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Spinal cord regeneration: a phenomenon unique to urodeles?

ELLEN A. G. CHERNOFF*

Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana, USA

ABSTRACT Studies of neuronal survival and axonal regeneration in birds and mammals have made it clear that the microenvironment of the CNS is critical to the failure of CNS regeneration in these animals. This environment includes growth and trophic factors, ECM components and matrix turnover enzymes, cytokines and other immune system contributions. Urodele amphibians (salamanders and newts) can regenerate spinal cord even as adults, and environmental contributions of glial populations are a major part of the difference between urodele and higher vertebrate spinal cord regeneration. In particular, the behavior of injury- reactive ependymal cells (radial glia) is critical to the regenerative capacity of urodele spinal cord. In this review we examine what is known about cell-cell interactions between ependymal cells and neurons and between ependymal cells and other glial populations. The known contributions of ependymal cell products such as matrix metalloproteinases and trophic factors are discussed. There is evidence in the literature that an ependymal response occurs during embryonic or fetal development in birds and mammals following spinal cord transection, and this review discusses the implications of such a process for future studies of spinal cord injury.

KEY WORDS: *spinal cord regeneration, spinal cord, ependymal cells, radial glia, tissue regeneration*

Introduction

Adult urodele amphibians regenerate their spinal cords following a cut or crush injury, achieving functional recovery. This process is intrinsically interesting to investigators interested in tissue regeneration (Stocum, 1995, for review), but a question which is often asked is whether understanding spinal cord regeneration in urodeles has relevance to spinal cord injury in higher (amniote) vertebrates, such as humans. This paper will discuss some of the phenomena that have been, are being, and should be studied in urodele spinal cord regeneration. We will explore some of the similarities and differences between lower and higher vertebrate responses to spinal cord injury and the implications of urodele spinal cord research for studies of mammalian spinal cord injury.

Urodeles are certainly not the only vertebrates that can regenerate injured spinal cord. Spinal cord regeneration occurs in teleost fish (Anderson *et al.*, 1986, 1994), in anuran amphibians before metamorphosis (Beattie *et al.*, 1990), in the tail spinal cord of lizards (Simpson, 1968; Duffy *et al.*, 1992), and during embryonic and fetal development in birds and mammals (Shimizu *et al.*, 1990; Hasan *et al.*, 1993; Iwashita *et al.*, 1994). In anuran amphibians, regeneration following spinal cord transection fails after metamorphic climax (Beattie *et al.*, 1990), and virtually all central nervous system (CNS) regeneration fails in mature higher vertebrates. Thus, urodele amphibians are the only tetrapod vertebrates that can regenerate all regions of the spinal cord as adults.

The different outcome of spinal cord injury in urodeles and higher vertebrates has several possible causes. Neurons might have inherently different capacities for axonal regrowth, glial populations might respond differently to injury, and the immune system might respond differently. It has been suggested that the urodele spinal cord retains embryonic characteristics, and that this permits regeneration. These points will be addressed in the following discussion of the processes of urodele spinal cord regeneration, the relationship between regeneration and embryonic processes, and the basis of the failure of higher vertebrate spinal cord regeneration.

General features of spinal cord regeneration

Stages of urodele spinal cord regeneration

The two best-studied aspects of urodele spinal cord regeneration are axonal regrowth and the ependymal response.

Studies of axonal regeneration in newts have shown that there is a range in the extent of functional recovery. The number of regenerated axons, the number of functional synapses, and the

Abbreviations used in this paper: CNS, central nervous system; CRBP, cellular retinol-binding protein; CRABP, cellular retinoic acid-binding protein; ECM, extracellular matrix; GFAP, glial fibrillary acidic protein; MMP, matrix metalloproteinase; NGF, nerve growth factor; PA, plasminogen activator; PS-N-CAM, polysialylated neural cell adhesion molecule; TIMP, tissue inhibitor of metalloproteinase.

***Address for reprints:** Department of Biology, Indiana University-Purdue University at Indianapolis (IUPUI), 723 W. Michigan St., Indianapolis, IN 46202-5132, USA. FAX: 317-274.2846. e-mail: echernof@indyvax.iupui.edu

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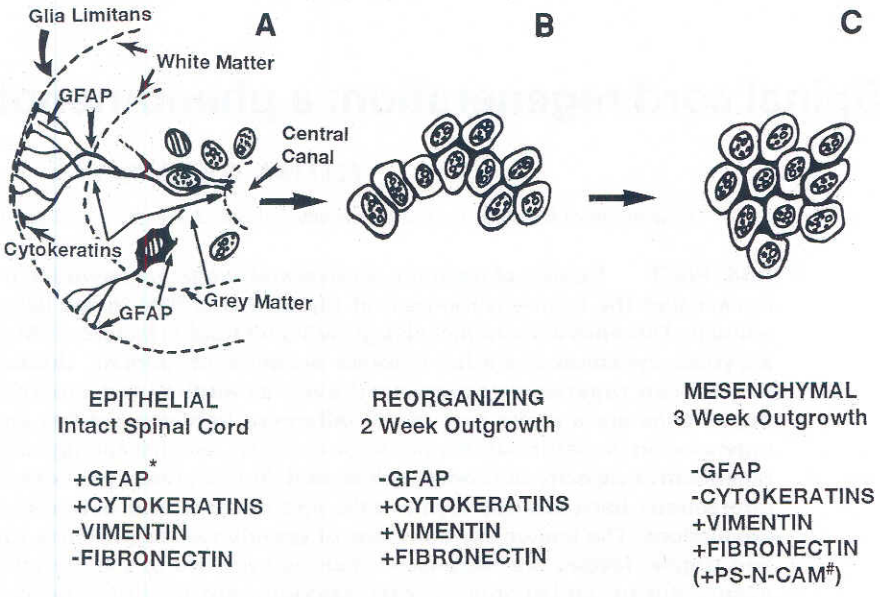

Fig. 1. Axolotl spinal cord ependymal reorganization. (A) *This cartoon illustrates the changes that occur in axolotl ependymal cells (radial glia) following transection of the spinal cord. In the intact spinal cord, ependymal cells with endfeet on the glia limitans and apical surfaces at the central canal express cytokeratins throughout (Holder et al., 1990; O'Hara et al., 1992) and GFAP in their outer processes. *GFAP has been localized to the outer (white matter) processes of radial glia (dotted nuclei) (Holder et al., 1990) and in astrocytic sub- ependymal zone cells in newt spinal cord, which have no luminal contact (striped nuclei) (Zamora and Mutin, 1988).* **(B)** *Following transection, the glia limitans is degraded, apical- basal polarity is lost and GFAP expression disappears from ependymal cells. Cytokeratins are still present and vimentin appears. Fibronectin also appears among the cells (O'Hara et al., 1992).* **(C)** *As the injury-reactive ependymal cells migrate across the lesion site, cytokeratins disappear, but vimentin and fibronectin are strongly expressed. #Polysialylated neural cell adhesion molecule (PS-N-CAM) is expressed in newt tail spinal cord injury- reactive ependymal cells (Caubit et al., 1993). Ependymal cells do not disorganize in the same manner after tail amputation as those in more caudal regions of the cord following injury, but we extrapolate that similar expression would be seen.*

extent to which coordinated movement is recovered can vary (Davis *et al.*, 1989, 1990). Not all of the connections made by regenerating axons are appropriate (Davis *et al.*, 1990). Even so, spinally projecting descending axons were found to regenerate through a complete transection lesion site for a distance of at least 10 mm in the newt *Notopthalmus viridescens*, and all regions of the brain stem that normally project to the lumbar spinal cord are able to regenerate (Davis *et al.*, 1989). Functional synaptic connections are formed by many regenerated axons, but the regenerated spinal cord is thinner than the intact cord and there are fewer axons (Stensaas, 1983; Davis *et al.*, 1989). The time course of regeneration in urodeles depends on the nature of the lesion and on the age of the animal. In adult axolotls (*Ambystoma mexicanum*, the Mexican salamander, >13 cm in length) newly myelinated axons are seen within a complete transection lesion site 4 weeks after lesioning (O'Hara *et al.*, 1992).

