In compliance with the
Canadian Privacy Legislation
some supporting forms
have been removed from
this dissertation.

While these forms may be included
in the document page count,
their removal does not represent
any loss of content from the dissertation.

Receptor Protein Tyrosine Phosphatase Sigma (RPTPσ) Inhibits Axonal Regeneration and the Rate of Axon Extension

Katherine M. Thompson
Department of Neurology and Neurosurgery
McGill University, Montreal

July 2002

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Master's of Science

Your file Votre référence
ISBN: 0-612-85829-4
Our file Notre référence
ISBN: 0-612-85829-4

Acknowledgements

A graduate student once told me that the lab would become my home and my labmates, my family. While at the time I thought he was crazy, I now know that he was correct. I owe many thanks to many people for their guidance and assistance in both academic and non-academic ways.

Firstly, I thank Tim, for providing me with the opportunity to study and for giving me both guidance and freedom to perform my research.

I am indebted to Colleen Manitt, who has spent countless hours showing me her technical expertise, with whom I have had many insightful discussions (academic and not), and from whom I have learned the meaning of research. Lolo – À ma première amie à Montréal, merci pour ton amitié et pour tout ton appui. To Cecilia, thank you for reminding me to always keep my goals in sight and for making me laugh. To the rest of the lab, Andrew, Jackie, Jean-François, Marc, Masoud, Nic, Ron and Simon, thanks for making the lab more than a place to work. I thank Noriko, whose contributions to this project are invaluable. To my peers in the CNS, thank you for listening and for taking me away from the lab and keeping me sane.

I thank Dr. Sam David for his advice as my committee member, and Dr. Michel Tremblay for his enthusiasm and support, and without whom this project would not exist.

I thank Mom, Dad, Christy, Terry, Nanny and Grandad, for their support in all my endeavors, however big or small.

Most of all, I thank Scot, who pushed me to pursue my love of research. At every step of this journey, you have been there, making the path so much easier.

Contributions of Authors

Chapter 2: "Receptor Protein Tyrosine Phosphatase Sigma (RPTPσ) inhibits axon growth during development and regeneration", a manuscript that has been submitted to the journal Molecular Cellular Neuroscience, is a collaboration between the labs of Dr. Tim Kennedy and Dr. Michel Tremblay and is co-authored by several individuals from these labs; Noriko Uetani [providing a continual supply of reagents, maintenance of the mouse colonies, mouse genotyping], Colleen Manitt [extensive technical assistance and *in situ* hybridization cRNA probe synthesis], Mounib Elchebly [monoclonal RPTPσ antibody 17G7.2 generation].

Abstract

Transgenic mice lacking RPTPσ, a type IIa receptor protein tyrosine phosphatase, exhibit severe neural-developmental deficits. Continued expression of RPTPσ in the adult suggests that it plays a functional role in the mature nervous system. To determine if RPTPσ might influence axonal regeneration, the time course of regeneration following facial nerve crush in wild type and RPTPσ (-/-) mice was compared. Mice lacking RPTPσ exhibited an accelerated rate of functional recovery, suggesting that RPTPσ slows the extension of regenerating axons. We detected a decrease in RPTPσ expression by facial motoneurons following nerve crush in wild type mice. Consistent with this, we show that the rate of axon extension is enhanced in neurons obtained from RPTPσ (-/-) mice. Furthermore, in wild type mice, RPTPσ is enriched in axonal growth cones. These findings indicate that RPTPσ slows axon growth via a mechanism intrinsic to the neuron and identify a role for RPTPσ in regulating axonal regeneration.

Résumé:

Les souris transgéniques dont le récepteur tyrosine phosphatase de type IIa RPTPσ est manquant manifestent des troubles sévères du développement neuronal. RPTPσ est également exprimé dans le système nerveux de l'animal adulte, ce qui suggère qu'il y joue un rôle fonctionnel. Afin de déterminer si RPTPσ peut influencer la régénération axonale, le décours temporel de la régénération du nerf facial après compression chez des souris sauvages et RPTP(-/-) a été comparé. Les souris ayant une délétion du gène codant pour RPTPσ ont une plus grande vitesse de régénération fonctionnelle, suggérant que RPTPσ ralentit la régénération axonale. Nous avons aussi détecté une baisse de l'expression de RPTPσ dans les motoneurones faciaux chez les souris sauvages ayant subit une compression du nerf facial. En accord avec cette observation, nous démontrons que la vitesse d'extension axonale est accélérée dans les neurones de souris RPTPσ(-/-). De plus, chez les souris sauvages, RPTPσ est concentré au niveau des cônes de croissance. Ces observations indiquent que RPTPσ ralentit la croissance axonale par un mécanisme neurone-spécifique et démontrent l'importance de RPTPσ dans la régulation de la régénération axonale.

(Traduction du résumé par Jean-François, Laurence, et Nic.)

Table of Contents

Introduction

During neural development neurons must extend axons great distances to establish functional connections. The motile tip of the extending axon, the neuronal growth cone, navigates using a host of cell surface receptors that allow it to respond to various cues in its environment. Signals from extracellular cues are transduced via intracellular signaling cascades and effector molecules to the growth cone cytoskeleton. The reorganization of the cytoskeleton underlies growth cone motility and guidance (reviewed by Tanaka and Sabry, 1995).

In the injured nervous system, functional recovery requires that axons regenerate and reestablish target connections. Mechanisms similar to those regulating axon growth and guidance during development likely regulate regenerative growth. In support of this, several factors that influence axon guidance in the developing nervous system continue to be expressed in the adult nervous system following injury.

Receptor protein tyrosine phosphatase sigma (RPTPσ) is a member of the type IIa family of RPTPs that is highly expressed in the developing mammalian nervous system (Schaapveld et al., 1998; Wang et al., 1995; Yan et al., 1993). Genetic analyses in *D. melanogaster* provided the initial evidence that type IIa receptor protein tyrosine phosphatases (RPTPs) influence axon outgrowth and guidance during development (Desai et al., 1997a; 1996; Krueger et al., 1996). Transgenic mice lacking RPTPσ expression exhibit severe neural defects, demonstrating the importance of this protein in nervous system development (Elchebly et al., 1999; Wallace et al., 1999). RPTPσ continues to be expressed in the adult, suggesting an additional role for this protein in the mature nervous system (Schaapveld et al., 1998; Wang et al., 1995; Yan et al., 1993). Of particular interest,

RPTPσ is expressed in regions of the adult nervous system associated with synaptic plasticity, sprouting, and regeneration.

Our goal was to investigate the influence of RPTPσ on axon growth and regeneration following injury. We used transgenic mice deficient in RPTPσ as a tool to investigate this question using both *in vivo* and *in vitro* assays. This thesis addresses the following specific aims: I) To investigate the influence of RPTPσ on the rate of nerve regeneration using a well-characterized *in vivo* model of PNS injury, facial nerve crush; II) To investigate whether RPTPσ expression is regulated following peripheral nerve injury (facial nerve crush); III) To determine whether RPTPσ directly influences axon growth by regulating the ability of a neuron to extend an axon.

Here, we report that RPTPσ delays functional recovery following injury. Furthermore, we provide evidence that RPTPσ regulates the rate at which a neuron extends an axon. Our results indicate that RPTPσ influences axon regeneration in the peripheral nervous system (PNS) and provide the basis for investigating the role of RPTPσ during axonal regeneration in the central nervous system (CNS).

Chapter 1. Literature Review

1. Axon Guidance

1.1 Axon Guidance Mechanisms

Development of the nervous system is dependent on the ability of neurons to extend axons over great distances to establish synaptic connections. The neuronal growth cone navigates using cell surface receptors that respond to extracellular guidance cues. Studies investigating the molecular basis of axon growth and guidance have identified a multitude of factors that can act as long-range diffusible cues to attract or repel growth cones, or as short range, contact dependent cues that are either permissive or inhibitory for growth (reviewed by Tessier-Lavigne and Goodman, 1996). While guidance cues were once characterized into distinct categories, it is now clear that the response of a growth cone to a particular extracellular cue is far more complex, and is dependent not only on the particular cue, but also on the intrinsic state of the growth cone. For example, the intracellular levels of adenosine 3'5'-monophosphate (cAMP) and guanosine 3'5'-monophosphate (cGMP) dictate the response of a cell to neurotrophic factors such as BDNF and NGF (Song et al., 1997). This is also the case for axon guidance cues such as netrins and semaphorins, and for factors that inhibit regeneration such as myelin and MAG (Cai et al., 2001; Song et al., 1998; Ming et al., 1997). Use of a competitive analogue of cAMP or an inhibitor of Protein Kinase A (PKA) converts attraction induced by netrin-1, BDNF, or NGF into repulsion; and conversely, elevation of cAMP or cGMP converts repulsion by MAG/myelin or SemaIII, respectively, into attraction (Cai et al., 2001; Song et al., 1998; 1997; Ming et al., 1997). Thus, the response of a growth cone to a particular

cue will depend not only on the extracellular guidance cues it encounters, but also on the presence of other factors in its environment that may modulate intracellular cAMP/cGMP levels. In support of this, treatment of neurons with neurotrophic factors elevates intracellular cAMP levels and allows these neurons to grow on substrates of myelin and MAG (Cai et al., 1999).

1.2 Growth Cone Cytoskeletal Dynamics

Substantial evidence indicates that extracellular cues influence motility by remodeling the actin cytoskeleton of the growth cone (reviewed by Suter and Forscher, 2000; Tanaka and Sabry, 1995). The neuronal growth cone can be divided into three cytoplasmic domains, the peripheral, the central, and the transitional domain. Finger-like projections, termed filopodia, and cytoplasmic-veils, called lamellipodia are present in the growth cone's peripheral domain. Filopodia and lamellipodia continuously extend and retract as they probe their environment for local growth and guidance cues. Contact of a single filopodium with an extracellular cue is sufficient to modulate the path of an extending axon (O'Connor et al., 1990; Chien et al., 1993). The transitional domain of the growth cone is located between the peripheral domain and the central domain, which contains the organelles of the growth cone.

Both filopodia and lamellipodia are composed of filamentous actin (F-actin), arranged in polarized bundles and interwoven networks. F-actin is highly dynamic. The assembly of F-actin occurs at the leading edge of growth cones, in lamellipodia and filopodia. Actin filaments are continuously translocated backwards towards the central domain via retrograde flow, powered by myosin motors. These filaments are

disassembled and F-actin is recycled in the transitional domain (reviewed in Suter and Forscher, 2000). Microtubules are the predominant cytoskeletal component of the central domain of the growth cone. Like F-actin, microtubules are also highly dynamic structures that extend and retract from the central to the peripheral domain of the growth cone (Tanaka et al., 1995).

1.3 Connecting Extracellular Cues into Directed Growth

Following the identification of a large number of extracellular guidance cues and their receptors, research has now focused on the intracellular signaling mechanisms that link the cell surface with the growth cone cytoskeleton. The "substrate-cytoskeleton coupling" model is a current theory for how extracellular substrate-bound cues can regulate axon growth (Suter and Forscher, 1998; Lin et al., 1994; Mitchison and Kirschner, 1988). In this model, the coupling of a fixed substrate via cell surface receptors to actomyosin motility allows for forward extension and growth. Functional linkage between the substrate and F-actin results in the attenuation of retrograde F-actin flow and the resulting tension pulls the central domain of the growth cone forward. Simultaneously, F-actin assembly continues at the leading edge resulting in forward growth. Microtubule extension occurs concurrently further promoting growth cone advance (Suter and Forscher, 1998).

Increasing evidence demonstrates that several growth and guidance factors may regulate the actin cytoskeleton via the Rho family of small GTPases. The Rho GTPases, RhoA, Rac, and Cdc42, are key regulators of the actin cytoskeleton in both non-neuronal and neuronal cells (reviewed in Dickson et al., 2001; Luo et al., 1997; Hall, 1998). RhoA is responsible for the formation of stress fibers and promotes

actin-myosin contractility resulting in growth cone collapse and repulsion. In contrast, the protrusive movement of the growth cone is mediated primarily by Rac and Cdc42, which regulate lamellipodia and filopodial extension, respectively (reviewed by Luo et al., 1997). The activity of the Rho GTPases has been implicated in the signaling pathways of several growth and guidance cues including the netrins (Shekerabi and Kennedy, 2002); semaphorins (reviewed in Liu and Strittmatter, 2001); ephrins (Wahl et al., 1999); the slit family (Wong et al., 2001), and the integrins (Schoenwaelder and Burridge, 1999). The mechanisms by which these cues and their receptors regulate Rho GTPase activity and the subsequent signaling to the actin cytoskeleton are presently being elucidated (reviewed by Dickson, 2001).

2. The Type IIa Family of Receptor Protein Tyrosine Phosphatases (RPTPs)

2.1 Protein Tyrosine Phosphatases (PTPs)

The protein tyrosine phosphatases (PTPs) represent a large family of structurally diverse enzymes that together with the protein tyrosine kinases (PTKs) regulate intracellular phosphotyrosine levels. All PTPs possess at least one conserved core of 240 amino acids, which defines their catalytic domain. A well-conserved signature motif within this core, (I/V)HCxAGxxR(S/T)G, contains a cysteine residue essential for phosphatase activity (Barford et al., 1994). The PTPs can broadly be divided into cytoplasmic and transmembrane receptor-like molecules (RPTPs). The receptor protein tyrosine phosphatases can further be classified into several subgroups (I, IIa, IIb, III, IV, and V; Figure 1) based on the structure of their extracellular domains (Brady-Kalney and Tonks, 1995).

