supraspinal input, *J. Neurotrauma.* 9:S119–S128.

Faissner, A., 1997, The tenascin gene family in axon growth and guidance, *Cell Tissue Res.* 290:331–341.

Forehand, C.J. and Farel, P.B., 1982, Anatomical and behavioral recovery from the effects of spinal cord transection: Dependence on metamorphosis in anuran larvae, *J. Neurosci.* 2:654–662.

Fouad, K., Metz, G.A., Merkler, D., Dietz, V., and Schwab, M.E., 2000, Treadmill training in incomplete spinal cord injured rats, *Behav. Brain Res.* 115:107–113.

Frostick, S.P., Yin, Q., and Kemp, G.J., 1998, Schwann cells, neurotrophic factors, and peripheral nerve regeneration, *Microsurgery* 18:397–405.

Fu, S.Y. and Gordon, T., 1997, The cellular and molecular basis of peripheral nerve regeneration, *Mol. Neurobiol.* 14:67–116.

Garcia-Verdugo, J.M., Ferron, S., Flames, N., Collado, L., Desfilis, E., and Font, E., 2002, The proliferative ventricular zone in adult vertebrates: A comparative study using reptiles, birds, and mammals, *Brain Res. Bull.* 57:765–775.

Gianola, S. and Rossi, F., 2002, Long-term injured purkinje cells are competent for terminal arbor growth, but remain unable to sustain stem axon regeneration, *Exp. Neurol.* 176:25–40.

Goldberg, J.L. and Barres, B.A. 2000, The relationship between neuronal survival and regeneration, *Annu. Rev. Neurosci.* 23:579–612.

Goldberg, J.L., Klassen, M.P., Hua, Y., and Barres, B.A., 2002, Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells, *Science* 296:1860–1864.

Golding, J.P., Bird, C., McMahon, S., and Cohen, J., 1999, Behaviour of DRG sensory neurites at the intact and injured adult rat dorsal root entry zone: Postnatal neurites become paralysed, whilst injury improves the growth of embryonic neurites, *Glia* 26:309–323.

Gomez, T.M. and Letourneau, P.C., 1994, Filopodia initiate choices made by sensory neuron growth cones at laminin/fibronectin borders in vitro, *J. Neurosci.* 14:5959–5972.

Grotewiel, M.S., Beck, C.D., Wu, K.H., Zhu, X.R., and Davis, R.L. 1998, Integrin-mediated short-term memory in Drosophila, *Nature* 391:455–460.

Harkema, S.J., 2001, Neural plasticity after human spinal cord injury: Application of locomotor training to the rehabilitation of walking, *Neuroscientist* 7:455–468.

Hedgecock, E.M., Culotti, J.G., and Hall, D.H., 1990, The unc-5, unc-6, and unc-40 genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans*, *Neuron* 4:61–85.

Hermanns, S., Reiprich, P., and Muller, H.W., 2001, A reliable method to reduce collagen scar formation in the lesioned rat spinal cord, *J. Neurosci. Meth.* 110:141–146.

Hildebrand, A., Romaris, M., Rasmussen, L.M., Heinegard, D., Twardzik, D.R., Border, W.A. *et al*., 1994, Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta, *Biochem. J.* 302:527–534.

Hopker, V.H., Shewan, D., Tessier-Lavigne, M., Poo, M., and Holt, C., 1999, Growth-cone attraction to netrin-1 is converted to repulsion by laminin-1, *Nature* 401:69–73.

Ito, J., Murata, M., and Kawaguchi, S., 1999, Regeneration of the lateral vestibulospinal tract in adult rats by transplants of embryonic brain tissue, *Neurosci. Lett.* 259:67–70.

Jones, F.S. and Jones, P.L., 2000, The tenascin family of ECM glycoproteins: Structure, function, and regulation during embryonic development and tissue remodeling, *Dev. Dyn.* 218:235–259.

Jones, L.S. and Grooms, S.Y., 1997, Normal and aberrant functions of integrins in the adult central nervous system, *Neurochem. Int.* 31:587–595.

Joosten, E.A., Dijkstra, S., Brook, G.A., Veldman, H., and Bar, P.R., 2000, Collagen IV deposits do not prevent regrowing axons from penetrating the lesion site in spinal cord injury, *J. Neurosci. Res.* 62:686–691.