An ependymal response (see below) occurs during spinal cord regeneration in all of the vertebrates that can regenerate injured spinal cord as adults: teleost fish, urodele amphibians, and lizards (in tail regeneration) (Simpson, 1968; Egar *et al.*, 1970; Egar and Singer, 1972; Anderson *et al.*, 1986, 1994; Alibardi and Meyer-Rochow, 1988; Duffy *et al.*, 1992). Ependymal cells line the central canal of the spinal cord in all vertebrates, but in regenerating spinal cord they either retain some of the developmental potential of the embryonic neuroepithelium, or they can be stimulated by injury to proliferate and remodel their tissue organization leading to the formation of a mesenchymal blastema-like outgrowth.

Following surgical transection of the spinal cord, the ependymal cells seal over the cut ends, form an ependymal bulb, and migrate into the lesion site from the cranial and caudal stumps (Singer *et al.*, 1979; Stensaas, 1983). During this process the ependymal cells become migratory and proliferative, and they engage in extracellular matrix (ECM) turnover (Simpson, 1968; Egar and Singer, 1972; Anderson *et al.*, 1986). Histological examination has shown that the cellular composition of the lesion site early in regeneration consists of ependymal cells, their progeny, some infiltrating macrophage-like cells, and debris from dead neuronal and other neural cells (Singer *et al.*, 1979; Stensaas, 1983; Chernoff *et al.*, 1990).

The urodele ependymal response to injury *in vivo* involves formation of a blastema by an epithelial-to-mesenchymal transformation with characteristic changes in intermediate filaments and extracellular matrix components (O'Hara *et al.*, 1992). By two weeks post-lesioning in adult axolotls the cranial and caudal ependymal outgrowths make contact, and by three weeks the outgrowths are firmly connected to each other. During the outgrowth process epithelial cytokeratin expression is lost and vimentin is produced. Fibronectin appears in the ECM of the ependymal mesenchyme cells. These changes are included in the diagram summarizing ependymal reorganization (Fig. 1). By four weeks the ependymal cells reform an epithelium and newly myelinated axons are seen within the lesion site (Chernoff *et al.*, 1990; O'Hara *et al.*, 1992). Following re-epithelialization, vimentin and fibronectin expression are lost and cytokeratins are re-expressed.

Cell-cell interactions in ependymal remodeling

As ependymal remodeling occurs there are interactions between ependymal cells and neurons and, possibly, between ependymal cells and fibrous astrocytes and oligodendrocytes.

Regenerating axons are found in association with terminal processes of re-epithelialized ependymal cells (Singer *et al.*, 1979). Studies of newt tail regeneration have shown that polysialylated-neural cell adhesion molecule (PS-N-CAM) is absent from normal adult urodele CNS, except for a low level on ependymal cell surfaces, but is upregulated in cells of the ependymal tube formed by injury-reactive ependymal cells, as well as on regenerating axons. Expression declines following completion of regeneration (Caubit *et al.*, 1993). This transient

expression of PS-N-CAM probably plays a role in axonal guidance (Singer *et al.,* 1979; Rutishauser, 1989).

During development, tenascin modulates cell-ECM interactions, and it is expressed throughout CNS development (reviewed in Riou, *et al.,* 1992). In normal adult newts, antibody localization shows little or no tenascin staining on or around the ependymal cells and periependymal cells, although the nerve tracts show strong staining (Caubit, *et al.,* 1994). In regenerating spinal cord the nerve tracts and pia mater show strong tenascin staining, and faint staining appears in the ependymal cells around the central canal. However, electron microscopic examination of tenascin antibody localization shows localization throughout the radial ependymal processes that contact the glia limitans, especially where axons and growth cones contact the radial ependymal processes. At the molecular level, *in situ* hybridization with antisense probes shows that tenascin transcripts increase following amputation and are in the ependymal tube lying within the regenerating tail mesenchyme and in ependymal cells of the distal regeneration vesicle. The presence of tenascin in radial processes strongly suggests an association with axonal regrowth (Caubit, *et al.,* 1994).

The relationship of the ependymal response in urodeles to the reactive gliosis of fibrous astrocytes in birds and mammals would be better understood if the following question was answered: *do amphibians have fibrous astrocytes?* There are glial fibrillary acidic protein (GFAP)-positive cells in urodele spinal cord, and there is reciprocal expression of GFAP-containing and vimentin-containing intermediate filaments during the regeneration process (Zamora and Mutin, 1988; Holder *et al.,* 1990; O'Hara *et al.,* 1992). GFAP-positive cells are absent from the regenerating region until the ependymal mesenchyme cells have re-epithelialized (O'Hara *et al.,* 1992), but it is not entirely clear how much of the GFAP visualized is in distinct astrocytes. There are two sets of conventional wisdom about the phylogenetic appearance of astrocytes. In one view, true astrocytes with pial endfeet (endfeet terminating on the glia limitans), no luminal contact, and participation in the blood-CNS barrier first appear in reptiles (Bodega *et al.* 1994). Bodega *et al.* (1994) have described the decrease in GFAP content within ependymal cells that is correlated with phylogenetic progression from fish and amphibians to reptiles, birds, and mammals. In reptiles (lizards, specifically) there are relatively few GFAP-positive tanycytes and a distinct population of astrocytes. The alternative point of view is that all vertebrates have astrocytes, but that the proportion of astrocytes to ependymal cells with GFAP-positive processes increases phylogenetically (Roots, 1986). The observations of Holder *et al.* (1990), showing GFAP localization in basal processes of radial glia that are also positive for cytokeratins, suggests that true astrocytes need not be present in axolotls. However, Zamora and Mutin (1988) have shown the existence of a population of GFAP-positive cells that have pial endfeet, but no luminal contact, in newt spinal cord. Both of these types of GFAP-positive cells are represented in Figure 1A. The observations of Zamora and Mutin (1988) fit an observation made in our own laboratory of a population of cells that are GFAP positive, but not cytokeratin positive, among the glial cells seen in dissociated adult axolotl spinal cord. These cells are smaller than ependymal cells and do not have the distinctive ring-shaped nucleoli found in axolotl ependymal cell nuclei (O'Hara and Chernoff, unpublished observation; Chernoff *et al.,* 1990). If these cells are fibrous astrocytes, then the observation that no GFAP localization is seen in regenerating axolotl spinal cord until ependymal cells have reepithelialized may reflect not only redifferentiation or re-organization of the reactive ependymal cells back into radial glia, but also the exclusion of fibrous astrocytes from the lesion site through the effects of ependymal cell products. Alternatively, the new astrocytes may be generated by re-epithelialized ependymal cells. The fate of the putative astrocytes during regeneration has not yet been determined, and requires further investigation.