2.2 The type IIa RPTP family

The type IIa family of RPTPs consists of three mammalian members, LAR (Streuli et al., 1988), RPTPσ (Ogata et al., 1994; Wagner et al., 1994; Pan et al., 1993; Walton et al., 1993; Yan et al., 1993; Zhang et al., 1994) and RPTPδ (Pulido et al., 1995a; Mizuno et al., 1994; 1993). Type IIa RPTPs identified in non-mammalian vertebrate species include chicken and *Xenopus* CRYPα (Johnson and Holt, 2000; Stoker et al., 1994). DLAR and DPTP69D are both type IIa RPTPs identified in *D. melanogaster* (Streuli et al., 1989). DLAR is homologous with all three mammalian type IIa RPTPs (Streuli et al., 1989). A mammalian homologue of DPTP69D has not been identified. Two type IIa RPTPs, HmLAR1 and HmLAR2 have been identified in *H. medicinalis*, the medicinal leech (Gershon et al., 1998a).

2.3 Type IIa RPTP structure, alternative splicing, proteolytic processing

Members of the type IIa family of RPTPs are characterized by an extracellular domain that consists of Ig-like domains in series with fibronectin type III (FN(III)) domains. Thus type IIa RPTPs are members of the Ig-superfamily and resemble cell adhesion molecules such as NCAM and L1 (reviewed by Walsh and Doherty, 1997). The extracellular domain of these proteins is connected via a transmembrane domain to an intracellular domain containing two phosphatase domains, termed membrane proximal D1 and membrane distal D2 (reviewed by Stoker and Dutta, 1998).

Multiple isoforms of mammalian type IIa RPTPs, LAR, RPTPσ, and RPTPδ, are generated as a result of alternative RNA splicing (Figure 2). Certain isoforms have a tissue-specific expression pattern that appears to be developmentally regulated. The presence of alternatively spliced segments may affect interactions with

extracellular ligands and binding proteins, as has been demonstrated for LAR-laminin-nidogen binding (see below), as well as interactions with intracellular proteins.

Mammalian type IIa RPTPs are expressed at the cell surface as a complex of two non-covalently associated subunits (Figure 3). Careful analysis of the biosynthesis and proteolytic processing of these proteins reveals that the precursor of all three mammalian family members is cleaved at a site amino terminal to the transmembrane domain to yield an extracellular E-subunit, and a P-subunit composed of a small portion of the extracellular domain, the transmembrane domain, and the intracellular domain (Aicher et al., 1997; Pulido et al., 1995a; Serra-Pages et al., 1994; Streuli et al., 1992). A penta-arginine site located at the cleavage site suggests that the precursor is processed by a furin or furin-like endoprotease (Streuli et al., 1992). Interestingly, the proteolytic processing is not required for cell surface expression, as LAR isoforms expressing mutations that inhibit proteolysis are still transported to the cell surface (Streuli et al., 1992). The extracellular domains of type IIa RPTPs can be shed via a second proteolytic event at a site amino terminal to the transmembrane domain within the small extracellular domain of the P-subunit (Aicher et al., 1997; Serra-Pages et al., 1994; Streuli et al., 1992). Shedding of the E-subunit is not simply the result of subunit dissociation (Serra-Pages et al., 1994) and appears to be a highly regulated process. Extracellular domain shedding occurs when cells are grown at high density or can be induced by treatment of cells with compounds that activate Protein Kinase C (PKC) (Aicher et al., 1997; Serra-Pages et al., 1994; Streuli et al., 1992). Internalization and redistribution of the P-subunit of

these phosphatases occurs following E-subunit shedding. Although the functional significance of the two-subunit structure and shedding of the extracellular domain has not been demonstrated, it is likely a means of regulating the activity of this family of phosphatases.

3. Receptor Protein Tyrosine Phosphatase Sigma (RPTPσ)

Receptor protein tyrosine phosphatase sigma (RPTPσ) was originally identified by several groups independently as a novel RPTP highly expressed in the nervous system. Orthologues have been identified in mouse (Ogata et al., 1994; Wagner et al., 1994), rat (Pan et al., 1993; Walton et al., 1993; Yan et al., 1993; Zhang et al., 1994), human (Pulido et al., 1995b), chicken (CRYPα) (Stoker et al., 1995; Stoker, 1994), and *Xenopus* (CRYPα) (Johnson and Holt, 2000).

3.1 RPTPσ mRNA Expression

During embryonic development, RPTPσ mRNA is highly expressed throughout the CNS and in specific regions in the PNS. The highest levels of RPTPσ mRNA expression in the CNS were observed in the cortical plate and the subventricular zone of the developing forebrain (Schaapveld et al., 1998; Wang et al., 1995). Expression was also observed in the developing retina and in the subventricular zone of the midbrain and hindbrain. In the PNS, RPTPσ is expressed in the DRGs, the olfactory epithelium, and in several cranial nerve ganglia, including the facial nucleus (Schaapveld et al., 1998; Wang et al., 1995). Outside the developing nervous system, RPTPσ is expressed at much lower levels in the developing lungs and kidneys (Wang et al., 1995).

RPTPσ mRNA continues to be expressed throughout the adult nervous system (Schaapveld et al., 1998; Wang et al., 1995). The highest areas of RPTPσ mRNA expression include the hippocampal formation, the olfactory bulb, and the cerebellum. RPTPσ mRNA expression was observed in the pyramidal cells of the hippocampus, the granule cell layer of the dentate gyrus, the mitral cell layer of the olfactory bulb, and the internal granule cell layer and Purkinje cell bodies of the cerebellum (Schaapveld et al., 1998; Wang et al., 1995). High to moderate levels of mRNA expression were observed in several basal forebrain nuclei and in the piriform cortex (Wang et al., 1995). Moderate levels of RPTPσ mRNA expression were observed in several other regions of the adult brain including the anterodorsal thalamic nucleus, the habenula and the posteromedial amygdala, and several brainstem nuclei, including the oculomotor nucleus, the red nucleus, the locus coeruleus, the lateral vestibular nucleus, the dorsal tegmental nucleus, the mesencephalic nucleus, the hypoglossal nucleus, the facial nucleus, and the trigeminal nuclei (Wang et al., 1995).

It was clear from the above studies that several populations of neurons express RPTPσ mRNA. Evidence has been provided that RPTPσ is also expressed by glial cells *in vitro*. Northern blot analyses revealed the presence of RPTPσ mRNA in primary mixed glial cells cultures from P4 rat forebrain, which contain astrocytes, O2A progenitors, and microglia (Wang et al., 1995). Further characterization of the glial cell populations expressing RPTPσ *in vivo* has not been reported.

The analyses described above demonstrate using *in situ* hybridization and Northern Blot analysis that RPTPσ mRNA is widely expressed throughout the

developing and mature nervous system. The distribution of RPTPσ protein has not yet been demonstrated.

3.2 A comparison of the expression of RPTPσ, LAR, and RPTPδ

All three mammalian members of the type IIa family of RPTPs are expressed in the developing and adult nervous system, however temporal and spatial differences in their expression profiles exist. The highest levels of LAR expression occurs in non-neuronal tissue such as the lung, liver, heart, muscle, kidney, pancreas, and thymus, although it is also expressed in the developing and adult nervous system (Schaapveld et al., 1998; Sahin et al., 1995; Wang et al., 1995; Longo et al., 1993). During embryonic development, LAR is expressed at high levels in the ventricular zone of the forebrain. Much lower levels of expression were observed in the cortical layers where postmitotic neurons are located (Sahin et al., 1995). LAR mRNA expression in the midbrain and the spinal cord was also observed. In the PNS, LAR mRNA expression was observed in the DRGs and the trigeminal ganglion (Wang et al., 1995). In the adult CNS, LAR mRNA expression was observed in neurons in the pyramidal cells of the hippocampus and the granule cell layer of the dentate gyrus; the internal granule cell layer of the olfactory bulb; the external and internal granule layers and Purkinje cell bodies of the cerebellum; deep cerebellar nuclei, subcortical nuclei, the basal forebrain nuclei, the oculomotor nucleus, and the vestibular nuclei (Schaapveld et al., 1998; Sahin et al., 1995; Wang et al., 1995; Longo et al., 1993). LAR was also expressed in non-neuronal cells in the ependymal layers lining the ventricular system and in astrocytes cultured from postnatal day 2 rat cortex (Schaapveld et al., 1998; Longo et al., 1993).

During embryonic development, strong RPTPδ mRNA expression was observed in the cortical layers of the forebrain, midbrain, and hindbrain, but not in the subventricular zone (Sommer et al., 1997). Expression was observed in the developing pituitary gland, the thalamic nuclei, and weakly in the developing hippocampus (Schaapveld et al., 1998; Sommer et l., 1997). In the PNS, low levels of RPTPδ expression were observed in the olfactory epithelium and expression was not detected in the DRGs (Schaapveld et al., 1998). Postnatally, RPTPσ was primarily detected in the pyramidal cell layer of the hippocampus, the mitral and glomerular layers of the olfactory bulb, the thalamic nucleus, and the pyramidal cell layer of the piriform cortex (Schaapveld et al., 1998; Sommer et al., 1997; Mizuno et al., 1993). Outside the nervous system, expression of RPTPδ was observed in heart, kidney, thymus, and spleen (Mizuno et al., 1994; 1993).

4. Axon Guidance and the Type IIa Family of RPTPs

4.1 Axon Guidance and Invertebrate Type IIa RPTPs

Initial evidence that type IIa RPTPs influence axon growth and guidance was obtained from genetic analyses in *D. melanogaster*. The type IIa family includes DLAR and DPTP69D in *Drosophila*. During embryonic development, DLAR and DPTP69D protein is distributed along axons in the *Drosophila* CNS (Desai et al., 1996; Tian et al., 1991). Transgenic loss-of-function mutants demonstrated that both DLAR and DPTP69D are required for normal motoneuron guidance (Figure 4). In wild type flies, the SNb defasciculates from the main ISN pathway and at a specific point turns to enter the ventral muscle regions to innervate a series of muscles (Figure 4a). In Dlar (-/-) embryos, SNb motor axons exit the ISN common pathway as a

distinct fascicle; however, they bypass their normal entry point to the ventral muscle region and instead continue parallel to ISN along its dorsal trajectory (Krueger et al., 1996). SNb axons then either terminate or make terminal arborisations with nearby muscles (Krueger et al., 1996; Figure 4b). The SNb phenotype observed in Dlar (-/-) flies demonstrates a specific axon guidance defect that suggests an inability of axons to read appropriate guidance cues.

DPTP69D (-/-) embryos exhibit a variety of SNb abnormalities that support a role for this RPTP in regulating axon guidance (Desai et al., 1996). The motor neuron projection defects observed resemble the following phenotypes. In the fusion bypass phenotype, SNb motor axons fail to defasciculate from the ISN, instead projecting dorsally (Figure 4c). In the stall phenotype, SNb motor axons defasciculate and leave the ISN at the appropriate choice point but stall over the ventral muscles without making functional synapses (Figure 4d). Finally, in the detour and U-turn phenotype, SNb axons fail to exit the ISN at the appropriate choice point. Instead they leave it more dorsally and turn back to innervate ventral muscles (Figure 4e). The results of these studies suggest that DLAR and DPTP69D are required to guide SNb motor axons to their appropriate target points

A role for *Drosophila* RPTPs in axon guidance in other systems has also been demonstrated. In the developing visual system, DPTP69D is required for photoreceptors to synapse with their specific targets in the lamina and medulla (Newsome et al., 2000; Garrity et al., 1999). In the wild type eye, photoreceptors extend axons through the optic nerve to visual centers in the brain, the lamina and the medulla. The pioneer R8 axon extends past the lamina to terminate in the medulla.

Photoreceptors R1-R6 defasciculate and terminate in the lamina. Photoreceptor R7 extends past R8 to terminate at a point more distal in the medulla. In DPTP69D (-/-) mutants, R1-6 axons fail to terminate in the lamina, instead continuing into the medulla (Newsome et al., 2000a; Garrity et al., 1999). Additionally, R7 axons stop short within the medulla, failing to reach their target (Newsome et al., 2000). The *Drosophila* RPTP, DPTP69D, is also required for appropriate CNS midline crossing (Sun et al., 2000a).

HmLAR2, a type IIa RPTP identified in *H. medicinalis*, is present in growth cones of the comb cell of the leech (Gershon et al., 1998b). Inactivation of HmLAR2 resulted in reduced outgrowth, guidance errors, and collapse of growth cones with reduced lamellipodial surface area and filopodia number (Baker et al., 2000a; 2000b; Gershon et al., 1998b).

4.2 Axon Guidance and Vertebrate Type IIa RPTPs

The generation of transgenic mice deficient in type IIa RPTPs allowed for investigation of the function of these proteins *in vivo*. The importance of RPTPσ in the development of the nervous system is demonstrated by the severe neural defects observed in RPTPσ (-/-) mice (Elchebly et al., 1999; Wallace et al., 1999). These mice exhibit growth retardation, a high mortality rate, delayed peripheral nerve development, and altered development of the olfactory bulb, pituitary gland, and hypothalamus (Elchebly et al., 1999; Wallace et al., 1999). The phenotypes observed in the developing pituitary gland and hypothalamus have been suggested to reflect innervation and migration defects. Analyses of other type IIa RPTP mutant mice indicate that the CNS of RPTPδ-deficient mice appears histologically normal

although these mice display altered learning and long term potentiation (Uetani et al., 2000). Transgenic mice deficient in LAR exhibit decreased basal forebrain cholinergic neuronal size and decreased hippocampal cholinergic innervation (Van Lieshout et al., 2001; Yeo et al., 1997).