Jung, M., Petrausch, B., and Stuermer, C.A., 1997, Axon-regenerating retinal ganglion cells in adult rats synthesize the cell adhesion molecule L1 but not TAG-1 or SC-1, *Mol. Cell. Neurosci.* 9:116–131.

Kamiguchi, H. and Lemmon, V., 2000, Recycling of the cell adhesion molecule L1 in axonal growth cones, *J. Neurosci.* 20:3676–3686.

Kamiguchi, H., Long, K.E., Pendergast, M., Schaefer, A.W., Rapoport, I., Kirchhausen, T. *et al*., 1998, The neural cell adhesion molecule L1 interacts with the AP-2 adaptor and is endocytosed via the clathrin-mediated pathway, *J. Neurosci.* 18:5311–5321.

Keino-Masu, K., Masu, M., Hinck, L., Leonardo, E.D., Chan, S.S., Culotti, J.G. *et al*., 1996, Deleted in Colorectal Cancer (DCC) encodes a netrin receptor, *Cell* 87:175–185.

Kennedy, T.E., Serafini, T., de la Torre, J.R., and Tessier-Lavigne, M., 1994, Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord, *Cell* 78:425–435.

Kirschstein, R. and Skirboll, L.R./National Institutes of Health, 2001, *Stem Cells: Scientific Progress and Future Research Directions* (May 2, 2002); http://www.nih.gov/news/stemcell/scireport.htm.

Kolodziej, P.A., Timpe, L.C., Mitchell, K.J., Fried, S.R., Goodman, C.S., Jan, L.Y. *et al*., 1996, Frazzled encodes a Drosophila member of the DCC immunoglobulin subfamily and is required for CNS and motor axon guidance, *Cell* 87:197–204.

Kuhn, T.B., Schmidt, M.F., and Kater, S.B., 1995, Laminin and fibronectin guideposts signal sustained but opposite effects to passing growth cones, *Neuron* 14:275–285.

Kury, P., Stoll, G., and Muller, H.W., 2001, Molecular mechanisms of cellular interactions in peripheral nerve regeneration, *Curr. Opin. Neurol.* 14:635–639.

Leonardo, E.D., Hinck, L., Masu, M., Keino-Masu, K., Ackerman, S.L., and Tessier-Lavigne, M., 1997, Vertebrate homologues of C. elegans UNC-5 are candidate netrin receptors, *Nature* 386:833–838.

Letourneau, P.C., Condic, M.L., and Snow, D.M., 1994, Interactions of developing neurons with the extracellular matrix, *J. Neurosci.* 14:915–928.

Li, M., Shibata, A., Li, C., Braun, P.E., McKerracher, L., Roder, J. *et al*., 1996, Myelin-associated glycoprotein inhibits neurite/axon growth and causes growth cone collapse, *J. Neurosci. Res.* 46:404–414.

Lim, D.A., Flames, N., Collado, L., and Herrera, D.G., 2002, Investigating the use of primary adult subventricular zone neural precursor cells for neuronal replacement therapies, *Brain Res. Bull.* 57:759–764.

Lindvall, O., 1998, Update on fetal transplantation: The Swedish experience, *Mov. Disord.* 13:83–87.

Logan, A., Baird, A., and Berry, M., 1999, Decorin attenuates gliotic scar formation in the rat cerebral hemisphere, *Exp. Neurol.* 159:504–510.

Long, K.E., Asou, H., Snider, M.D., and Lemmon, V., 2001, The role of endocytosis in regulating L1-mediated adhesion, *J. Biol. Chem.* 276:1285–1290.

Lundberg, C., Martinez-Serrano, A., Cattaneo, E., McKay, R.D., and Bjorklund, A., 1997, Survival, integration, and differentiation of neural stem cell lines after transplantation to the adult rat striatum, *Exp. Neurol.* 145:342–360.

Mason, M.R., Campbell, G., Caroni, P., Anderson, P.N., and Lieberman, A.R., 2000, Overexpression of GAP-43 in thalamic projection neurons of transgenic mice does not enable them to regenerate axons through peripheral nerve grafts, *Exp. Neurol.* 165:143–152.