About possible interactions between ependymal cells and oligodendrocytes during regeneration, nothing is known.

Intermediate filament content in the ependymal response

In addition to the presence of GFAP in intact ependymal cells and vimentin in mesenchymal cells discussed earlier, the cytokeratin content of axolotl ependymal cells has also been characterized. Ependymal cells in intact spinal cord contain intermediate filament proteins that cross-react with antibodies to cytokeratins 8 and 18, simple epithelial cytokeratins (Holder *et al.,* 1990) and with antibody that reacts with tonofilaments (O'Hara *et al.,* 1992).

Is the intermediate filament content significant in the differential response to injury of urodele and mammalian ependymal cells? Is expression of epithelial cytokeratins or vimentin associated with a differentiative state that permits an ependymal response to injury? Bodega *et al.* (1994) surveyed 11 vertebrate species from fish to mammals, examining GFAP and vimentin expression in ependymal cells. They found that lower vertebrates had more GFAP in ependymal cells than higher vertebrates. Vimentin, on the other hand, was present in ependymal cells in intact spinal cord with no phylogenetic correlation. It was present only in fish and rats and abundant only in rat ependymal cells (Bodega *et al.,* 1994). A phylogenetic study of cytokeratin 8 (type II cytokeratin) showed stronger expression in the spinal cords in lower vertebrates, principally in the ependymal cells or radial ependymoglia (described as radial astrocytes). Faint cytokeratin II expression was found in mammalian spinal cord, also in the ependymal cells (Bodega *et al.,* 1995). In humans, fetal ependymal cells express cytokeratins 8 and 18, but in adult spinal cord expression is restricted to only a few ependymal cells, possibly tanycytes (Kasper *et al.,* 1991). The expression of epithelial cytokeratins is not, per se, associated with regenerative capacity, however. In frogs, epithelial cytokeratins are present in adult spinal cord, which does not regenerate (Godsave *et al.,* 1986).

Growth and trophic factors in CNS regeneration

Preliminary experiments in our laboratory have shown that nerve growth factor (NGF) enhances the survival of dissociated larval axolotl CNS neurons *in vitro.* The survival-promoting effect is much greater, however, when the neurons are co-cultured with adult axolotl ependymal mesenchyme cells, suggesting that the mesenchyme produces other neurotrophic factors in addition to NGF. Physical contact between the two cell populations is not required for the effect, suggesting that the mesenchymal factors are diffusable. These putative ependymal-derived neurotrophic factors await chemical characterization.

There is an increasing body of information which addresses the following question: *to what extent are neurotrophic factors active*

in regenerating systems? These include the neuro-trophins: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5 (NT-4); the neuropoietic factors, ciliary neurotrophic factor (CNTF) and cholinergic neuronal differentiation factor/leukemia inhibitory factor (CDF/LIF or LIF) (Lindsay *et al.,* 1994; Qiu *et al.,* 1994); and growth factors such as fibroblast growth factors (FGF-1, FGF-2) and TGF-ß. These factors can be produced by target cells, neighboring glia, the neurons themselves, and immune system cells. The pathway used may be paracrine, autocrine, or intracrine, depending on the trophic factor. CNTF, for example lacks a signal peptide sequence that would indicate targeting to a secretory pathway. CNTF and some of the FGF's are probably released from cells when they are damaged, IL-1 is produced by recruited macrophages, and NGF is produced by Schwann cells and fibroblasts (Blottner and Baumgarten, 1994 for review).

Following axotomy of adult rodent motor neurons, retrograde transport of the neurotrophic factors either appears or increases, according to different "schedules" for different neurotrophic factors (Lindsay *et al.,* 1994). Pairs of neurotrophic factors have been found to act synergistically (Mitsumoto *et al.,* 1994; Nishi, 1994). The location and morphogenetic behavior of the ependymal cells make them a good candidate as a source of neurotrophic factors in the injured urodele spinal cord.

It will be important to separate the effects of ECM components produced by ependymal cells from those of trophic factors. In our preliminary experiments we have found no significant difference in neuronal survival on either fibronectin- or laminin-coated substrata, and addition of soluble fibronectin or laminin to the culture medium has no effect, suggesting that these two ECM components are not the critical products supplied by the ependymal cells (Chernoff, unpublished results). Other ECM components have not yet been tested. Of particular interest is proteoglycan production: in embryonic chick the permissive period for spinal cord repair is associated with a high ratio of heparan sulfate proteoglycan to chondroitin sulfate proteoglycan (Dow *et al.,* 1994).

It is not yet known whether ependymal mesenchyme cells exert a directional influence on axonal outgrowth. The configuration of the co-culture system (solid 2-dimensional substratum, liquid culture medium) would not permit maintenance of a directional signal. It is also unknown whether re-epithelialized ependymal cells produce a directional signal to guide axonal outgrowth through the ependymal channels.

The potential exists for an ependymal-derived chemoattractant effect on axon regrowth during spinal cord regeneration. Retinoic acid has been shown to elicit axonal outgrowth from axolotl spinal cord explants *in vitro* in a directional fashion (Hunter *et al.,* 1991), but an *in vivo* effect in spinal cord regeneration has not yet been identified. In the intact spinal cord of juvenile axolotls (4-8 cm long), cellular retinol-binding protein (CRBP) is found in the ependymal (radial glial) cell bodies surrounding the central canal as well as in the ventral floor plate (Hunter *et al.,* 1991). It is proposed that the radial glial cells (as well as floor plate glia acting on commissural neurons) sequester retinol and metabolize it to retinoic acid to promote neurite outgrowth. CRAPB (cellular retinoic acid-binding protein) is present in most axons within the axolotl spinal cord white matter, suggesting their susceptibility to retinoic acid.

The axolotl ependymal cell response to exogenous growth factors has been characterized *in vitro.* Our *in vitro* studies have shown a differential response of ependymal cells to a variety of growth factors. Fibroblast growth factor (FGF-2) had no effect, while EGF proved to be essential for proliferation and migration. Platelet-derived growth factor (PDGF) improves long- term survival in combination with EGF. Transforming growth factor-ß1 (TGF-ß1), as the sole trophic factor, eliminated even baseline levels of cell proliferation. The insulin-like growth factors (IGF-1 and 2) proved toxic in the insulin-containing medium used in these studies (Chernoff *et al.,* 1990; O'Hara and Chernoff, 1994). EGF is the most critical growth factor in ependymal mesenchyme proliferation and migration in culture, but the source of this factor for ependymal cells *in vivo* is not yet known.

Adult brain ependymal cells in humans and other mammals show very limited ability to regenerate following injury (Del Bigio, 1995; Sarnat, 1995), but mammalian (primarily rodent) ependymal cells are known to express receptors for a number of growth and trophic factors (reviewed in Del Bigio, 1995). Mammalian ependymal cells have receptors for basic fibroblast growth factor (bFGF), EGF, insulin-like growth factor and BDNF. Following injury, rodent ependymal cells upregulate bFGF production (reviewed in Del Bigio, 1995).

The involvement of immune system-derived cytokines is not known for urodele spinal cord regeneration. In teleost fish, however, an interaction involving injured neurons in the optic nerve and cytokines has been characterized. In fish optic nerve, the number of oligodendrocytes is down-regulated after injury due to processing of blood-borne factors. The fish neurons produce a transglutaminase that causes dimerization of the fish IL-2-like molecule. The dimer is toxic to oligodendrocytes, so the inhibition of axonal regrowth by oligodendrocytes seen in mammalian CNS injury does not occur (reviewed in Lotan and Schwartz, 1994). It is not clear whether this neuronal response is triggered directly in injured neurons or elicited by interaction with other neural cell populations.