Evidence demonstrating the type IIa RPTPs influence axon growth comes from several *in vitro* studies. RPTPδ promotes and directs axon extension by cultured mouse basal forebrain neurons (Sun et al., 2000b; Wang and Bixby, 1999) and *Xenopus* retinal ganglion cells (Johnson et al., 2001). CRYPα, a homologue of RPTPσ identified in chicken (Stoker, 1994) and *Xenopus* (Johnson et al., 2000), regulates retinal ganglion cell (RGC) axon growth in these species. However, it is not clear if CRYPα activity promotes or inhibits outgrowth. Disruption of the interaction between CRYPα and a ligand associated with the glial endfeet of retinal basement membrane reduced RGC axon growth by ~60% *in vitro*, leading to the hypothesis that CRYPα promotes axon growth (Ledig et al., 1999). In contrast, expression of a putative dominant negative CRYPα construct in *Xenopus* RGCs increased axon outgrowth by ~60% under similar culture conditions (Johnson et al., 2001). It is difficult to infer the mechanism of action of CRYPα from these findings as little is known about how type IIa RPTP activity is regulated. Unlike the well-characterized model of PTK activation by dimerization, the enzymatic activity of some RPTPs is inhibited by dimerization (Wallace et al., 1998; Bilwes et al., 1996), but dimerization does not affect the activity of others (Hoffmann et al., 1997). Additionally, heterotypic ligand binding has been demonstrated to influence phosphatase activity both in a positive (Sorby et al., 2001) and negative (Meng et al., 2001) manner. It is

not currently clear how binding of CRYPα or RPTPσ to a ligand influences either dimerization or activity. Consistent with a role in mediating axon growth, CRYPα and LAR protein is present in neuronal growth cones (Gershon et al., 1998; Zhang et al., 1998; Stoker et al., 1995)

5. Type IIa RPTPs and Downstream Signaling

Although several recent studies clearly demonstrate a role for type IIa RPTPs in the regulation of axon growth and guidance, the mechanisms through which these proteins signal are unknown. Ligands that modulate phosphatase activity or physiological phosphatase substrates have not been identified. Several extracellular and intracellular binding proteins have, however, been identified, although the functional consequences of binding are unclear.

5.1 Extracellular Binding Proteins

The similarity between the extracellular domains of type IIa RPTPs and cell adhesion molecules (CAMs) suggests that these RPTPs may interact with ligands on the surface of other cells or in the extracellular matrix via homophilic and/or heterophilic binding. The extracellular matrix laminin-nidogen complex has recently been demonstrated to bind the extracellular domain of LAR (O'Grady et al., 1998). The binding site mapped to LAR FN(III) domain 5, previously demonstrated to contain a 27-bp LASE-c insert (Zhang and Longo, 1995). The presence of the LASE-c insert completely inhibits LAR- laminin-nidogen binding (O'Grady et al., 1998). In the nervous system, the predominant form of LAR contains the LASE-c insert, suggesting that other ligands for LAR exist (O'Grady et al., 1998; Zhang et al., 1998; Zhang and Longo, 1995). Interestingly, following sciatic nerve crush, there is an

increase in the protein expression of the LAR isoform lacking the LASE-c insert, in dorsal root ganglia neurons (Xie et al., 2001). Alternative splicing of exon 13 within the fifth FN(III) domain is conserved among other type IIa RPTPs family members, RPTPσ and RPTPδ (Pulido et al., 1995a; b). Binding of these family members to laminin-nidogen has not been demonstrated. Initial experiments demonstrate that LAR-laminin binding regulates changes in cell morphology via reorganization of the cytoskeleton (O'Grady et al., 1998).

In a study aiming to identify ligands for CRYPα, a fusion protein made up of the extracellular domain of CRYPα and alkaline phosphatase was found to bind to the glial endfeet of basement membranes in the retina (Haj et al., 1999; Ledig et al., 1999). This binding was recently shown to be due to an interaction between heparan sulfate proteoglycans (HSPGs) and the first immunoglobulin domain of CRYPα (Aricescu et al., 2002). The functional significance of this interaction has not been demonstrated.

The above studies demonstrate heterophilic binding interactions for the type IIa RPTPs, LAR and CRYPα. In addition to heterophilic binding, cell adhesion molecules can exhibit homophilic binding. RPTPδ binds homophilically to promote cell adhesion and neurite outgrowth (Wang and Bixby, 1999). These findings do not rule out the possibility that heterophilic ligands for RPTPδ exist.

5.2 Intracellular Binding Proteins

Liprins:

Type IIa RPTPs interact with a family of coiled-coil proteins known as the liprins (Serra-Pages et al., 1998; 1995; Pulido et al., 1995b). Liprin-α1 was originally named LIP.1 because it was identified as a LAR interacting protein (Serra-Pages et al., 1995). Liprins consist of an N-terminal coiled-coil domain and a C-terminal liprin homology (LH) domain. Based on sequence homology and binding properties, this family can be divided into two groups, the α-liprins and the β-liprins. The C-terminus of α-liprins bind to the membrane distal D2 domain of type IIa RPTPs as well as to the C-terminal domain of β-liprins (Serra-Pages et al., 1998). It is unknown at present whether α-liprins can bind both type IIa RPTPs and β-liprins simultaneously, or whether competitive binding takes place. Additional interactions between β-liprins and other signaling proteins is also unclear. The N-terminal coiled-coil domains of liprins facilitate α- and β- homodimerization (Serra-Pages et al., 1998).

The α-liprins modify the cellular distribution of type IIa RPTPs such that they co-localize at the disassembling edges of focal adhesions (Serra-Page et al., 1998; 1995). Phosphorylation on serine residues suggest that liprin α1 is not a substrate for LAR. It is believed that the liprins function to recruit type IIa RPTPs to discrete locations in the plasma membrane where they may be able to interact with other signaling molecules and potential substrates.

Cadherin-catenin complex:

Intercellular adhesion can be mediated by cadherin mediated cell-cell adhesion. Cadherins are calcium-dependent transmembrane cell adhesion molecules.

The intracellular domain of cadherins binds β-catenin, that in turn binds α-catenin, which serves to link the complex to the actin cytoskeleton (reviewed by Juliano, 2002).

The distribution of LAR and RPTPσ at sites of cell-cell contact and the co-localization with plakoglobin, a component of desmosomes, prompted investigators to determine whether these phosphatases interacted with proteins of these complexes. Using a LAR intracellular domain GST fusion protein, it was demonstrated that in HEK 293 cells, there was an association between the LAR ICD and β-catenin and plakoglobin (Aicher et al., 1997). This association appeared to be independent of cellular phosphotyrosine levels, as there was no difference in the association with or without pervanadate treatment of the cells before they were lysed. In contrast, there was no specific association between the ICD of LAR and α-catenin, or E-cadherin (Aicher et al., 1997). These results indicate that LAR and RPTPσ co-localize and interact in a functional manner with the cadherin-catenin complex at adhesion sites between cells.

The Guanine Nucleotide Exchange Factor Trio:

Interestingly, type IIa RPTPs may influence the organization of F-actin by signaling through the Rho family of small GTPases. Members of the Rho subfamily of small GTPases, RhoA, Rac, and Cdc42, are key components of intracellular signaling cascades that regulate the organization of the actin cytoskeleton in both neuronal and non-neuronal cells (Dickson et al., 2001; Luo et al., 1997; Hall, 1998). Rac and cdc42 are responsible for the formation of lamellipodia and filopodia, respectively, while RhoA promotes the formation of stress fibers and potentiates

actin-myosin contractility, thereby mediating growth cone collapse (reviewed by Luo et al., 1997).

The Rho GTPases cycle between an active GTP bound state and an inactive GDP bound state (reviewed by Hall, 1998). When active, these proteins bind to specific downstream effector molecules that alter the actin cytoskeleton. Once GTP is hydrolysed to GDP, the GDP must be replaced with free cytoplasmic GTP in order to be reactivated. Three classes of proteins directly regulate the activity of Rho GTPases. Rho GTPase activating proteins (RhoGAPs) "switch" off these enzymes by stimulating their intrinsic GTPase activity; guanine nucleotide dissociation inhibitors (GDIs) stabilize GTPases in their inactive GDP-bound state; and the Rho guanidine nucleotide exchange factors (RhoGEFs) activate Rho GTPases by facilitating the exchange of GDP with GTP (Van Aelst and D'Souza-Schorey, 1997). The Dbl family of GEFs is specific for the Rho family of small GTPases. Dbl GEFs have a Dbl domain (DH) that is the GDP-GTP exchange site as well as many other protein interaction domains that allow these GTPases to be involved in multiple signal transduction pathways (reviewed in Bateman and Van Vactor, 2001).

Trio is a novel member of the Dbl GEF family that was identified on the basis of its interaction with the intracellular domain (PTP D2) of LAR (Debant et al., 1996). It is a multidomain protein containing 2 GEF domains; GEF-D1 is specific for Rac and GEF-D2 is specific for Rho (Debant et al., 1996). Human Trio has, in addition to its GEF domains, a series of spectrin-like repeats, 2 SH3 domains, an Ig-domain, and a C-terminal serine threonine kinase domain (STK) (Seipel et al., 1999; Debant et al., 1996). The binding of LAR to hTrio occurs at the C-terminus of hTrio.

Trio homologues have been identified in *C.elegans* (Unc73; Steven et al., 1998) and *D. melanogaster* (Dtrio; Awasaki et al., 2000; Bateman et al., 2000; Liebl et al., 2000; Newsome et al., 2000b). Like hTrio, the first DH-PH element of both DTrio and Unc73 contains Rac GEF activity. The specific substrate for the second DH-PH element has not yet been identified for either homologue(Newsome et al., 2000b). Interestingly, the C-terminal domains of Trio family members are not conserved. Neither Dtrio nor *C. elegans* Unc73 contains the C-terminal sequence responsible for LAR binding, suggesting a direct interaction does not occur (Bateman et al., 2000, Debant et al., 1996). Genetic interactions, however, suggest Dtrio and Dlar mediate axon guidance in motor neuron projections (Bateman et al., 2000). Further investigation is needed to determine whether this is the case. It is clear that Dtrio/Unc73 is involved in the control of axon growth and guidance in *C.elegans* and *D. melanogaster,* possibly via interactions with Abl, Pak and Dock (reviewed by Batemann and Van Vactor, 2001; Lin and Greenberg, 2000). Interactions between Dlar and Abl and enabled have been demonstrated (Wills et al., 1999).

6. Regeneration in the Adult Nervous System

Many neurons in the adult central nervous system do not regenerate following injury. Understanding the reasons behind this phenomenon has been a focus of research for many years (reviewed in Schwab, 2002; Horner and Gage, 2000).

6.1 The Damaged Adult CNS is an Unfavourable Environment for Regeneration

Evidence that CNS neurons were capable of regenerating their axons if provided with a permissive environment such as a peripheral nerve graft (David and Aguayo, 1981), and that cultured neurons were able to extend axons into explants of

sciatic nerve but not optic nerve (Schwab and Theonen, 1985), led to the hypothesis that the adult CNS was an unfavourable environment for neurite outgrowth.

Myelin Associated Inhibitors: A correlation between the loss of the ability of an axon to regenerate and the onset of myelination suggested that injured myelin had an inhibitory function in the adult CNS (Keirstead et al., 1992). Indeed, multiple factors that inhibit axon growth have been identified in myelin including Nogo (reviewed by Huber and Schwab, 2000), myelin associated glycoprotein (MAG) (McKerracher et al., 1994; Mukhopadhyay et al., 1994) and chondroitin sulfate proteoglycans (CSPGs) (Niederost et al., 1999). Blocking Nogo using the IN-1 antibody has allowed for improved regeneration *in vivo* following corticospinal and brainstem spinal lesions (Bregman et al., 1995; Schnell and Schwab, 1993). Similarly, the neutralization of myelin through the use of a therapeutic vaccine allows for extensive regeneration *in vivo* following corticospinal tract lesion (Huang et al., 1999). Results from studies examining regeneration following CNS lesions in MAG (-/-) mice are conflicting at present (Li et al., 1996; Bartsch et al., 1995). In addition to Nogo, MAG, and CSPGs, several other inhibitory factors in CNS myelin remain to be identified (McKerracher et al., 1994).

The Glial Scar: Damage to the CNS results in the formation of the glial scar, which acts as a barrier for extending axons (reviewed by Fawcett and Asher, 1999; Stichel et al., 1998). The glial scar is a dynamic structure that consists of a variety of cellular components, including reactive astrocytes, microglia/macrophages, meningeal cells, and oligodendrocytes, as well as several extracellular matrix components. In addition to acting as a physical barrier, the glial scar contains several proteins known to inhibit

axon growth *in vitro*, including tenascin (reviewed by Faissner, 1997), keratin (Canning et al., 1996), and CSPGs (Chen et al., 2002).

Repulsive Axon Guidance Factors: Several guidance factors identified for their chemorepulsive roles in axon pathfinding and target recognition during development may also contribute to the nonpermissive environment of the CNS. Following several forms of CNS injury, there is an upregulation of semaphorin-3A by fibroblasts of meningeal origin in the glial scar (reviewed by Pasterkamp and Verhaagen, 2001). The expression of Eph B3 is upregulated in reactive astrocytes in the epicenter of the lesion as well as by neurons in the ventral and intermediate zone, rostral and caudal to the lesion site (Miranda et al., 1999). In the adult CNS, netrin-1 is expressed by oligodendrocytes in the CNS and is enriched in periaxonal myelin (Manitt et al., 2001). How netrin-1 may influence CNS regeneration requires further investigation (reviewed by Manitt and Kennedy, in press)

6.2 Intrinsic Factors Affecting CNS Regeneration

Lesioning the central branch of DRG neurons in the dorsal column of the spinal cord does not normally result in regenerative growth. However, a conditioning lesion to the peripheral branch of DRGs allows for regenerative growth to occur following dorsal column lesions without the neutralization of CNS inhibitory factors (Neumann and Woolf, 1999). This study suggests that the intrinsic state of injured neurons influences their ability to regenerate. As a result, recent strategies to improve CNS regeneration have focused on the intrinsic state of regenerating neurons. As previously stated, the intracellular levels of cAMP/cGMP can dictate the response of a growth cone to guidance cues, neurotrophic factors, as well as inhibitory factors in

the CNS such as MAG/myelin (Qiu et al., 2002; Cai et al., 2001; 1999; Song et al., 1998; 1997; Ming et al., 1997). Recent evidence demonstrates that the loss of the ability of a neuron to regenerate also correlates with a developmental drop in endogenous cAMP levels; that is, embryonic neurons have higher intracellular cAMP levels when compared to adult neurons (Cai et al., 2001). These observations suggest that increasing cAMP levels could result in improved CNS regeneration following injury. Evidence supporting this theory comes from two recent studies demonstrating that microinjection of a membrane-permeable analog of cAMP into lumbar dorsal root ganglia results in extensive regeneration of dorsal column axons following lesion (Qiu et al., 2002; Neumann et al., 2002). Furthermore, Qiu et al., (2002) demonstrate that lesioning the peripheral branch of the DRGs, previously demonstrated to allow for regeneration following dorsal column lesion, elevates cAMP levels in these neurons.