McFarlane, S., 2000, Attraction vs. repulsion: The growth cone decides, *Biochem. Cell Biol.* 78:563–568.

McKenna, M.P. and Raper, J.A., 1988, Growth cone behavior on gradients of substratum bound laminin, *Dev. Biol.* 130:232–236.

McKeon, R.J., Hoke, A., and Silver, J., 1995, Injury-induced proteoglycans inhibit the potential for laminin-mediated axon growth on astrocytic scars, *Exp. Neurol.* 136:32–43.

Meiners, S., Mercado, M.L., and Geller, H.M., 2000, The multi-domain structure of extracellular matrix molecules: Implications for nervous system regeneration, *Prog. Brain Res.* 128:23–31.

Meyer, R.L., Miotke, J.A., and Benowitz, L.I., 1994, Injury induced expression of growth-associated protein-43 in adult mouse retinal ganglion cells in vitro, *Neuroscience* 63:591–602.

Miranda, J.D., White, L.A., Marcillo, A.E., Willson, C.A., Jagid, J., and Whittemore, S.R., 1999, Induction of Eph B3 after spinal cord injury, *Exp. Neurol.* 156:218–222.

Moon, L.D., Asher, R.A., Rhodes, K.E., and Fawcett, J.W., 2001, Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC, *Nat. Neurosci.* 4:465–466.

Moreno-Flores, M.T. and Wandosell, F., 1999, Up-regulation of Eph tyrosine kinase receptors after excitotoxic injury in adult hippocampus, *Neuroscience* 91:193–201.

Nakamura, F., Kalb, R.G., and Strittmatter, S.M., 2000, Molecular basis of semaphorin-mediated axon guidance, *J. Neurobiol.* 44:219–229.

Neugebauer, K.M. and Reichardt, L.F., 1991, Cell-surface regulation of beta 1-integrin activity on developing retinal neurons, *Nature* 350:68–71.

Neumann, S., Bradke, F., Tessier-Lavigne, M., and Basbaum, A.I., 2002, Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation, *Neuron* 34:885–893.

Neumann, S. and Woolf, C.J., 1999, Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury, *Neuron* 23:83–91.

Nishimura, S.L., Boylen, K.P., Einheber, S., Milner, T.A., Ramos, D.M., and Pytela, R., 1998, Synaptic and glial localization of the integrin alphavbeta8 in mouse and rat brain, *Brain Res.* 791:271–282.

Nogradi, A. and Vrbova, G., 1994, The use of embryonic spinal cord grafts to replace identified motoneuron pools depleted by a neurotoxic lectin, volkensin, *Exp. Neurol.* 129:130–141.

Palecek, S.P., Horwitz, A.F., and Lauffenburger, D.A., 1999, Kinetic model for integrin-mediated adhesion release during cell migration, *Ann. Biomed. Eng.* 27:219–235.

Palecek, S.P., Loftus, J.C., Ginsberg, M.H., Lauffenburger, D.A., and Horwitz, A.F., 1997, Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness, *Nature* 385:537–540.

Parent, J.M. and Lowenstein, D.H., 2002, Seizure-induced neurogenesis: Are more new neurons good for an adult brain? *Prog. Brain Res.* 135:121–131.

Pasterkamp, R.J., De Winter, F., Holtmaat, A.J., and Verhaagen, J., 1998, Evidence for a role of the chemorepellent semaphorin III and its receptor neuropilin-1 in the regeneration of primary olfactory axons, *J. Neurosci.* 18:9962–9976.

Pasterkamp, R.J., Giger, R.J., Ruitenberg, M.J., Holtmaat, A.J., De Wit, J., De Winter, F. *et al*., 1999, Expression of the gene encoding the chemorepellent semaphorin III is induced in the fibroblast component of neural scar tissue formed following injuries of adult but not neonatal CNS, *Mol. Cell. Neurosci.* 13:143–166.

Pasterkamp, R.J. and Verhaagen, J., 2001, Emerging roles for semaphorins in neural regeneration, *Brain Res. Brain Res. Rev.* 35:36–54.

Pinkstaff, J.K., Detterich, J., Lynch, G., and Gall, C., 1999, Integrin subunit gene expression is regionally differentiated in adult brain, *J. Neurosci.* 19:1541–1556.