The role of matrix degrading enzymes in spinal cord regeneration

Ultrastructural examination of regenerating lizard tail *in vivo* suggests that ependymal cells secrete a variety of materials during spinal cord regeneration (Alibardi and Sala, 1989). *Do these materials include proteases for ECM remodeling, as well as new ECM components and growth factors that are necessary to stimulate axon regrowth?* In order to understand the role of ependymal cells in urodele spinal cord regeneration it is necessary to characterize the materials that injury- reactive ependymal cells contribute to the regenerative environment.

One set of materials that is under analysis is the matrix metalloproteinases (MMP's), an important class of matrix-degrading enzymes. The MMP's require zinc for activity and calcium for substrate binding. They degrade extracellular matrix components, including collagens, fibronectin, laminin, and proteoglycans (Matrisian, 1990). MMP's are secreted in proenzyme form and must be activated by other proteases. Enzyme activity is affected by post-translational modification of the proenzymes and is inhibited by a class of glycoproteins called tissue inhibitors of metalloproteinases (TIMP's). The structure of MMP's, their substrate specificities, and specific TIMP's are

reviewed by Birkedal-Hansen *et al.* (1993) and by Woessner (1991).

In neural systems, the role of MMP's and their activators, such as plasminogen activator, has been described best *in vitro* in peripheral nervous system neurite outgrowth (Pittman, 1985; Pittman and Buettner, 1989; Muir, 1994), but these enzymes are present in the CNS as well. The first indication of the ability of ependymal cells to produce proteolytic enzymes was a set of experiments in which embryonic chick brain ependymal explants were observed to liquefy plasma clots in culture (Weiss, 1934). This proteolytic activity did not inhibit neurite outgrowth. In retrospect, digestion of the plasma clot suggests the production of plasminogen activator (PA). PA often works in concert with MMP's, but PA production has not yet been examined in regenerating spinal cord.

There is strong evidence from other amphibian tissue systems that suggests the involvement of MMP's in remodeling of the lesion site in the injured urodele spinal cord. The existence of MMP's was first described in the resorption of frog tadpole tail during metamorphosis (Gross and Lapiere, 1962; Grillo *et al.,* 1968). More recently these MMP's have been isolated and biochemically characterized (Oofusa and Yoshizato, 1991). The removal of the glia limitans, and the remodeling of meningeal collagen, strongly suggests a role for these enzymes in spinal cord regeneration.

Our laboratory is examining ependymal MMP production *in vivo* and *in vitro*. We are using a tissue culture system for ependymal mesenchyme cells (Chernoff *et al.,* 1990; O'Hara *et al.,* 1992) to detect secreted forms of the enzymes and tissue isolated *in situ* to examine zymogen forms of the enzymes. Culture on a fibronectin substratum in the presence of epidermal growth factor maintains the ependymal cells as a proliferating, migratory mesenchymal cell population (O'Hara and Chernoff, 1994). MMP activity is present in the ependymal outgrowth *in situ* as well as in cultured ependymal cells and ependymal cell conditioned medium. By contrast, MMP activity is undetectable in unlesioned adult cord (unpublished results). Provisional assignments have been made based on molecular weight and substrate specificity which suggest that MMP-2 (type IV collagenase, gelatinase A), MMP-9 (gelatinase/type IV collagenase) and MMP-1 (interstitial collagenase) are produced by ependymal mesenchyme cells. MMP-2 and MMP-9 could be degrading glia limitans components, while MMP-1 could be acting on meningeal matrix. Preliminary experiments suggest that MMP levels decline after axonal regrowth has occurred, and that TIMP levels rise at the time of, or just following, re- epithelialization of the ependymal cells. If the astrocytes described in urodele spinal cord by Zamora and Mutin (1988) share more than GFAP content with mammalian astrocytes, then the possibility exists that ependymal cells may inhibit reactive gliosis of astrocytes by removing chondroitin sulfate proteoglycan (CSPG) and cytotactin-tenascin associated with glial scars in higher vertebrates (McKeon *et al.,* 1991).

Removal of the meningeal matrix may involve the action of other cells in addition to the ependymal cells. Osteoclasts produce acid hydrolases and metalloproteinases (Everts *et al.,* 1992; Reponen *et al.,* 1994), and are involved in the remodeling of the damaged vertebral column in spinal cord-lesioned axolotls. Osteoclasts appear to be associated with the meningeal matrix within the lesion site: when meningeal matrix is not completely removed from ependymal cells in culture, osteoclasts attach to the culture substratum and form characteristic syncytia (Chernoff *et al.,* 1990). These cells may be involved in removal of the meningeal matrix, although this has not yet been tested.

Contributions from the leptomeningeal cells have also not yet been examined. In the later stages of the regeneration process the leptomeningeal cells become a multilayered sheath around the regenerated spinal cord (unpublished observation), presumably involved in rebuilding the meningeal matrix. They are a potential source of proteinase inhibitors.

Differences between tail amputations and tail cord regeneration

Are there significant differences in regeneration of the spinal cord following tail amputation and regeneration of spinal cord transected more caudally? Changes in ependymal cell organization and in the inductive effects of the spinal cord on surrounding tissue differ (Piatt, 1955; Egar and Singer, 1972; Stensaas, 1983). In regenerating tail the ependymal cells form a hollow vesicle that is continuous with the central canal of the more cranial intact spinal cord. The ependymal outgrowth occurs in the form of a tubular extension that does not lose its apical/basal polarity. The epithelial intercellular junctional complexes and basal lamina are maintained (Egar and Singer, 1981). Contact between the regenerating spinal cord and the tail stump wound epithelium result in induction of an epimorphic regeneration process similar to limb regeneration.

Arsanto *et al.* (1992) report that during newt tail regeneration, the ependymal cells in the ependymal tube of the regenerate are GFAP positive in immunohistochemical localization. Their Western Blot analysis with GFAP antibody shows bands at 52 kDa and 46 kDa, consistent with the molecular weight of GFAP in mammals. They suggest the possibility that the 52 kDa band may correspond to another intermediate filament protein. Holder *et al.* (1990) report GFAP from axolotls to produce bands at 64 kDa (major band) and 67 kDa (minor band), consistent with GFAP from other amphibia and from reptiles. They found cytokeratin antibody-binding bands at 58 kDa, 55 kDa, and 45 kDa (Holder *et al.,* 1990), consistent with the bands identified as GFAP by Arsanto *et al.* (1992). The absence of GFAP we see in regenerating lumbar spinal cord could thus be due to differences between regeneration of tail cord and other regions of the spinal cord, to differences between axolotls and newts, or to cross- reaction of the GFAP antibody in the newt studies with cytokeratins

It is highly likely that the studies of tail cord regeneration showing up-regulation of PS-N-CAM (Caubit *et al.,* 1993) and tenascin (Caubit *et al.,* 1994) will prove applicable to non- tail spinal cord regeneration in whole or part. Since the regenerating tail spinal cord remains epithelial, the major question is whether the expression of these two molecules is up-regulated in migrating mesenchymal ependymal cells in non-tail spinal cord, or only up-regulates following re-epithelialization. The possibility exists that the expression of one or both of these molecules will only occur when the injury-reactive ependymal cells have reepithelialized.