In summary, research demonstrates that the failure of CNS to regenerate following injury is due to both inhibitory environmental factors in the adult CNS as well as intrinsic properties of CNS neurons.

7. Type IIa RPTPs and Regeneration

A role for type IIa RPTPs in axon growth and guidance during development and their continued expression in the adult nervous system raises the possibility that this family of phosphatases influences plasticity and/or regenerative neurite outgrowth in the adult mammalian nervous system. However, evidence supporting such a role is limited at present. The expression of LAR and RPTPσ by neurons has been demonstrated to be responsive to peripheral nerve injury (Haworth et al., 1998).

In situ hybridization demonstrated that three days following sciatic nerve crush, the expression of LAR mRNA in DRG neurons decreases, while that of RPTPσ increases (Haworth et al., 1998). A direct involvement of LAR in regenerative growth was demonstrated by Xie et al., (2001) who found that LAR deficient mice exhibit delayed nerve regeneration following sciatic nerve crush. Furthermore, increased LAR protein expression was observed in DRG neurons two weeks following injury (Xie et al., 2001). This discrepancy between differences in mRNA and protein expression has not been addressed. A functional role for other mammalian type IIa RPTPs has not been reported.

Figure 1. Receptor Protein Tyrosine Phosphatases. The protein tyrosine phosphatase gene family consists of both cytoplasmic and receptor-like molecules. All protein tyrosine phosphatases share at least one conserved core of ~ 200 amino acids that defines their catalytic domain, the phosphatase domain. The receptor protein tyrosine phosphatases (RPTPs) have an intracellular domain consisting either one or two tandem phosphatase domains, a single transmembrane domain, and a variable extracellular domain. The RPTPs have been classified into 5 subclasses based on the structure of their extracellular domain. The type II family of RPTPs are members of the Ig-superfamily and as such have extracellular domains containing Ig-like domains. Type IIa RPTPs have Ig-like domains in combination with fibronectin type III (FN(III)) domains and resemble classical cell adhesion molecules like NCAM and L1. Type IIb RPTPs have MAM (mephrin/A5/PTPμ) domains in addition to Ig-like domains and FN(III) repeats.

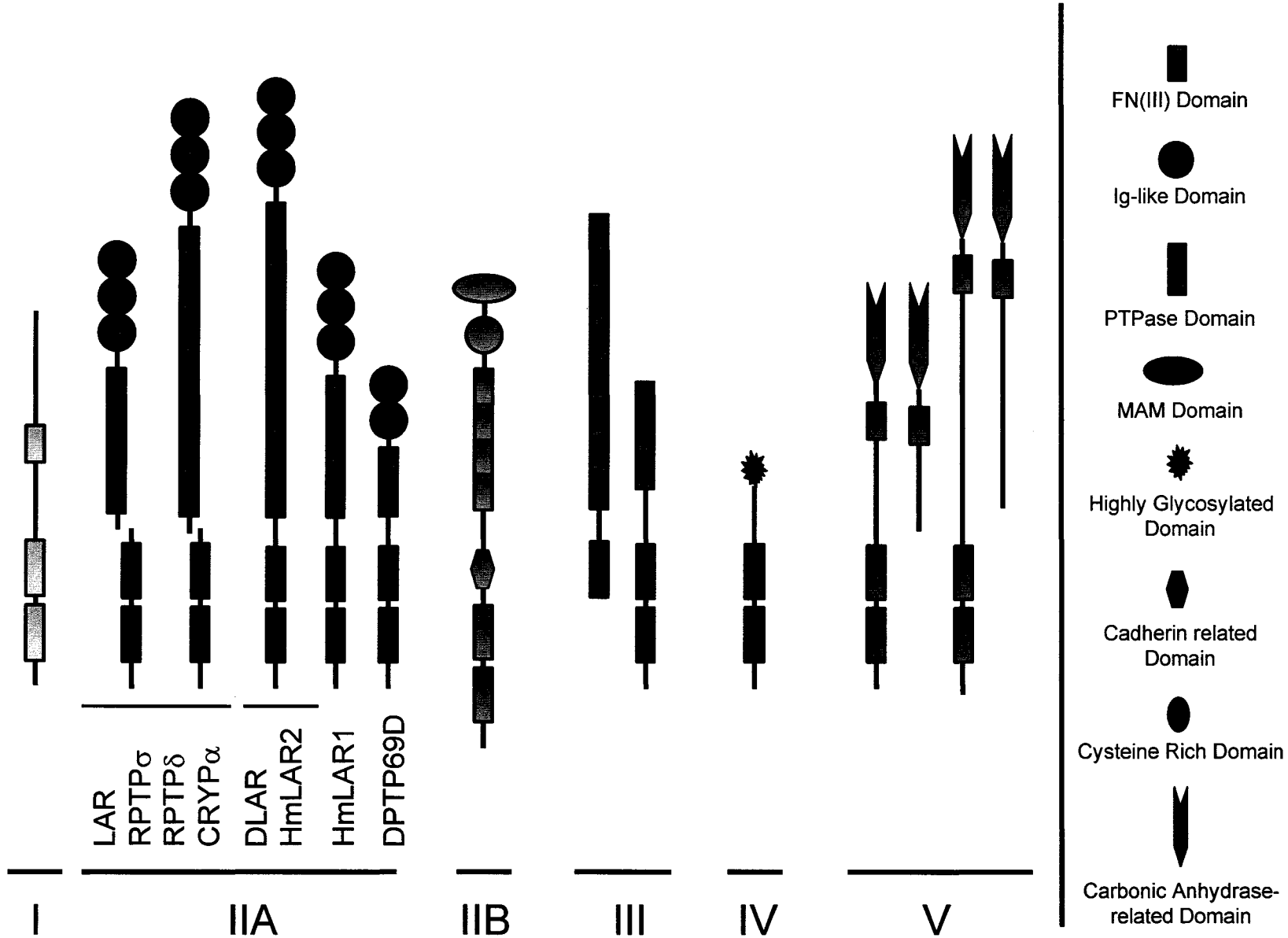

LAR
RPTPσ
RPTPδ
CRYPα
DLAR
HmLAR2
HmLAR1
DPTP69D
I
IIA
IIB
III
IV
V
FN(III) Domain
Ig-like Domain
PTPase Domain
MAM Domain
Highly Glycosylated Domain
Cadherin related Domain
Cysteine Rich Domain
Carbonic Anhydrase-related Domain

Figure 2. Alternative Splicing of Type IIa RPTPs. Multiple isoforms of type IIa RPTPs are generated as a result of alternative splicing. In addition to full length (fl) LAR, isoforms of LAR lacking FN(III) domain 4, domains 6 and 7, and domains 4, 6, and 7, have been reported (Zhang and Longo, 1995; O'Grady et al., 1994). RPTPσ and RPTPδ are present as two major isoforms, full length (fl) isoforms with an extracellular domain consisting of 3 Ig-like repeats in series with 8 FN(III) repeats, and shortened isoforms in which FN(III) domains are lacking (Pulido et al., 1995a; Mizuno et al., 1994; 1993; Wagner et al., 1994; Ogata et al., 1994; Zhang et al., 1994; Yan et al., 1993; Walton et al., 1993). Additional splicing of the first two Ig-like repeats in RPTPδ have also been reported (Pulido et al., 1995a; Mizuno et al., 1994; 1993). The splicing of mini exons, termed LASE (LAR alternatively spliced element) inserts occurs in all three mammalian family members (Pulido et al., 1995a; 1995b; Zhang and Longo, 1995; O'Grady et al., 1994). The location of these small alternatively spliced sequences (arrowheads) are as follows; LASE-a is located within the second Ig-like repeat, LASE-b is located between the second and third Ig-like repeats, LASE-c is located within FN(III) domain 5, and LASE-d is located between the transmembrane region and the first phosphatase domain D1 (Pulido et al., 1995a; 1995b).