Poppel, E., Held, R., and Frost, D., 1973, Residual visual function after brain wounds involving the central visual pathways, *Nature* 243: 295–296.

Qiu, J., Cai, D., Dai, H., McAtee, M., Hoffman, P.N., Bregman, B.S. *et al*., 2002, Spinal axon regeneration induced by elevation of cyclic AMP, *Neuron* 34:895–903.

Ramon y Cajal, S., 1991, Cajal's degeneration and regeneration of the nervous system. In *History of Neuroscience* (J. Defelipe and E.G. Jones, eds.), Oxford University Press, New York, p. 769.

Reichardt, L.F. and Tomaselli, T.J., 1991, Extracellular matrix molecules and their receptors, *Ann. Rev. Neurosci.* 14:531–570.

Richardson, P.M., McGuinness, U.M., and Aguayo, A.J., 1980, Axons from CNS neurons regenerate into PNS grafts, *Nature* 284:264–265.

Romero, M.I., Rangappa, N., Li, L., Lightfoot, E., Garry, M.G., and Smith, G.M., 2000, Extensive sprouting of sensory afferents and hyperalgesia induced by conditional expression of nerve growth factor in the adult spinal cord, *J. Neurosci.* 20:4435–4445.

Rossi, F., Bravin, M., Buffo, A., Fronte, M., Savio, T., and Strata, P., 1997, Intrinsic properties and environmental factors in the regeneration of adult cerebellar axons, *Prog. Brain Res.* 114:283–296.

Rossi, F. and Cattaneo, E., 2002, Opinion: Neural stem cell therapy for neurological diseases: Dreams and reality, *Nat. Rev. Neurosci.* 3:401–409.

Ruoslahti, E., Yamaguchi, Y., Hildebrand, A., and Border, W.A., 1992, Extracellular matrix/growth factor interactions, *Cold Spring Harb. Symp. Quant. Biol.* 57:309–315.

Salinero, O., Garrido, J.J., and Wandosell, F., 1998, Amyloid precursor protein proteoglycan is increased after brain damage, *Biochim. Biophys. Acta.* 1406:237–250.

Salinero, O., Moreno-Flores, M.T., and Wandosell, F., 2000, Increasing neurite outgrowth capacity of beta-amyloid precursor protein proteoglycan in Alzheimer's disease, *J. Neurosci. Res.* 60:87–97.

Sango, K., Horie, H., Inoue, S., Takamura, Y., and Takenaka, T., 1993, Age-related changes of DRG neuronal attachment to extracellular matrix proteins in vitro, *NeuroReport* 4:663–666.

Schmalfeldt, M., Bandtlow, C.E., Dours-Zimmermann, M.T., Winterhalter, K.H., and Zimmermann, D.R., 2000, Brain derived versican V2 is a potent inhibitor of axonal growth, *J. Cell. Sci.* 113:807–816.

Schreyer, D.J. and Skene, J.H., 1991, Fate of GAP-43 in ascending spinal axons of DRG neurons after peripheral nerve injury: Delayed accumulation and correlation with regenerative potential, *J. Neurosci.* 11:3738–3751.

Shimizu, I., Oppenheim, R.W., O'Brien, M., and Shneiderman, A., 1990, Anatomical and functional recovery following spinal cord transection in the chick embryo, *J. Neurobiol.* 21:918–937.

Shirasaki, R., Katsumata, R., and Murakami, F., 1998, Change in chemoattractant responsiveness of developing axons at an intermediate target, *Science* 279:105–107.

Sholomenko, G.N. and Delaney, K.R., 1998, Restitution of functional neural connections in chick embryos assessed in vitro after spinal cord transection in Ovo, *Exp. Neurol.* 154:430–451.

Singer, W., 1982, Recovery mechanisms in the mammalian brain. In *Repair and Regeneration of the Nervous System* (J.G. Nicholls, ed.), Springer-Verlag, New York, pp. 203–226.

Snow, D.M., Atkinson, P.B., Hassinger, T.D., Letourneau, P.C., and Kater, S.B., 1994, Chondroitin sulfate proteoglycan elevates cytoplasmic calcium in DRG neurons, *Dev. Biol.* 166:87–100.

Stein, E. and Tessier-Lavigne, M., 2001, Hierarchical organization of guidance receptors: Silencing of netrin attraction by slit through a Robo/DCC receptor complex, *Science* 291:1928–1938.