Triggering the ependymal response

What is the specific triggering mechanism for the urodele ependymal response? The signal for the ependymal cell reaction must lie in properties of the physiological microenvironment in the

lesion site following injury. As yet unidentified processes following injury must trigger changes within the ependymal cells leading to the onset of matrix remodeling, cell migration, and increased proliferation. The physiology of injured spinal cord has been extensively studied in mammals, and it is likely that many, if not all, of the events following the initial damage to cells in spinal cord injury in mammals also occur in urodeles: these include changes in calcium levels and in excitatory amino acids. It has been shown that extracellular calcium levels decrease and intracellular calcium levels increase following higher vertebrate spinal cord injury (Happel *et al.,* 1981; Stokes *et al.,* 1983). Electrophysiological examination of calcium activity in injured rat spinal cord suggests that cells within the dorsal grey matter are strongly implicated in the ionic events following injury (Moriya *et al.,* 1994). Using an *in vitro* system, Nedergaard (1994) showed that stimulation of increases in cytosolic calcium in individual astrocytes is propagated to neighboring astrocytes and to adhering neurons, providing an additional path for calcium-mediated toxic events following CNS injury. This astrocyte-propagated increase in cytosolic calcium is independent of extracellular calcium levels and is stimulated by glutamate. One major difference in the urodele and mammalian responses to spinal cord injury may lie in the reaction of ependymal cells to the lesion site micro-environment. As ependymal cells respond to the early events following injury, they may become a buffer between the neurons and the processes that trigger secondary cell death and axonal degeneration.

The embryonic character of urodele spinal cord

To what extent does urodele spinal cord regeneration depend on retention of embryonic characteristics? Holder and Clarke (1988) have suggested that spinal cord regeneration does indeed depend on embryonic characteristics. The most notable "embryonic" characteristic retained by ependymal cells in the intact cord of urodeles is the continued contact of ependymal endfeet with the pial basal lamina (the glia limitans). The epithelial character of salamander and newt ependymal cells is further emphasized by their epithelial cytokeratin content, including cytokeratins 8 and 18 (Holder *et al.,* 1990; O'Hara *et al.,* 1992). The pial contact is lost during embryonic or fetal development in mammals, and cytokeratin expression is found only in tanycytes in adult human spinal cord (Kasper *et al.,* 1991), and, faintly, in sheep and rat spinal cord (Bodega *et al.,* 1995).

Embryonic character has also been suggested from the ability of injury-reactive ependymal cells to re-express vimentin (O'Hara *et al.,* 1992) and polysialylated-N-CAM (Caubit *et al.,* 1993). The expression of vimentin in intact adult spinal cords is peculiarly species specific, with ependymal expression having no apparent phylogenetic pattern (Bodega *et al.,* 1994). In urodeles vimentin is reported to be mainly in tanycytes in newt spinal cord ependymal cells by Zamora and Mutin (1988), but not present at all by Bodega *et al.* (1994). No one proposes that expression of one or another intermediate filament protein is directly associated with spinal cord regeneration capacity, but these cytoskeletal proteins are useful markers within species for changes in response to injury.

Studies of clonal cultures of ependymal cells from regenerating newt spinal cord suggest embryonic character in the sense that the ependymal cells in this situation appear to be pluripotent (Benrais *et al.,* 1996). When lineage labeled regenerate tail ependymoglia were reimplanted into regenerating newt tail, the cells could differentiate into melanocytes and Schwann cells, which are normally of neural crest origin.

The role of continued neurogenesis in CNS regeneration is still debatable. There is a strong correlation in a variety of organisms between the ability of neural systems to regenerate and the persistence of neurogenesis (Holder and Clarke, 1988, for review). Newly born neurons are common in larval axolotls up to 7 cm in length, but are rare in unlesioned axolotls greater than 7.5 cm in length (juvenile to adult stages) (Holder *et al.,* 1991). *Does neurogenesis reappear during spinal cord regeneration in adult urodeles?* In teleost fish, studies of changes in intermediate filament content in regenerating tail found neurofilament containing, neurite-bearing cells from ependymal precursors (Anderson *et al.,* 1994). In urodeles, studies of regenerating adult newt spinal cord describe the presence of mitotic cells in a subependymal layer, presenting the possibility that ependymal cell progeny could form neuroblasts (Stensaas, 1983), but the ependymal cell progeny could also be glial populations such as the GFAP-positive sub-ependymal zone cells described by Zamora and Mutin (1988). During embryonic development, radial glia generate progeny that differentiate into neurons (Gray and Sanes, 1992, chick optic tectum). The persistence of contact of ependymal endfeet with the glia limitans, characterizing these cells as radial glia, has sustained the proposal that the adult urodele radial glia generate new neurons during regeneration (Holder *et al.,* 1990). The problem of neurogenesis was addressed directly in lizard tail spinal cord regeneration in metabolic radiolabeling studies, and no neurogenesis was observed (Duffy *et al.,* 1992). While there is a lingering possibility that new neurons are generated from ependymal cells during spinal cord regeneration, the major role of these cells in urodeles appears to be one of providing an environment that fosters axonal regeneration.

Relationship of urodele responses to those in higher vertebrates

A combination of factors prevents regeneration of CNS axons in neonates and adults of higher vertebrates. These factors include: toxic myelin breakdown products (Schwab, 1990); calcium influx and associated toxic reactions (Stokes *et al.,* 1983); glutamate excitotoxicity (Panter *et al.,* 1990); cholesterol depletion (arachidonic acid pathway; Saunders and Horrocks, 1987); and glial scars (Reier and Houle, 1988). Together, the extracellular environment and glial responses following CNS injury prevent axonal regrowth. Experimental evidence suggests that the CNS neurons of mammals, like their urodele counterparts, are capable of axonal regrowth in an environment with the appropriate adhesion molecules and free from toxic materials (reviewed in Bahr and Bonhoeffer, 1994). Urodele spinal cord regeneration can make an important contribution by defining the requirements for successful CNS regeneration through experimental manipulation of a regenerating adult system that allows examination of the cell interactions that elicit regeneration and suppress toxic reactions.

The following examples about lipid recycling, reactive astrocytic gliosis, oligodendrocytes, matrix turnover enzymes, and a possible ependymal response in embryonic birds and mammals, illustrate

some areas in which comparison of avian and mammalian CNS injury responses with parallel responses in lower vertebrates produces a more complete picture of the nature of a toxic reaction and strategies for combating it.

In mammals lipid recycling associated with regeneration occurs in the PNS (distal segment of sciatic nerve in rat). It is mediated by synthesis and secretion of the lipid-binding glycoprotein apolipoprotein E (apo E) by infiltrating macrophages. Apo E appears to be involved in formation of endoneural lipoprotein complexes mediating lipoprotein uptake into growth cones. In the spontaneously regenerating fish visual system, apolipoprotein A-I is produced in CNS in a process similar to the apo E response in mammals and plays a similar role to apo E in mammalian PNS. (See Lotan and Schwartz, 1994, for review).