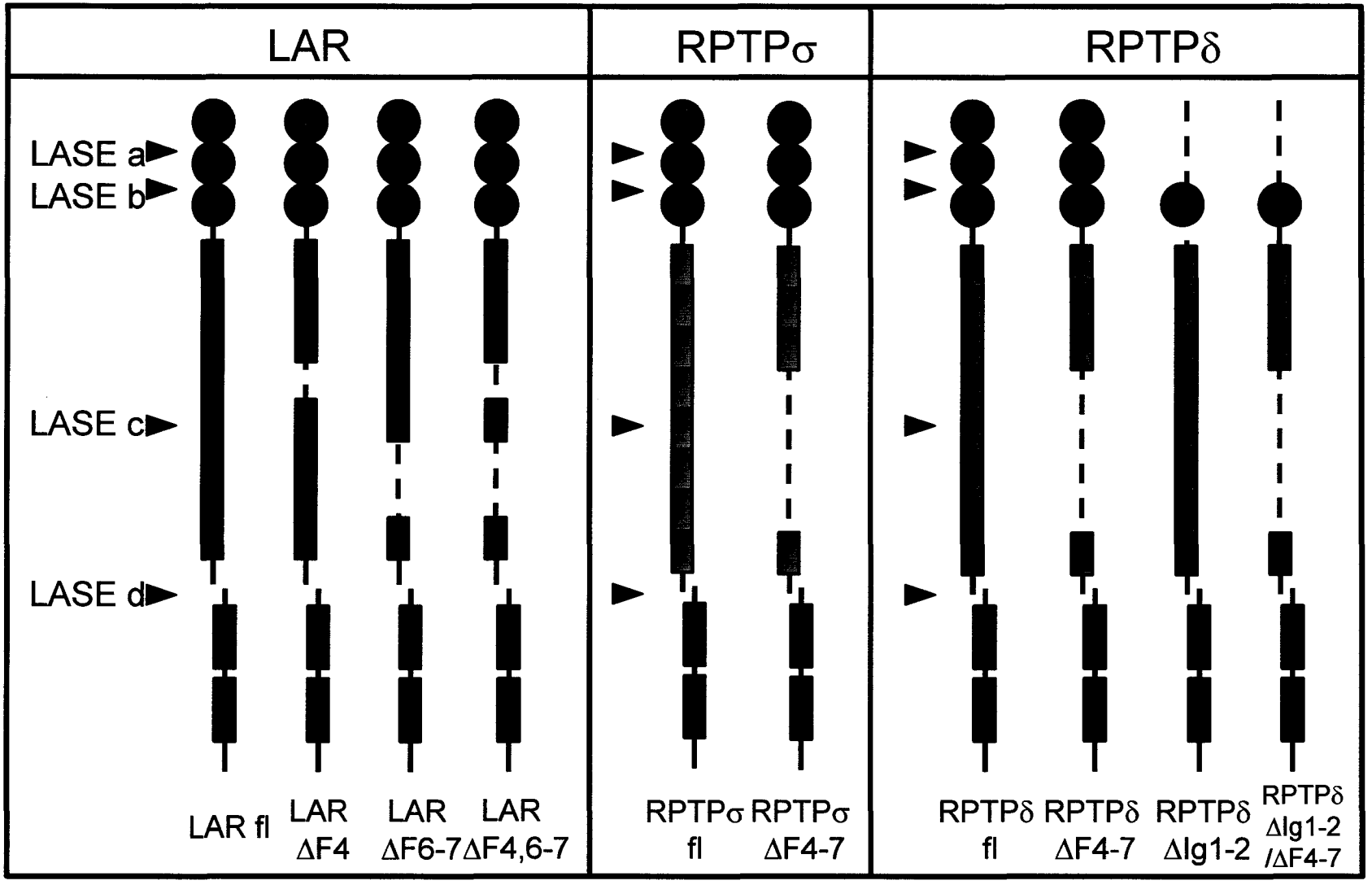
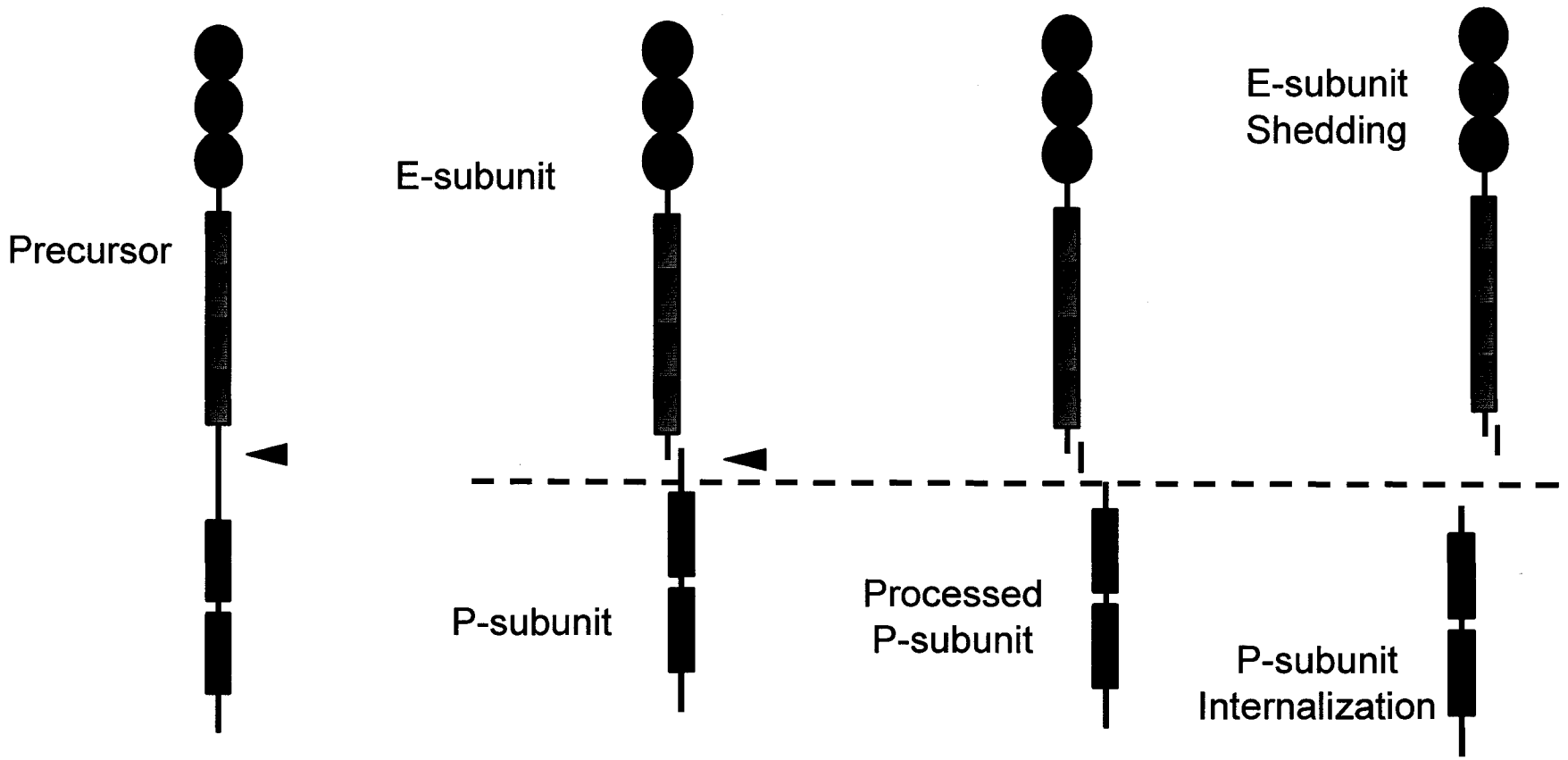
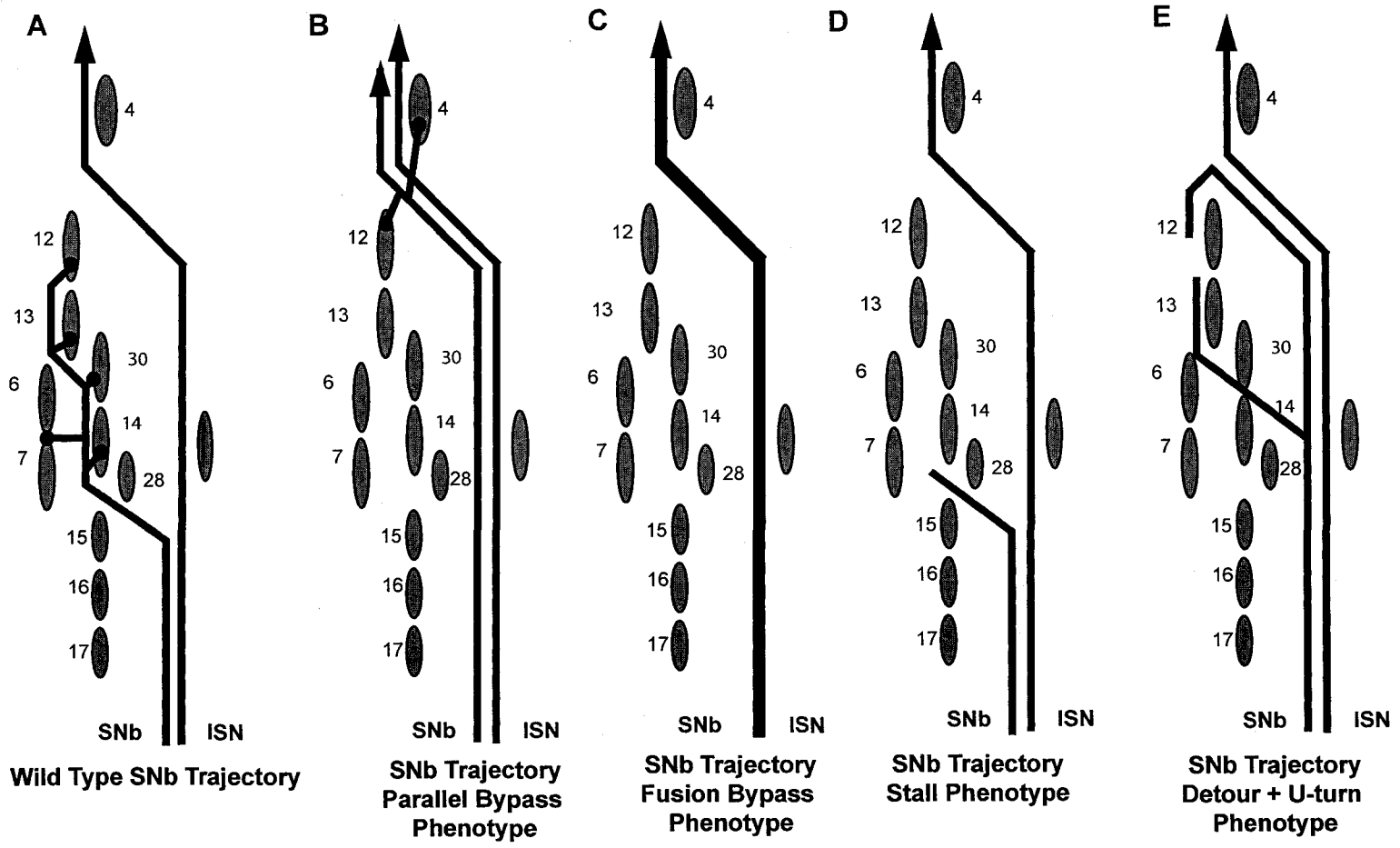

LAR
RPTPσ
RPTPδ
LASE a
LASE b
LASE c
LASE d
LAR fl
LAR ΔF4
LAR ΔF6-7
LAR ΔF4,6-7
RPTPσ fl
RPTPσ ΔF4-7
RPTPδ fl
RPTPδ ΔF4-7
RPTPδ Δlg1-2
RPTPδ Δlg1-2 /ΔF4-7

Figure 3. The Proteolytic Processing of Type IIa RPTPs, RPTPσ, LAR, and RPTPδ. Vertebrate type IIa RPTPs are expressed at the cell surface as a complex of two non-covalently linked subunits. The following processing occurs for LAR, RPTPσ, and RPTPδ (Aicher et al., 1997; Pulido et al., 1995a; Serra-Pages et al., 1994; Streuli et al., 1992). The precursor protein is synthesized intracellularly and is subsequently cleaved at a site amino terminal to the transmembrane domain to yield an extracellular E-subunit, and a P-subunit composed of a small region of the extracellular domain, the transmembrane domain, and the intracellular domain. The extracellular domains of type IIa RPTPs can be shed via a second proteolytic event at a site amino terminal to the transmembrane domain (see text). The sizes of the precursor protein, the E-subunit, the P-subunit, and the processed P-subunit (where available) for human LAR transiently expressed in HEK293 cells (Aicher et al., 1997; Serra-Pages et al., 1994; Streuli et al., 1992), rat RPTPσ transiently expressed in HEK293 cells (Aicher et al., 1997), and human RPTPδ transiently expressed in COS-7 cells (Pulido et al., 1995a) are indicated in the chart.



	Precursor	E-subunit	P-subunit	Pro.P-s.
LAR fl	~ 205 kDa	~ 150 kDa	~85 kDa	~70 kDa
RPTPσ ΔF4-7	~ 158 kDa	~ 97 kDa	~80 kDa	~72 kDa
RPTPδ fl	~ 220 kDa	~ 150 kDa	~85 kDa	NA

Figure 4. Segmental Nerve b (SNb) trajectories in *Drosophila* Mutants. A) Schematic diagram of a cross section of the wild type SNb branching. The SNb defasciculates from the main ISN pathway and at a specific point (muscle 28) turns to enter the ventral muscle region to innervate a series of muscles (muscles 6,7,12,13,14,28,30) B) In the bypass phenotype, SNb motor axons exit the ISN common pathway as a distinct fascicle; however, they bypass their normal entry point to the ventral muscle region and instead continue parallel to ISN along its dorsal trajectory where they either terminate or make terminal arborisations with nearby muscles. C) In the fusion bypass phenotype, SNb motor axons fail to defasciculate from the ISN, instead following a dorsal projection. D) In the stall phenotype, SNb motor axons defasciculate and leave the ISN at the appropriate choice point but stall over the ventral muscles without making functional synapses. E) In the detour and U-turn phenotype, SNb axons fail to exit the ISN at the appropriate choice point. Instead they leave it more dorsally and turn back to innervate ventral muscles.


A
4
12
13
30
6
14
7
28
15
16
17
SNb
ISN
Wild Type SNb Trajectory
B
4
12
13
30
6
14
7
28
15
16
17
SNb
ISN
SNb Trajectory
Parallel Bypass
Phenotype
C
4
12
13
30
6
14
7
28
15
16
17
SNb
ISN
SNb Trajectory
Fusion Bypass
Phenotype
D
4
12
13
30
6
14
7
28
15
16
17
SNb
ISN
SNb Trajectory
Stall Phenotype
E
4
12
13
30
6
14
7
28
15
16
17
SNb
ISN
SNb Trajectory
Detour + U-turn
Phenotype

Chapter 2. Receptor protein tyrosine phosphatase sigma (RPTPσ) inhibits axonal regeneration and the rate of axon extension *

ABSTRACT

Transgenic mice lacking RPTPσ, a type IIa receptor protein tyrosine phosphatase, exhibit severe neural-developmental deficits. Continued expression of RPTPσ in the adult suggests that it plays a functional role in the mature nervous system. To determine if RPTPσ might influence axonal regeneration, the time course of regeneration following facial nerve crush in wild type and RPTPσ (-/-) mice was compared. Mice lacking RPTPσ exhibited an accelerated rate of functional recovery, suggesting that RPTPσ slows the extension of regenerating axons. We detected a decrease in RPTPσ mRNA expression by facial motoneurons following nerve crush in wild type mice. Consistent with this, we show that the rate of axon extension is enhanced in neurons obtained from RPTPσ (-/-) mice. Furthermore, in wild type mice, RPTPσ protein is enriched in axonal growth cones. These findings indicate that RPTPσ slows axon growth via a mechanism intrinsic to the neuron and identify a role for RPTPσ regulating axonal regeneration.

* Thompson, K.M., Uetani, N., Manitt, C., Elchebly, M., Tremblay, M.L., and Kennedy, T.E. Submitted to Molecular Cellular Neuroscience June 2002.

INTRODUCTION

The neuronal growth cone is a highly motile structure that explores its environment using a host of cell surface receptors. The ability of the growth cone to respond to multiple guidance cues and direct axon extension is essential for the establishment of appropriate synaptic connections in the developing nervous system. Following axotomy, similar mechanisms regulate axonal growth cones as they attempt to regenerate and re-establish connections with their targets. Tyrosine phosphorylation plays an essential role in growth cone function (reviewed by Desai et al., 1997b). Cellular phosphotyrosine levels are regulated by the opposing activities of two gene families, the protein tyrosine kinases (PTKs) and the protein tyrosine phosphatases (reviewed by Stoker, 2001; Hunter, 1989).

Genetic analyses in *D. melanogaster* provided the initial evidence that type IIa receptor protein tyrosine phosphatases (RPTPs) influence axon outgrowth and guidance during development. Mutation of *Drosophila* type IIa RPTPs, Dlar and DPTP69D, caused errors in axon extension by motoneurons (Desai et al., 1997a; 1996; Krueger et al., 1996), photoreceptors (Newsome et al., 2000; Garrity et al., 1999) and commissural neurons (Sun et al., 2000a).

RPTPσ is a type IIa RPTP that is highly expressed in the mammalian nervous system (Schaapveld et al., 1998; Wang et al., 1995; Yan et al., 1993). The type IIa family of RPTPs includes two other members in mammals: LAR and RPTPδ (reviewed by Stoker, 2001). Transgenic mice lacking RPTPσ exhibit growth retardation, a high mortality rate, delayed peripheral nerve development, and altered development of the olfactory bulb, pituitary gland, and hypothalamus (Elchebly et al.,

1999; Wallace et al., 1999). Analyses of other RPTP type IIa transgenic mice indicate that the CNS of RPTPδ deficient mice appears histologically normal but these mutants exhibit impaired learning and enhanced long-term potentiation (Uetani et al., 2000). Interestingly, although LAR (-/-) mice exhibit a relatively mild neural phenotype that includes reduced forebrain neuronal number and decreased cholinergic innervation of the dentate gyrus (Van Lieshout et al., 2001; Yeo et al., 1997), the absence of LAR delays axonal regeneration *in vivo* following sciatic nerve injury (Xie et al., 2001).