Stichel, C.C., Niermann, H., D'Urso, D., Lausberg, F., Hermanns, S., and Muller, H.W., 1999, Basal membrane-depleted scar in lesioned CNS: Characteristics and relationships with regenerating axons, *Neuroscience* 93:321–333.

Subramanian, T., 2001, Cell transplantation for the treatment of Parkinson's disease, *Semin. Neurol.* 21:103–115.

Sunderland, S., 1965, The connective tissues of peripheral nerves, *Brain* 88:841–854.

Sunderland, S., 1970, Anatomical features of nerve trunks in relation to nerve injury and nerve repair, *Clin. Neurosurg.* 17:38–62.

Sunderland, S., 1990, The anatomy and physiology of nerve injury, *Muscle Nerve* 13:771–784.

Tanelian, D.L., Barry, M.A., Johnston, S.A., Le, T., and Smith, G.M., 1997, Semaphorin III can repulse and inhibit adult sensory afferents in vivo, *Nat. Med.* 3:1398–1401.

Temple, S., 2001, The development of neural stem cells, *Nature* 414:112–117.

Terenghi, G., 1999, Peripheral nerve regeneration and neurotrophic factors, *J. Anat.* 194:1–14.

Turlejski, K. and Djavadian, R., 2002, Life-long stability of neurons: A century of research on neurogenesis, neuronal death and neuron quantification in adult CNS, *Prog. Brain Res.* 136:39–65.

Tzeng, S.F., Cheng, H., Lee, Y.S., Wu, J.P., Hoffer, B.J., and Kuo, J.S., 2001, Expression of neural cell adhesion molecule in spinal cords following a complete transection, *Life Sci.* 68:1005–1012.

Vaudano, E., Campbell, G., Anderson, P.N., Davies, A.P., Woolhead, C., Schreyer, D.J. *et al*., 1995, The effects of a lesion or a peripheral nerve graft on GAP-43 upregulation in the adult rat brain: An in situ hybridization and immunocytochemical study, *J. Neurosci.* 15:3594–3611.

Vielmetter, J., Kayyem, J.F., Roman, J.M., and Dreyer, W.J., 1994, Neogenin, an avian cell surface protein expressed during terminal neuronal differentiation, is closely related to the human tumor suppressor molecule deleted in colorectal cancer, *J. Cell Biol.* 127:2009–2020.

Wang, X.M., Basso, D.M., Terman, J.R., Bresnahan, J.C., and Martin, G.F., 1998a, Adult opossums (Didelphis virginiana) demonstrate near normal locomotion after spinal cord transection as neonates, *Exp. Neurol.* 151:50–69.

Wang, X.M., Terman, J.R., and Martin, G.F., 1998b, Regeneration of supraspinal axons after transection of the thoracic spinal cord in the developing opossum, Didelphis virginiana, *J. Comp. Neurol.* 398:83–97.

Weidner, N., Grill, R.J., and Tuszynski, M.H., 1999, Elimination of basal lamina and the collagen "scar" after spinal cord injury fails to augment corticospinal tract regeneration, *Exp. Neurol.* 160:40–50.

Wictorin, K. and Bjorklund, A., 1992, Axon outgrowth from grafts of human embryonic spinal cord in the lesioned adult rat spinal cord, *Neuroreport* 3:1045–1048.

Wilkinson, D.G., 2001, Multiple roles of EPH receptors and ephrins in neural development, *Nat. Rev. Neurosci.* 2:155–164.

Wu, A., Pangalos, M.N., Efthimiopoulos, S., Shioi, J., and Robakis, N.K., 1997, Appican expression induces morphological changes in C6 glioma cells and promotes adhesion of neural cells to the extracellular matrix, *J. Neurosci.* 17:4987–4993.

Yick, L.W., Wu, W., So, K.F., Yip, H.K., and Shum, D.K., 2000, Chondroitinase ABC promotes axonal regeneration of Clarke's neurons after spinal cord injury, *NeuroReport* 11:1063–1067.

Yin, Q., Kemp, G.J., and Frostick, S.P., 1998, Neurotrophins, neurones and peripheral nerve regeneration, *J. Hand Surg. [Br.]* 23:433–437.