In mammals, oligodendrocytes inhibit regeneration, and mammalian CNS myelin produces toxic breakdown products following injury (Schwab, 1990). It has been suggested that the myelin produced by fish oligodendrocytes does not have the toxic properties of mammalian CNS myelin. Fish myelin *in vitro* has little or no toxic effect on axonal regrowth (reviewed in Bahr and Bonhoeffer, 1994). In frogs, postmetamorphic spinal cord regeneration fails (Beattie, *et al.,* 1990), and it has recently been shown that frog CNS myelin (but not optic nerve myelin) is inhibitory to axonal outgrowth (Lang *et al.,* 1995). Comparable experiments have not yet been done in urodeles.

The inhibitory properties of mammalian reactive astrocytes include the lack of permissive cell-surface adhesion molecules, the absence of permissive extracellular matrix components, and the appearance of specific inhibitory proteoglycans following lesioning (reviewed in Bahr and Bonhoeffer, 1994). It is not clear whether reactive astrocytic gliosis or inhibition by oligodendrocytes is the most critical factor. Both cell populations probably are involved. To understand the fundamental differences in urodeles, it will be necessary to define the similarities and differences between avian or mammalian astrocytes and oligodendrocytes and urodele amphibian astrocyte-like cells and oligodendrocytes, and the cell-cell interactions they engage in during regeneration.

Further comparison of pre- and post-metamorphic anurans with and adult urodeles would be valuable with regard to many parameters of the regeneration process. There is a profound change in CNS regeneration capacity between urodele and anuran amphibians, and knowledge of the mechanism underlying that difference might be directly applicable to understanding the failure of CNS regeneration in birds and mammals. In particular, it would be useful to know whether the reactive gliosis seen in post-metamorphic frogs is truly astrocytosis (Beattie *et al.,* 1990). GFAP in frogs was found to be localized to peripheral processes of radial glia by Miller and Liuzzi (1986). Studies of intact adult toad spinal cord by Bodega *et al.* (1994) show GFAP positive cells surrounding blood vessels, which may be astrocytes, as well as some GFAP-positive ependymal cells.

Beneficial involvement of matrix degrading enzymes in higher vertebrate neurite outgrowth has been demonstrated using embryonic PNS neurons *in vitro* (Pittman, 1985; Muir, 1994). However, most examples of MMP or PA involvement in avian or mammalian CNS are deleterious effects following injury. Rosenberg *et al.* (1994) describe the induction of MMP-9 collagenase/gelatinase and urokinase (PA) production following hemorrhagic injury to rat brain. The resulting degradation of ECM compromises the blood-brain barrier. The source of the matrix degrading enzymes described in this study is not yet known. In non-neural cells in culture, calcium influx has been shown to up-regulate MMP-2 expression (Kohn *et al.,* 1994). This suggests a potential link between the known deleterious effects of calcium in higher vertebrate spinal cord with undesirable MMP production following injury. The sharp contrast this makes with the remodeling events in urodele spinal cord regeneration suggests that tissues beyond the immediate lesion site in the urodele spinal cord are protected from the effects of the MMP's produced by the ependymal cells. TIMP production in the cranial and caudal wound stumps should be investigated.

Urodeles display properties similar to those described in fish spinal cord regeneration. Injured axolotl cord does not form a glial scar, and there may be differences in the inflammatory response, as urodeles are slow to mount an immune response in a number of situations (such as graft rejection). Finally, there is the ependymal response in urodele amphibians that is a positive driving force, as there is in fish spinal cord and lizard tail regeneration.

An ependymal response in birds and mammals

Examination of published histological sections of transected avian and mammalian spinal cord suggests that there is an ependymal response during embryonic or fetal life. Shimizu *et al.* (1990) showed that spinal cord regeneration could occur in the chick until approximately day 15 of development. Hasan *et al.* (1993) identifies the end-point for regeneration of brainstem-spinal neurons projecting to the lumbar spinal cord as day 13 in chick embryos. In studies of axonal regrowth, Shimizu *et al.* (1990) described what appears to be an ependymal response among the non-neuronal cells at the lesion site. When embryonic chick spinal cord was transected at E5, nerve fibers crossed the transection 48 hours later. The transected cord fused back together. Reactive gliosis was absent but "a slightly disorganized architecture" was observed in the grey matter (Shimizu *et al.,* 1990). In cross sections this "disorganization" included loss of the central canal, suggesting changes in ependymal organization. In parasagittal sections a band of stained nuclei was seen at the site of fusion of the transected cord. We suggest that these are nuclei of injury-reactive ependymal cells. In experiments where embryonic rat spinal cord was transplanted into neonatal rats, Iwashita *et al.* (1994) described the healing-in of fetal cord grafts in which the central canal "disappears". The transformation of the ependymal cells from their normal epithelial form into a mesenchyme would account for this event. Together these studies suggest that an ependymal response may be involved in regeneration of embryonic spinal cord in amniote vertebrates.

Research to determine whether there is an ependymal response following transection of embryonic higher vertebrate spinal cord would be valuable to our understanding of both higher vertebrate and urodele spinal cord injury responses. Examination of changes in gene expression in ependymal cells and fibrous astrocytes during development and following injury would help our understanding of the onset of astrocyte reactive gliosis in birds and mammals. If an ependymal response can be confirmed in birds and mammals, this would also strengthen the view that urodele spinal cord regeneration as involves the retention or re-activation of embryonic potential in the ependymal population

would be strengthened. Examination of changes in cell-cell interactions, growth and trophic factor production, ECM, matrix turnover enzymes, and cell-cell adhesion components throughout the ependymal response in both lower and higher vertebrates would produce a more complete picture of the nature of an environment permissive for axonal regeneration. Understanding the ependymal response in adult urodeles would present the possibility of new experimental strategies in the treatment of mammalian spinal cord injury centered around the induction of an ependymal response or supplying materials produced by reactive ependymal cells.

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References

ALIBARDI, L. and MEYER-ROCHOW, V.B. (1988). Ultrastructure of the neural component of the regenerating spinal cord in the tails of three species of New Zealand lizards. *New Zealand J. Zool. 15:* 535-550.

ALIBARDI, L. and SALA, M. (1989). Ependymal fine structure and secretory activity during early phases of tail regeneration in lizard. *Arch. Ital. Anat. Embryol. 94:* 55-69

ANDERSON, M.J., CHOY, C.Y. and WAXMAN, S.G. (1986). Self-organization of ependyma in regenerating teleost spinal cord: Evidence from serial section reconstructions. *J. Embryol. Exp. Morphol. 96:* 1-18.

ANDERSON, M.J., ROSSETTO, D.L. and LORENZ, L.A. (1994). Neuronal differentiation *in vitro* from precursor cells of regenerating spinal cord of the adult teleost *Apteronotus albifrons. Cell Tissue Res. 278:* 243-248.

ARSANTO, J-P., KOMOROWSKI, T.E., DUPIN, F., CAUBIT, X., DIANO, M., GÉRAUDIE, J., CARLSON, B. and THOUVENY, Y. (1992). Formation of the peripheral nervous system during tail regeneration in urodele amphibians: ultrastructural and immuno- histochemical studies of the origin of the cells. *J. Exp. Zool. 264:* 273-292.

BAHR, M. and BONHOEFFER, F. (1994). Perspectives in axonal regeneration in the mammalian CNS. *Trends Neurosci. 17:* 473- 479.

BEATTIE, M.S., BRESNAHAN, J.C. and LOPATE, G. (1990). Metamorphosis alters the response to spinal cord transection in *Xenopus laevis* frogs. *J. Neurobiol. 21:* 1108-1122.