In the adult nervous system, RPTPσ mRNA is expressed at high levels in regions of the brain associated with sprouting, synaptic plasticity, and regeneration (Schaapveld et al., 1998; Wang et al., 1995; Yan et al., 1993). Increasing evidence indicates that factors that influence axonal growth during development can play a significant role in the adult nervous system following injury. Here, we report that following facial nerve crush, RPTPσ (-/-) mice recover significantly faster than wild type mice, indicating that RPTPσ slows the regeneration and functional recovery of facial motoneurons. We detect a decrease in RPTPσ mRNA expression by facial motoneurons following nerve crush in wild type mice raising the possibility that decreased expression of RPTPσ may facilitate regeneration. Conversely, we detect an increase in RPTPσ protein in the facial nerve during regeneration. Because we observe decreased mRNA expression of RPTPσ by facial motor neurons, the increased protein expression in the nerve raises the possibility that non-neuronal cells in the nerve express RPTPσ. To determine if RPTPσ directly affects the ability of a neuron to extend an axon, we first examined the subcellular distribution of RPTPσ.

We show that RPTPσ protein is enriched in neuronal growth cones. Furthermore, we report enhanced axonal extension in low-density cultures of embryonic cortical neurons derived from RPTPσ (-/-) mice compared to wild type littermates. These results indicate that RPTPσ influences the neuronal response to injury and demonstrate that RPTPσ slows axon outgrowth.

RESULTS

Accelerated regeneration in RPTP σ (-/-) mice.

The cell bodies of facial motoneurons are located in the brainstem and they project an axon out of the CNS, through the ipsilateral peripheral facial nerve, to the musculature of the face. We used facial nerve crush, an extensively studied model of nerve injury, to investigate a possible role for RPTPσ in regeneration *in vivo*. Axotomy of facial motoneurons causes ipsilateral paralysis of the whiskers in mice. As the injured axons regenerate and reinnervate the facial musculature, whisker movement gradually returns. To investigate the functional role of RPTPσ during nerve regeneration, we compared the time course of functional regeneration in RPTPσ (-/-) mice and wild type age-matched control animals. The time course of functional regeneration was assessed by monitoring the recovery of whisker movement using a well established behavioural score: 0 corresponds to no whisker movement, 1 to slight whisker movement, 2 to strong but asymmetrical whisker movement, and 3 to strong symmetrical whisker movement, indicating complete functional regeneration.

Functional recovery occurred approximately one day earlier in RPTPσ (-/-) mice (Fig. 1). In these mice, limited whisker movement was first detected ~8 days after injury and functional regeneration was achieved in all animals by 10 days following injury. In wild type animals, whisker movement was first observed on the ninth day following injury and complete recovery of function was achieved in all animals 11 days post-injury (Appendix). The time required to achieve each level of behavioural recovery was significantly earlier in RPTPσ (-/-) mice than in wild type

mice (score of 1, $p < 0.001$; score of 2, $p < 0.01$; score of 3, $p < 0.001$, t-test). The length of the facial nerve, as measured from the point at which it exits the stylomastoid foramen to the end of the snout, was not significantly different between these two groups (data not shown). The time course of functional recovery observed in wild type animals was consistent with previous reports (Ferri et al., 1998; Chen and Bisby, 1993). Our results demonstrate that the loss of RPTPσ results in enhanced functional regeneration following facial nerve crush.

Characterization of a monoclonal antibody against RPTPσ.

RPTPσ is a type I transmembrane protein that is proteolytically processed and presented at the cell surface as two non-covalently linked subunits: an extracellular subunit and an intracellular subunit (Aicher et al., 1997). We examined the distribution of RPTPσ protein using a monoclonal antibody (17G7.2) raised against the intracellular subunit of RPTPσ. Western blot analysis of homogenates of newborn mouse brain identified an ~80 kDa band consistent with the molecular weight of the intracellular P-subunit of RPTPσ (Fig. 2; Aicher et al., 1997). A less abundant ~72 kDa band was detected using 17G7.2, consistent with a previous report of further proteolysis of the intracellular subunit (Aicher et al., 1997). Both immunoreactive bands were absent from brain homogenates derived from RPTPσ (-/-) mice, demonstrating the specificity of the antibody (Fig. 2). The full length precursor protein is not detected by our antibody. The multiple lower molecular weight bands in our blot are likely the result of blood derived immunoglobulins in our samples, as

these bands are detected when blots are incubated with donkey anti-mouse secondary antibody alone.

Altered RPTPσ expression during regeneration.

To examine RPTPσ gene expression following injury, facial motoneurons of adult mice were unilaterally crushed and coronal sections through the facial nucleus were processed for *in situ* hybridization. The axons of facial motoneurons exit the CNS exclusively via the ipsilateral facial nerve, allowing the motoneurons in the nucleus contralateral to the crushed nerve to be used as a within animal uninjured control. Figure 3, panels C and D, illustrates the hybridization signal detected using an RPTPσ antisense riboprobe in a single section of the brainstem of an adult mouse 3 days after unilateral facial nerve crush. No signal was detected using the corresponding sense riboprobe (not shown). Panel 3C shows facial motoneuron cell bodies projecting to the intact nerve and panel 3D the signal detected in motoneurons projecting to the crushed nerve. RPTPσ hybridization is detected in both uninjured and regenerating motoneurons; however, we consistently detected less RPTPσ mRNA expression in motoneurons regenerating an axon.

We then examined the relative amount of RPTPσ protein in control and injured facial nerves isolated from animals 3 days after crush injury. Approximately 5 mm of nerve centered around the site of crush was removed, homogenized, and protein content examined by western blot analysis. GAP-43, an axonal protein that increases in expression during regeneration (Skene and Willard, 1981) was used as a positive control. After nerve crush, the amount of RPTPσ protein in the facial nerve

at the lesion site increased approximately 3.4 fold (Fig. 3A and 3B: n = 7 pairs of nerves; control nerves, relative mean densitometric value = 102.75 ± 18.24 SEM; crushed nerves, relative mean densitometric value = 345.85 ± 58.60 SEM, *$p<0.005$; t-test). The increased amount of RPTPσ protein we observe at the site of injury may be due to increased RPTPσ expression by non-neuronal cells in the nerve, such as Schwann cells, or by cells that invade the peripheral nerve following injury, such as macrophages (reviewed by Fawcett and Keynes, 1990).

RPTPσ protein is enriched in neuronal growth cones.

The neuronal growth cone contains proteins important for axon growth and guidance that include cell adhesion molecules, receptors for guidance cues and intracellular signaling molecules. The enhanced functional recovery in RPTPσ (-/-) mice described above might be caused by changes in neuronal or glial responses to injury. As a first step toward identifying the role of RPTPσ in neurons, we set out to determine if it is present in neuronal growth cones. We performed a subcellular fractionation (Fig. 4) of E18 mouse brain homogenate that enriches for growth cone particles as described (Gordon-Weeks, 1987). Low speed centrifugation of embryonic brain homogenate produced a low speed pellet (P1) that contains a mixture of nuclei, intact cells, large cellular debris, and insoluble extracellular matrix, and a low speed supernatant (S1) that includes cellular organelles, soluble proteins, and membrane fragments (Fig. 4A). Medium speed centrifugation of S1 then separates membrane fragments and organelles (P2) from soluble proteins and membrane microsomes (S2). Layering the P2 fraction onto a 7% Ficoll cushion followed by

high-speed centrifugation, isolates a membrane fraction enriched for growth cone particles (GCP), while plasma membrane fragments, mitochondria, and ER membranes are found in the pellet (P). Following fractionation and western blot analysis, GAP-43 and DCC were used as markers for fractions enriched with membranes derived from growth cones (Fig. 4B; Shekarabi and Kennedy, 2002; Meiri et al., 1986; Skene et al., 1986). RPTPσ was enriched in the fraction containing membranes derived from neuronal growth cones (Fig. 4B, lane 7).

We next examined the distribution of RPTPσ protein in embryonic day 15 (E15) mouse cortical neurons, cells that express high levels of RPTPσ (Wang et al., 1995), following two days in culture. Immunohistochemical analysis revealed RPTPσ immunoreactivity along neurites and in growth cones. RPTPσ immunoreactivity was more abundant in the central domain of the growth cone and was not detected at the tips of filopodia or the leading edges of lamellipodia (Fig. 5). As the antibody recognizes an epitope in the intracellular domain of the protein, cells were permeabilized prior to incubation with the antibody. As a result, the staining we observe represents all intracellular pools of RPTPσ, and thus does not represent only cell surface expression. Close inspection of RPTPσ and F-actin revealed intermixed, but largely non-overlapping distributions within the growth cone (Fig. 5D). The localization of RPTPσ protein in the neuronal growth cone places it in an appropriate location to mediate growth.

RPTPσ (-/-) cortical neurons exhibit enhanced axon outgrowth.

To test the hypothesis that RPTPσ regulates axon growth, we measured axon length in low density neuronal cultures derived from the cortex of E15 RPTPσ (-/-) or

wild type littermates (Fig. 6). After 48 hours *in vitro*, neurons cultured from RPTPσ (-/-) mice extended significantly longer axons than neurons cultured from wild type littermates. When cultured on a substrate of poly-D-lysine (PL), we observed an ~7% increase in axon length in RPTPσ (-/-) neurons as compared to wild type (Fig. 5C, Table 1, $p < 0.05$, Rank Sum Test). Interestingly, when cultured on a substrate of poly-D-lysine plus laminin-1 (PLL), the mean axon length of RPTPσ (-/-) neurons was ~21% longer than axons of wild type (Fig. 6C, $p < 0.005$, Rank Sum Test, Table 1, Fig. 6C).

RPTPσ might produce this effect on axon length by increasing the rate of either axon extension or axon initiation. To address the possibility that RPTPσ might influence the rate of axon initiation, the number of cells having neurites of a given length after a brief period in culture were counted. Cells isolated from E15 mouse cortex were cultured for 16 hours on glass coverslips coated with PL. Following immunostaining for tau, each coverslip was divided into 4 quadrants and the number of cells with tau positive neurites were counted in each quadrant. Tau immuno-positive cells attached to the substrate but lacking a neurite were included in the quantification. There was no significant difference in the percentage of tau positive cells derived from RPTPσ (+/+), (+/-), or (-/-) mice that extended a short neurite, at least 20 μm long. However, significantly more neurons from RPTPσ (-/-) mice extended at least one tau immuno-positive neurite that was greater than 30 μm long compared to the RPTPσ (+/+) mice (Fig. 7). Together with the findings indicating a change in axon length after 48 hours *in vitro*, these results indicate that in these growth conditions, RPTPσ does not affect the rate of axon initiation and support the

conclusion that RPTPσ slows axon growth via a mechanism that is intrinsic to the neuron.

DISCUSSION

RPTPσ is essential for normal neural development, but its role in the adult nervous system is not known (Elchebly et al., 1999; Wallace et al., 1999). Here we investigated the possibility that RPTPσ might regulate axon growth. We report that RPTPσ delays functional regeneration of facial motoneurons following axotomy and that RPTPσ mRNA expression is downregulated in facial motor neurons following injury. Interestingly, the expression of RPTPσ protein in the nerve increases following injury, raising the possibility that RPTPσ is expressed by non-neuronal cells at the site of lesion. Furthermore, we demonstrate that RPTPσ is enriched in neuronal growth cones, and that axon extension is enhanced in cultures of dissociated embryonic cortical neurons lacking RPTPσ. These findings indicate that RPTPσ slows axon growth through a mechanism that is intrinsic to the neuron and identify a role for RPTPσ regulating axonal regeneration.

Role of type IIa RPTPs in axon extension and axon guidance.

Initial evidence that type IIa RPTPs influence axon extension was obtained from genetic analysis in *D. melanogaster*. During embryonic development, both DPTP69D and DLAR protein are distributed along axons (Desai et al., 1996; Tian et al., 1991) and their absence causes defects in the axonal projections of motoneurons (Krueger et al., 1996; Desai et al., 1996). DPTP69D is also required for the repulsion of growth cones from the CNS midline (Sun et al., 2000a) and for photoreceptor synaptic targeting (Newsome et al., 2000; Garrity et al., 1999). In addition, HmLAR2, a DLAR homologue in leech, has been shown to be required for appropriate axon

guidance and extension (Baker and Macagno 2000; Baker et al., 2000; Gershon et al., 1998).

Our findings indicate that RPTPσ inhibits axon extension. Although CRYPα, the orthologue of RPTPσ in chicken (Stoker, 1994) and *Xenopus laevis* (Johnson and Holt, 2000) has been shown to regulate retinal ganglion cell (RGC) axon growth, it was not clear from the results of these studies if CRYPα activity promotes or inhibits outgrowth. Disruption of the interaction between CRYPα and a ligand associated with the glial endfeet of retinal basement membrane reduced RGC axon growth by ~60% *in vitro*, leading to the hypothesis that CRYPα promotes axon growth (Ledig et al., 1999). In contrast, expression of a putative dominant negative CRYPα construct in *Xenopus* RGCs increased axon outgrowth by ~60% under similar culture conditions (Johnson et al., 2001). It is difficult to infer the mechanism of action of CRYPα from these findings as little is known about how type IIa RPTP activity is regulated. Unlike the well-characterized model of PTK activation by dimerization, the enzymatic activity of some RPTPs is inhibited by dimerization (Wallace et al., 1998; Bilwes et al., 1996), but dimerization does not affect the activity of others (Hoffmann et al., 1997). It is not clear how binding of CRYPα or RPTPσ to a ligand influences either dimerization or activity. However, Johnson et al., (2001) propose a model in which the interaction of CRYPα and its ligand inactivates CRYPα phosphatase activity, resulting in the promotion of axon growth. This interpretation is consistent with our findings.

Role of type IIa RPTPs in axon regeneration

Following injury, multiple factors influence the ability of an axon to regenerate in the adult nervous system (reviewed by Schwab, 2002). Here, we demonstrate that RPTPσ expression delays functional regeneration following peripheral nerve injury. Our analysis of axon extension in dissociated cell culture supports the hypothesis that the enhanced functional recovery observed in RPTPσ (-/-) mice is the result of an affect of RPTPσ on the intrinsic ability of a injured neuron to extend an axon. It has been suggested that following sciatic nerve injury, RPTPσ mRNA expression increases in DRG sensory neurons regenerating an axon (Haworth et al., 1998). In contrast, we provide evidence that expression of RPTPσ mRNA decreases in facial motoneurons following injury. The regulated expression of RPTPσ protein following injury in these systems remains to be determined.

LAR has been recently reported to regulate peripheral nerve regeneration (Xie et al., 2001). While we demonstrate enhanced axonal regeneration in RPTPσ (-/-) mice, axonal regeneration in the sciatic nerve is delayed in LAR (-/-) mice (Xie et al., 2001). Immediately following sciatic nerve injury, LAR expression decreases in DRG sensory neurons (Haworth et al., 1998). Two weeks following sciatic nerve injury; however, LAR protein expression increases (Xie et al., 2001). These findings indicate that although members of the type IIa RPTP family share a high degree of sequence similarity, they are differently regulated following injury and may have different functional consequences for regenerating axons.

RPTPσ is widely expressed by neurons in the adult CNS (Schaapveld et al., 1998; Wang et al., 1995; Yan et al., 1993). It remains to be determined how RPTPσ

may influence the capacity of neurons to regenerate an axon in the non-permissive environment of the mammalian CNS.

Type IIa RPTP signaling.

The mechanism by which RPTPσ influences growth cone motility is not known. Genetic analyses of DLAR function in *D. melanogaster* suggest that type IIa RPTPs regulate the organization of actin through proteins that include Trio (Awasaki et al., 2000; Batemann et al., 2000; Liebl et al., 2000; Debant et al., 1996), Abl (Wills et al., 1999a,b) and Mena (Wills et al., 1999a). Downstream effectors of type IIa RPTP signaling in mammals are not known. The interaction between type IIa RPTPs and several intracellular binding proteins may suggest possible functional roles for this family of proteins.

Integrins link the extracellular matrix to F-actin at points of cell-substrate attachment called focal adhesions (FA, reviewed by Sastry and Burridge, 2000). During cell migration, FAs are assembled at the leading edge of the cell and then disassembled to release the trailing edge. Tyrosine phosphorylation regulates focal adhesion remodeling. Type IIa RPTPs interact with a family of coiled-coil proteins known as the liprins (Serra Pages et al., 1998; 1995; Pulido et al., 1995b). This interaction is thought to localize the RPTPs to focal adhesions, bringing them into contact with potential substrates and signaling proteins (Serra-Pages et al., 1998; 1995). The LAR-liprin α1 complex is associated with focal adhesions at the trailing end of motile cells, suggesting that LAR plays a role in focal adhesion disassembly (Serra-Pages et al., 1995). Interestingly, RPTPσ protein is enriched in the central

region of the neuronal growth cone and appears to be absent from the leading edge of lamellipodia and the tips of filopodia.

We report that the absence of RPTPσ has a greater effect on axon extension when cortical neurons are plated on poly-D-lysine and laminin-1, compared to poly-D-lysine alone. Binding of LAR to a complex of laminin-nidogen has been proposed to modulate its function (O'Grady et al., 1998). As such, it may be the case that the difference we report is the result of a direct interaction between RPTPσ and laminin-1; however, such an interaction has not been demonstrated. Alternatively, RPTPσ may influence laminin-induced neurite outgrowth by modulating integrin signal transduction (reviewed by Powell and Kleinman, 1997), which is regulated by tyrosine phosphorylation (reviewed by Schlaepfer and Hunter, 1998). Furthermore, the Rho GTPase Rac1 is required for integrin mediated axon extension on laminin, but not on poly-D-lysine (Kuhn et al., 1998). Trio regulates Rac1 and RhoA activity (Debant et al., 1996). An attractive hypothesis is that RPTPσ influences axon growth on laminin-1 via an effect of Trio on Rac1 and RhoA; however, this remains to be tested.

Protein tyrosine phosphatases play key roles regulating cell motility (reviewed by Angers-Loustau et al., 1999). Here, we demonstrate that functional recovery is accelerated in RPTPσ (-/-) mice following facial nerve crush. We show that RPTPσ is enriched in axonal growth cones and provide evidence that RPTPσ slows the outgrowth of extending axons. These results indicate that RPTPσ inhibits axon extension via a mechanism intrinsic to the neuron and identify RPTPσ as a protein that regulates axonal regeneration following injury. Our findings identify RPTPσ as a

target for the development of inhibitors that aim to facilitate nerve regeneration following injury.

MATERIALS AND METHODS

Animals

Transgenic mice lacking receptor tyrosine phosphatase sigma, RPTPσ (-/-), were generated and bred in a Balb/C background as described (Elchebly et al., 1999). E15 and E18 CD-1 mouse embryos were obtained from Charles River Canada (QC). All procedures were performed in accordance with the "Canadian Council on Animal Care" guidelines for the use of animals in research.

Facial nerve crush.

Wild type and RPTPσ (-/-) knockout mice 3 months of age were anaesthetized using ketamine and xylazine (1:1; 0.04ml/20g mouse; Centre de Médecine Vétérinaire, St-Hyacinthe, Quebec) and the facial nerve on the left side of the animal exposed through a small incision dorsal and caudal to the external ear. The nerve trunk was released from the surrounding connective tissue by gentle dissection and crushed using fine forceps for three times for thirty sec distal to the point where it exits the stylomastoid foramen. Following 24 hr of post-operative recovery, the completeness of lesions were assessed prior to behavioral scoring. Whisker movement was monitored twice daily following surgery to observe the progression of regeneration and was compared between the injured and contralateral uninjured side. A well established behavioral scoring system was used to assess whisker movement (Ferri et al., 1998): 0 is no whisker movement, 1 is slight whisker movement, 2 is strong but asymmetrical whisker movement, and 3 is strong symmetrical whisker

movement, corresponding to complete functional recovery. The observer was unaware of the genotype of the mice being scored.

***In situ* hybridization.**

Sense and antisense cRNA probes corresponding to 478 bases of mouse RPTP-σ (nucleotides 3461-3939) were used. Transcription was carried out using T7 (New England Biolabs, MA) or T3 polymerases (Promega, WI) and digoxigenin (DIG) RNA labeling mix (Roche, QC). *In situ* hybridization was carried out on the facial nuclei of adult mice following unilateral facial nerve crush. Three days following surgery animals were anesthetized as described above and perfused transcardially with 50 ml of PBS (pH=7.5) and heparin (1 unit/ml) at 4°C. CNS tissue was then rapidly dissected and frozen by immersion in 2-methyl butane (Fisher) chilled in liquid nitrogen. Six μm cryostat sections were mounted onto slides (Superfrost Plus, Fisher), briefly dried, and fixed by immersion in 4% paraformaldehyde, 15% picric acid (pH 8.5) in PBS for 1 hour at room temperature. Following fixation, sections were rinsed in DEPC-treated 2X SSC and equilibrated for 5 min in DEPC-treated 10 mM triethanolamine (Fisher). Tissue sections were acetylated by incubation with 0.25% acetic anhydride (Sigma) in DEPC-treated 10 mM triethanolamine for 10 minutes at room temperature. *In situ* hybridization was carried out as described (Braissant and Wahli, 1998) using DIG-labeled probes. Sections were transferred to pre-hybridization solution (50% formamide, 5X SSC, 5X Denhardt's, 1% SDS, 40 μg/ml single-stranded salmon sperm DNA) for 30 min at room temperature. Hybridization was carried out overnight at 57°C, in 100 μl of

solution containing 200 ng of probe in 50% formamide, 5X SSC, 40 μg/ml single-stranded salmon sperm DNA. Sections were rinsed in 2X SSC at room temperature followed by a stringent wash in 2X SSC for 1 hr at 65°C. Hybridization was detected using an anti-DIG peroxidase-coupled antibody (Roche, QC), amplified using the TSA-Indirect (ISH) Tyramide Signal Amplification kit (NEN, MA), and visualized with peroxidase/DAB detection (Vector Laboratories).

Culture of dissociated embryonic cortical neurons.

Neocortices from RPTPσ (+/+), (+/-), and (-/-) E15 littermates were dissected in Hanks Balanced Salt Solution (HBSS; Invitrogen, ON) and diced into small pieces using a razor blade. Tail samples were collected for genotyping by polymerase chain reaction (PCR). Tissue was incubated in 0.25% trypsin (Invitrogen) in S-MEM (Invitrogen) at 37°C for 25 min, then washed in Neurobasal medium (Invitrogen) supplemented with 10% heat inactivated fetal bovine serum (iFBS, Biomedia, QC) 2 mM Glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were dissociated by trituration and plated at a density of twenty-five thousand cells per glass coverslip (Carolina Biological Supply, NC). Coverslips were precoated by incubation with 20 μg/ml poly-D-lysine (PL; Sigma, MO) in Hank's Balanced Salt Solution (HBSS; Invitrogen,ON) overnight at 4°C, washed with sterile water and allowed to dry. They were then coated with 10μg/ml laminin-1 (Collaborative, MA) in HBSS at 37°C overnight. Coverslips were then washed with water and allowed to dry prior to cell plating. Cells were allowed to adhere for 2 hrs at 37 °C after which the medium was replaced with serum-free Neurobasal medium supplemented with

1% B27 (Invitrogen), 0.5% N2 (Invitrogen), 0.4 mM glutamine, 100 units/ml penicillin, and 100μg/ml streptomycin. Cultures were maintained at 37°C for 48 hrs.

Antibodies, immunocytochemistry, and western blot analysis.

RPTPσ immunoreactivity was detected using a monoclonal antibody (17G7.2) raised against a purified recombinant protein corresponding to the intracellular domain of mouse RPTPσ. To examine the subcellular distribution of RPTPσ, cortical cultures were washed with phosphate buffered saline (PBS) and fixed with 4% PFA, 4% sucrose (Fisher, QC) at rt for 10 min. Cells were washed several times in PBS and blocked in 2% Bovine Serum Albumin (BSA) (Fisher), 0.2% Tween-20 (Fisher) in PBS for 1 hr prior to incubation with primary antibody (17G7.2) at a dilution of 1:10 overnight at 4°C. Coverslips were then washed in blocking solution and immunofluorescence visualized using an Alexa 488 coupled secondary antibody (Calbiochem, CA) in blocking solution.

For analysis of axon extension *in vitro*, axons were visualized using an antibody against the microtubule associated protein tau (1:500, Chemicon, CA). Cultures were fixed for 20 min with 4% PFA in PBS (pH 7.5) with 4% sucrose. Cells were permeablized with PBS, 0.25% triton-X 100 (Fisher) for 4 min prior to incubation in blocking solution (3% heat-inactivated normal goat serum, 0.125% Triton X-100 in PBS). Immunoreactivity was visualized using a Cy3-coupled secondary antibody (Jackson ImmunoResearch, PA). F-actin was visualized using 0.5 μg/ml rhodamine conjugated phalloidin (Molecular Probes, OR) and cell nuclei labeled with Hoescht dye. Epifluorescent and phase-contrast images were captured

using a Carl Zeiss Axiovert microscope and a MagnaFire CCD camera (Optronics, CA). Axon length was measured from phase-contrast images using Northern Eclipse software (Empix Imaging, ON). Only isolated cells from randomly selected fields were analyzed. Statistical analysis was performed using SigmaStat (SPSS Inc., IL).

For western blot analysis the brains of wild type or RPTPσ (-/-) embryos were homogenized in lysis buffer (10mM Tris HCl, pH 7.8; 1% NP-40; 150mM NaCl; 1mM EDTA; 2mM sodium orthovanadate; 10 mM NaF; and protease inhibitors: 2μg/ml leupeptin, 1μg/ml pepstatin, and 2μg/ml aprotinin). Samples were centrifuged at 15,000 X g for 5 min at 4°C and the supernatant collected.

For western blot analysis of RPTPσ protein in the facial nerve, animals were sacrificed 3 days following injury and perfused transcardially with phosphate buffered saline (PBS) containing 1 U/ml heparin (Fisher). Nerves were rapidly dissected and frozen in liquid nitrogen. Individual nerves were homogenized using a Dounce tissue grinder in 50 μl of high detergent RIPA buffer (150 mM NaCl, 1% NP-40, 1% deoxycholate, 0.5% SDS, 50 mM Tris pH 8.0, 2μg/ml aprotinin, 2μg/ml leupeptin, and 1μg/ml pepstatin). Samples were sonicated at 40% amplitude (VibraCell, Sonics and Materials Inc., CT) for three times ten seconds at 4°C after which they were centrifuged at 15,000 X g for 5 min and the supernatant collected.

Protein content of homogenates was quantified using the BCA protein assay kit (Pierce, IL) and proteins separated using 7.5% SDS-PAGE and transferred to nitrocellulose membrane (Amersham Pharmacia Biotech, NJ). Membranes were stained with Ponceau S to visualize total protein and then washed and incubated in 5% milk, 2% BSA, 0.1% tween-20 in 10 mM Tris HCl pH 7.5 (TBST + 2% BSA) for

1 hr at rt. Membranes were briefly washed and incubated with primary antibody overnight in TBST plus 2% BSA at 4°C. Immunoreactivity was visualized using a peroxidase conjugated secondary antibody (1:4000; Jackson ImmunoResearch, PA) and the Chemiluminescence Reagent Plus protein detection kit (NEN Life Science Products, MA). Following immunoblotting, membranes were stained with India Ink (0.1%) in PBS with 0.4% tween-20. Densitometry and quantification of the relative levels of RPTPσ protein was performed on scanned images of western blots (HPScanJet 3300C) using NIH Image Software (National Institutes of Health). Protein size standards correspond to 116, 97.4, 66.2, 45, and 31 kDa (Bio-Rad). The molecular weight of each band was calculated by generating a plot of distance from the bottom of the gel versus size of the molecular marker.