BENRAISS, A., CAUBIT, X., ARSANTO, J-P., COULON, J., NICOLAS, S., LE PARCO, Y. and THOUVENY, Y. (1996). Clonal cell cultures from adult spinal cord of the amphibian urodele *Pleurodeles waltl* to study the identity and potentialities of cells during tail regeneration. *Dev. Dynamics 205:* 135-149.

BIRKEDAL-HANSEN, H., MOORE, W.G.I., BODDEN, M.K., WINDSOR, L.J., BIRKEDAL-HANSEN, B., DECARLO, A. and ENGLER, J.A. (1993). Matrix metalloproteinases: a review. *Crit. Rev. Oral Biol. Med. 4:* 197-250.

BLOTTNER, D. and BAUMGARTEN, H.G. (1994). Neurotrophy and regeneration *in vivo. Acta Anat. 150:* 235-245.

BODEGA, G., SUAREZ, I., RUBIO, M. and FERNANDEZ, B. (1994). Ependyma: phylogenetic evolution of glial fibrillary acidic protein (GFAP) and vimentin expression in vertebrate spinal cord. *Histochemistry 102:* 113-122.

BODEGA, G., SUAREZ, I., RUBIO, M. and FERNANDEZ, B. (1995). Type II cytokeratin expression in the adult vertebrate spinal cord. *Tissue Cell 27:* 555-559.

CAUBIT, X., ARSANTO, J-P., FIGARELLA-BRANGER, D. and THOUVENY, Y. (1993). Expression of polysialylated neural cell adhesion molecule (PSA-N-CAM) in developing, adult and regenerating caudal spinal cord of the urodele amphibians. *Int. J. Dev. Biol. 37:* 327-336.

CAUBIT, X., RIOU, J.-F., COULON, J., ARSANTO, J-P., BENRAISS, A., BOUCAUT, J-C. and THOUVENY, Y. (1994). Tenascin expression in developing, adult and regenerating caudal spinal cord in the urodele amphibians. *Int. J. Dev. Biol. 38:* 661-672.

CHERNOFF, E.A.G., MUNCK, C.M., EGAR, M.W. and MENDELSOHN, L.G. (1990). Primary cultures of axolotl spinal cord ependymal cells. *Tissue Cell 5:* 601-613.

DAVIS, B.M., AYERS, J.L., KORAN, L., CARLSON, J., ANDERSON, M.C. and SIMPSON, S.B. Jr. (1990). Time course of salamander spinal cord regeneration and recovery of swimming: HRP retrograde pathway tracing and kinematic analysis. *Exp. Neurol. 108:* 198-213.

DAVIS, B.M., DUFFY, M.T. and SIMPSON, S.B. Jr. (1989). Bulbospinal and intraspinal connection in normal and regenerated salamander spinal cord. *Exp. Neurol. 103:* 41-51.

DEL BIGIO, M. (1995). The ependymal: a protective barrier between brain and cerebrospinal fluid. *Glia 14:* 1-13.

DOW, K.E., ETHELL, D.W., STEEVES, J.D. and RIOPELLE, R.J. (1994). Molecular correlates of spinal cord repair in the embryonic chick: heparan sulfate and chondroitin sulfate proteoglycans. *Exp. Neurol. 128:* 233-238.

DUFFY, M.T., LIEBICH, D.R., GARNER, L.K., HAWRYCH, A., SIMPSON, S.B. Jr. and DAVIS, B.M. (1992). Axonal sprouting and frank regeneration in the lizard tail spinal cord: correlation between changes in synaptic circuitry and axonal growth. *J. Comp. Neurol. 316:* 363-374.

EGAR, M. and SINGER, M. (1972). The role of ependyma in spinal cord regeneration in the urodele, *Triturus. Exp. Neurol. 37:* 422-430.

EGAR, M. and SINGER, M. (1981). The role of ependyma in spinal cord regrowth. In *Mechanisms of Growth Control* (Ed. R.O. Becker). Charles C. Thomas Publisher, Springfield, IL, pp. 93-106.

EGAR, M., SIMPSON S.B. and SINGER, M. (1970). The growth and differentiation of the regenerating spinal cord of the lizard, *Anolis carolinensis. J. Morphol. 131:* 131-152.

EVERTS V., DELAISSE J-M. KORPER W., NIEHOF, A., VAES, G., and BEERTSEN, W. (1992). Degradation of collagen in the bone-resorbing compartment underlying the osteoclast involves both cysteine-proteinases and matrix metalloproteinases. *J. Cell Physiol. 150:* 221-231.

GODSAVE, S.F., ANDERTON, B.H. and WYLIE, C.C. (1986). The appearance and distribution of intermediate filament proteins during differentiation of the central nervous system, skin and notochord of *Xenopus laevis. J. Embryol. Exp. Morphol. 97:* 201-223.

GRAY, G.E. and SANES, J.R. (1992). Lineage of radial glia in the chick optic tectum. *Development 114:* 271-383.

GRILLO, H.C., LAPIERE, C.M., DRESDEN, M.H. and GROSS, J. (1968). Collagenolytic activity in regenerating forelimb of the adult newt *(Triturus viridescens). Dev. Biol. 17:* 571-583.

GROSS, J. and LAPIERE, C.M. (1962). Collagenolytic activity in amphibian tissues: a culture assay. *Proc. Natl. Acad. Sci. USA 48:* 1014-1022

HAPPEL, R.D., SMITH, K., BANIK, N.L., POWERS. J.A., HOGAN, E.L. and BALENTINE, J.D. (1981). Ca^{2+}-accumulation in experimental spinal cord trauma. *Brain Res. 211:* 476-479.

HASAN, S.J., KEIRSTEAD, H.S., MUIR, G.D. and STEEVES, J.D. (1993). Axonal regeneration contributes to repair of injured brainstem-spinal neurons in embryonic chick. *J. Neurosci. 13:* 492-507.

HOLDER, N. and CLARKE, J.D.W. (1988). Is there a correlation between continuous neurogenesis and directed axon regeneration in the vertebrate nervous system? *Trends Neurosci. 11:* 94-99.

HOLDER, N., CLARKE, J.D.W., KAMALATI, T. and LANE, E.B. (1990). Heterogeneity in spinal radial glia demonstrated by intermediate filament expression and HRP labelling. *J. Neurocytol. 19:* 915- 928.

HOLDER, N., CLARKE, J.D.W., STEPHENS, N., WILSON, S.W., ORSI, C., BLOOMER, T. and TONGE, D.A. (1991). Continuous growth of the motor system in the axolotl. *J. Comp. Neurol. 303:* 534-550.

HUNTER, K., MADEN, M., SUMMERBELL, D., ERIKSSON, U., and HOLDER, N. (1991). Retinoic acid stimulates neurite outgrowth in the amphibian spinal cord. *Proc. Natl. Acad. Sci. USA 88:* 3666-3670.

IWASHITA, Y., KAWAGUCHI, S. and MURATA, M. (1994). Restoration of function by replacement of spinal cord segments in the rat. *Nature 367:* 167-170.

KASPER, M., PERRY, G. and STOSIEK, P. (1991). Cytokeratin expression in human spinal meninges and ependymal cells. *J. Hirnforsch. 32:* 19-25.

KOHN, E.C., JACOBS, W., KIM, Y.S., ALESSANDRO, R., STETLER-STEVENSON, W.G. and LIOTTA, L.A. (1994). Calcium influx modulates expression of matrix metalloproteinase-2 (72 kDa type IV collagenase/gelatinase A). *J. Biol. Chem. 269:* 21505-21511.

LANG, D.M., RUBIN, B.P., SCHWAB, M.E., and STUERMER, C.A.O., (1995). CNS myelin and oligodendrocytes of the *Xenopus* spinal cord — but not optic nerve — are non-permissive for axon outgrowth. *J. Neurosci. 15:* 99-109.

LINDSAY, R.M., WIEGAND, S.J., ALTAR, C.A. and DISTEFANO, P.S. (1994). Neurotrophic factors: from molecule to man. *Trends Neurosci. 17:* 182-189.

LOTAN, M. and SCHWARTZ, M. (1994). Cross talk between the immune system and the nervous system in response to injury: implications for regeneration. *FASEB J. 8:* 1026-1033.

MATRISIAN, L.M. (1990). Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet. 6:* 121-125.

MCKEON, R.J., SCHREIBER, R.C., RUDGE, J.S. and SILVER, J. (1991). Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of two inhibitory molecules in reactive astrocytes. *J. Neurosci. 11:* 3398-3411.

MILLER, R.H. and LIUZZI, F.J. (1986). Regional specialisation of the radial glial cells of the adult frog spinal cord. *J. Neurocytol. 15:* 187-196.

MITSUMOTO, H., IKEDA, K., KLINKOSZ, B., CEDARBAUM, J.M., WONG, V. and LINDSAY, R.M. (1994). Arrest of motor neuron disease in wobbler mice co-treated with CNTF and BDNF. *Science 265:* 1107-1110.

MORIYA, T., HASSAN, A.Z., YOUNG, W. and CHESLER, M. (1994). Dynamics of extracellular calcium activity following contusion of the rat spinal cord. *J. Neurotrauma 11:* 255-263.

MUIR, D. (1994). Metalloproteinase-dependent neurite outgrowth within a synthetic extracellular matrix is induced by nerve growth factor. *Exp. Cell Res. 210:* 243-252.

NEDERGAARD, M. (1994). Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science 263:* 1768-1171.

NISHI, R. (1994). Neurotrophic factors: two are better than one. *Science 265:* 1052-1053.

O'HARA, C.M. and CHERNOFF, E.A.G. (1994). Growth factor modulation of injury-reactive ependymal cell proliferation and migration. *Tissue Cell 26:* 599-611.

O'HARA, C.M., EGAR, M.W. and CHERNOFF, E.A.G. (1992). Reorganization of the ependyma during axolotl spinal cord regeneration: changes in intermediate filament and fibronectin expression. *Dev. Dynamics 193:* 103-115.

OOFUSA, K. and YOSHIZATO, K. (1991). Biochemical and immunological characterization of collagenase in tissues of metamorphosing bullfrog tadpoles. *Dev. Growth Differ. 33:* 329-339.

PANTER, S.S., YUM, S.W. and FADEN, A.I. (1990). Alteration in extracellular amino acids after traumatic spinal cord injury. *Ann. Neurol. 27:* 96-99.

PIATT, J. (1955). Regeneration in the central nervous system of amphibia. In *Regeneration in the Central Nervous System* (Ed. W.F. Windle). C. Thomas Publishing Co., Springfield, IL, pp. 20-46.

PITTMAN, R.N. (1985). Release of plasminogen activator and a calcium-dependent metalloproteinase from cultured sympathetic and sensory neurons. *Dev. Biol. 110:* 91-101.

PITTMAN, R.N. and BUETTNER, H.M. (1989). Degradation of extracellular matrix by neuronal proteases. *Dev. Neurosci. 11:* 361-375.

QIU, L., BERND, P. and FUKADA, K. (1994). Cholinergic neuronal differentiation factors (CDF)/Leukemia Inhibitory Factor (LIF) binds to specific region of the developing nervous system *in vivo. Dev. Biol. 163:* 516-520.

REIER, P.J. and HOULE, J.D. (1988). The glial scar: its bearing on axonal elongation and transplantation approaches to CNS repair. *Adv. Neurol. 47:* 87-138.

REPONEN, P., SAHLBERG, C., MUNAUT, C., THESLEFF, I. and TRYGGVASON, K. (1994). High expression of a 92kD type IV collagenase (gelatinase B) in the osteoclast lineage during mouse development. *J. Cell Biol. 124:* 1091-1102.

RIOU, J-F., UMBAUER, M., SHI, D-L. and BOUCAUT, J-C. (1992). Tenascin: a potential modulator of cell-extracellular matrix interactions during vertebrate embryogenesis. *Biol. Cell 75:* 1-9

ROOTS, B.I. (1986). Phylogenetic development of astrocytes. In *Astrocytes. Development, Morphology and Regional Specialization of Astrocytes*, Vol. 1 (Eds. S. Federoff and A. Vernadakis). Academic Press, New York, pp. 1-34.

ROSENBERG, G.A., DENCOFF, J.E., MCGUIRE, P.G., LIOTTA, L. and STETLER-STEVENSON, W.C. (1994). Injury-induced 92-kilodalton gelatinase and urokinase expression in rat brain. *Lab. Invest. 71:* 417-422.

RUTISHAUSER, U. (1989). Polysialic acid as a regulator of cell interactions. In *Neurobiology of Glycoconjugates* (Eds. R.U. Margolis and R.K. Margolis). Plenum Publishing, New York, pp. 367-382.

SARNAT, H.B. (1995). Ependymal reactions to injury: a review. *J. Neuropathol. Exp. Neurol. 54:* 1-15.

SAUNDERS, R. and HORROCKS, L.A. (1987). Eicosanoids, plasma membranes, and molecular mechanisms of spinal cord injury. *Neurochem. Pathol. 7:* 1-22.

SCHWAB, M.E. (1990). Myelin-associated inhibitors of neurite growth and regeneration in the CNS. *Trends Neurosci. 13:* 452- 456.

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.

SIMPSON, S.B. Jr. (1968). Morphology of the regenerated spinal cord in the lizard, Anolis carolinensis. *J. Comp. Neurol. 134:* 193-210.

SINGER, M., NORDLANDER, R.H. and EGAR, P. (1979). Axonal guidance during embryogenesis and regeneration in the spinal cord of newt: the blueprint hypothesis of neuronal pathway patterning. *J. Comp. Neurol. 185:* 1-22.

STENSAAS, L.J. (1983). Regeneration in the spinal cord of the newt *Notopthalmus (Triturus) pyrrhogaster.* In *Spinal Cord Reconstruction* (Eds. C.C. Kao, R.P. Bunge and P.J. Reier). Raven Press, New York, pp. 121-149.

STOCUM, D.L. (1995). Tissue regeneration. In *Wound Repair, Regeneration and Artificial Tissues.* R.G. Landes Publishing Company, Austin, Texas, pp. 21-50.

STOKES, B.T., FOX, P. and HOLLINDEN, G. (1983). Extracellular calcium activity in the injured spinal cord. *Exp. Neurol. 80:* 561-572.

WEISS, P. (1934). Secretory activity of the inner layer of the embryonic mid-brain of the chick as revealed by tissue culture. *Anat. Rec. 58:* 299-302.

WOESSNER, J.F. Jr. (1991). Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J. 5:* 2145- 2154.

ZAMORA, A.J. and MUTIN, M. (1988). Vimentin and glial fibrillary acidic protein in radial glia of the adult urodele spinal cord. *Neuroscience 27:* 279-288.