Subcellular fractionation.

Fractionation of E18 mouse brain to produce a membrane preparation enriched with growth cone particles was carried out as described (Gordon-Weeks, 1987). Whole brains were dissected from 6 E18 mice and homogenized in 4 mls of ice-cold homogenization buffer (0.32M sucrose, 10mM HEPES, pH 7.5, 2μg/ml aprotinin, 2μg/ml leupeptin, and 1μg/ml pepstatin) in a Potter-Elvehjem homogenizer with 8 strokes. This crude homogenate was centrifuged at 1000 X g_{av} for 5 min at 4°C. The supernatant (S1a) was collected and the pellet resuspended in homogenization buffer and recentrifuged at 1000 X g_{av} for 5 min at 4°C. This supernatant (S1b) was removed and combined with S1a. Pooled S1 was centrifuged at 13,300 X g_{av} for 15 min at 4°C to obtain a second pellet (P2) and supernatant (S2). P2

was washed twice by recentrifugation at 13,300 g_{av} for 20 min at 4°C. P2 was then resuspended in 4 mls of homogenization buffer. This was then loaded onto 30 mls of a 7% Ficoll solution and centrifuged 58,800 X g_{av} for 20 min at 4°C. The fraction enriched with growth cone particles (GCP) was recovered from the sucrose/Ficoll interface and resuspended in 10mM HEPES plus protease inhibitors. The pellet at the bottom of the 7% Ficoll cushion (P) was recovered and resuspended in 10mM HEPES with protease inhibitors. The growth cone particle fraction was centrifuged at 97,000 X g_{av} for 60 min at 4°C after which the pellet was resuspended in approximately 300 µl of 10 mM HEPES with protease inhibitors. Samples were sonicated at 40% amplitude for 3 X 10 second pulses and then analyzed by western blot.

FIGURES

Figure 1. Accelerated functional recovery in RPTPσ (-/-) mice following facial nerve crush. Recovery of function occurs approximately one day earlier in RPTPσ (-/-) mice as compared to age-matched controls. Vertical axis: behavioral score: 0 = lack of whisker movement, 1 = slight whisker movement, 2 = strong asymmetrical whisker movement, 3 = strong, symmetrical movement. At each time point, one score was given per animal. The mean of the scores +/- SEM is plotted here. Horizontal axis: days following crush injury. (○ = RPTPσ (-/-); n = 8; ● = RPTPσ (+/+) wild type mice; n = 7).

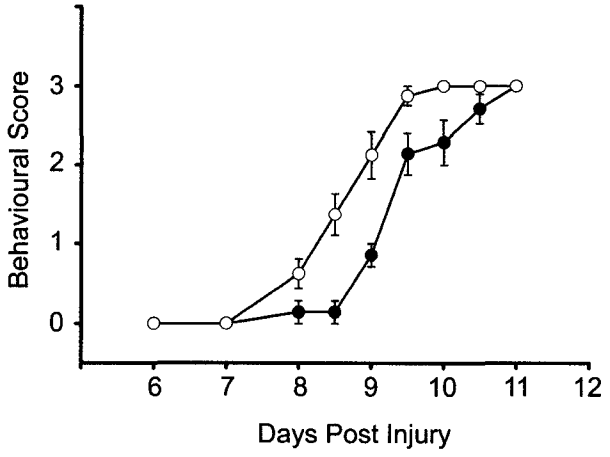

Behavioural Score
0
1
2
3
6
7
8
9
10
11
12
Days Post Injury

Figure 2. Monoclonal antibody 17G7.2 binds RPTPσ.

Western blot analyses of protein present in newborn mouse whole brain homogenate using a monoclonal antibody 17G7.2 against the intracellular domain of RPTPσ. Immunoreactivity reveals an ~80 kDa immunoreactive band in wild type mouse brain homogenate (+/+), consistent with the molecular weight of the intracellular subunit of RPTPσ. A less abundant ~72 kDa band is also detected. Neither band was detected in RPTPσ (-/-) E13 whole brain homogenate. The non-specific lower molecular weight bands are due to the anti-mouse IgG secondary antibody binding to mouse immunoglobulins present in blood in the whole brain homogenate. The right hand panel illustrates Ponceau S staining of the same blot indicating that equal amounts of protein were loaded in each lane. Protein size standards correspond to 116, 97.4, 66.2, 45, and 31 kDa (Bio-Rad).

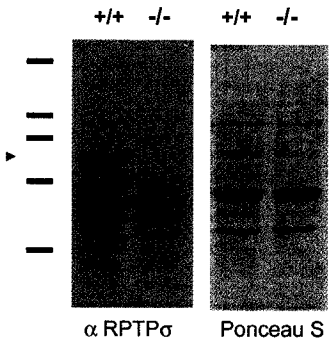

+/+
-/-
+/+
-/-
α RPTPσ
Ponceau S

Figure 3. Altered RPTPσ expression during regeneration.

Protein extracts were prepared from injured and contralateral uninjured facial nerves isolated 3 days after facial nerve crush. Panel A illustrates western blot analysis for RPTPσ in homogenates of uninjured control facial nerve and nerve centered on the site of the crush. Increased GAP-43 protein is shown on the same blot as a positive control. India Ink staining of total protein indicates that similar amounts of total protein were loaded in each lane. Protein size standards correspond to 116, 97.4, 66.2, 45, and 31 kDa (Bio-Rad). Panel B illustrates that RPTPσ protein increased approximately 3.4-fold in the injured nerve (n = 7 pairs of control and crushed nerve; control nerves, relative densitometric values = 102.75 ± 18.24 (SEM); crushed nerves, relative densitometric values = 345.85 ± 58.60 (SEM) *$p<0.005$; t-test). Panels C and D illustrate RPTPσ expression using *in situ* hybridization on a single section of brainstem containing the two facial motor nuclei. Panel C illustrates RPTPσ expression in the nucleus projecting to the uninjured facial nerve and panel D shows expression in the nucleus projecting to a nerve that had been crushed three days before. Photomicrographs were taken using the same light intensity and exposure time (phase contrast optics, 10X objective).

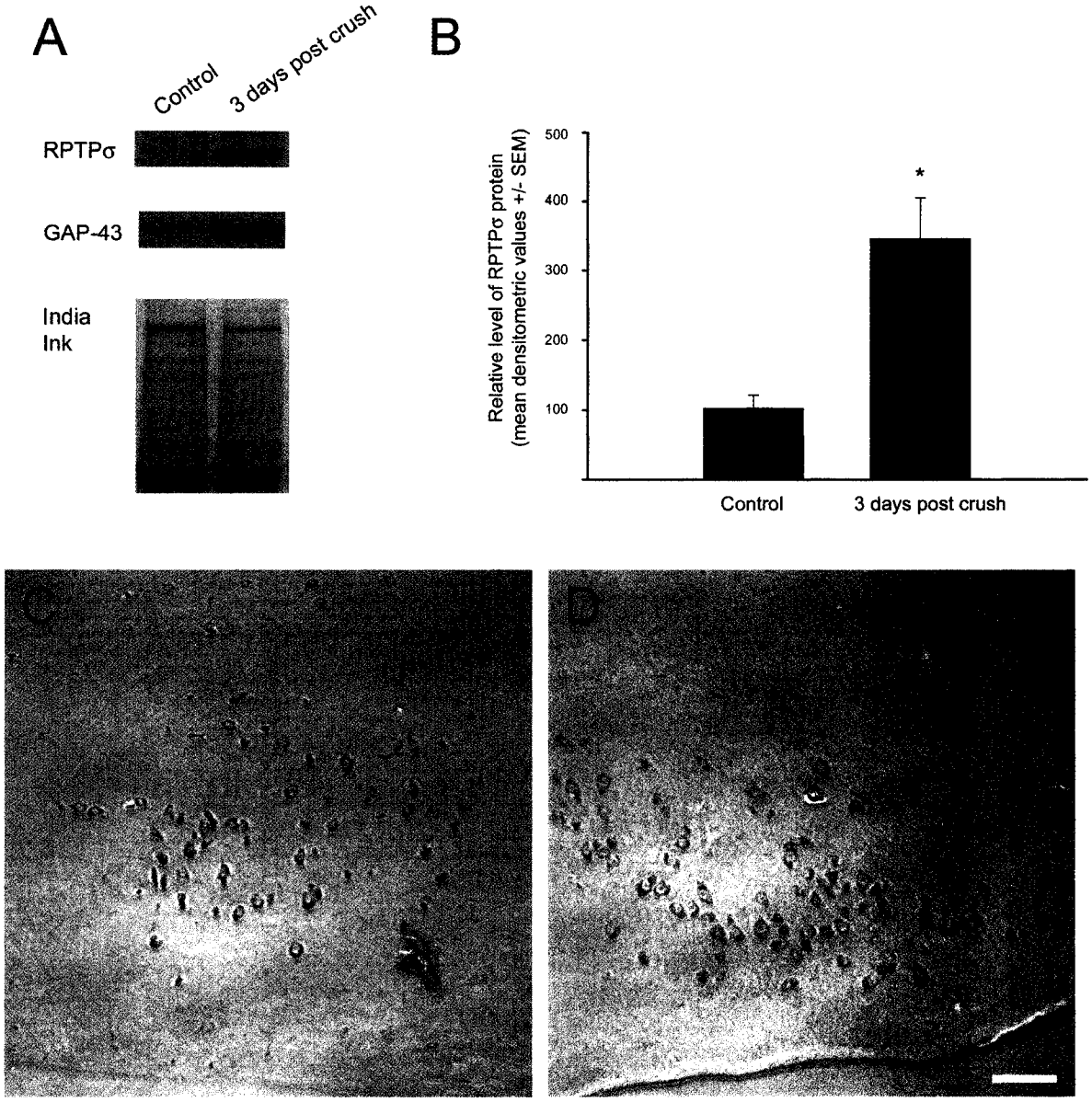
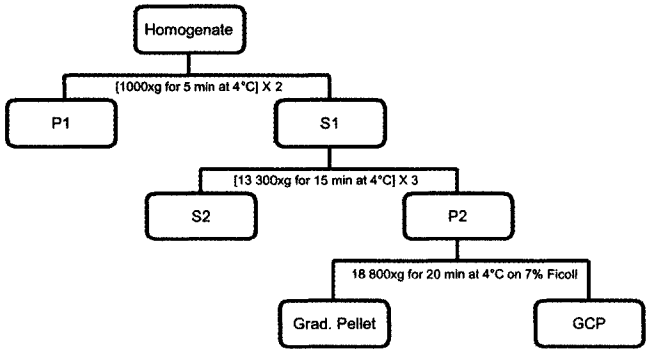

A
Control
3 days post crush
RPTPσ
GAP-43
India
Ink
B
Relative level of RPTPσ protein
(mean densitometric values +/- SEM)
500
400
300
200
100
*
Control
3 days post crush
C
D

Figure 4. Association of RPTPσ protein with growth cone membranes following subcellular fractionation of E18 mouse brain.

A) Schematic illustrating the protocol used to isolated growth cone particle (GCP) fractions from whole brain homogenate. Fractions include: TH total homogenate; S1 low speed supernatant; P1 nuclear fraction; S2 soluble fraction; P2 membrane fraction; P pellet at bottom of Ficoll gradient; GCP growth cone fraction. B) Western blot analysis was used to examine the distribution of RPTPσ in subcellular fractions. RPTPσ protein partitions to the membrane fractions (S1 and P2) and then to the growth cone particle fraction, where it is enriched. The proteins GAP-43 (~43 kDa) and DCC (~ 180 kDa) are used as markers for enrichment of growth cone particles. SDS-PAGE (7.5%) was used to separate 20 μg of total protein loaded per lane. The same nitrocellulose membrane was reprobed several times to visualize the different markers shown. Below the immunoblots, the same blot is shown stained with India Ink revealing the total protein content in each lane. Protein size standards correspond to 116, 97.4, 66.2, 45, and 31 kDa (Bio-Rad).

A


Homogenate
[1000xg for 5 min at 4°C] X 2
P1
S1
[13 300xg for 15 min at 4°C] X 3
S2
P2
18 800xg for 20 min at 4°C on 7% Ficoll
Grad. Pellet
GCP

B

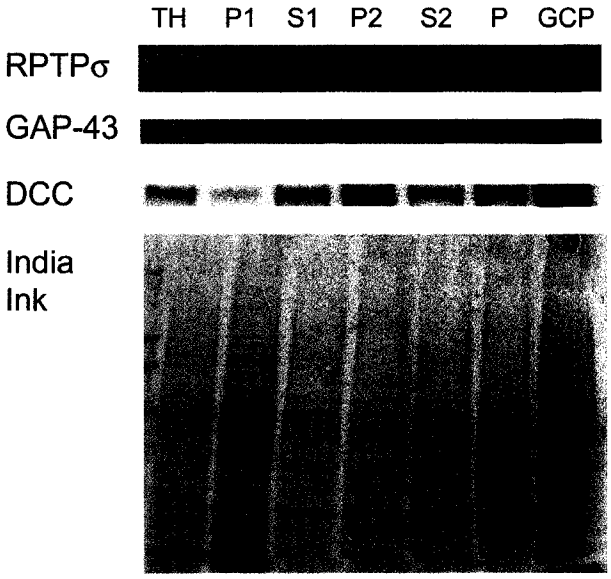

TH
P1
S1
P2
S2
P
GCP
RPTPσ
GAP-43
DCC
India
Ink

Figure 5. Distribution of RPTPσ and F-actin in growth cones of embryonic cortical neurons.

Panel A shows the growth cone of a neuron isolated from E15 Balb/c mouse cortex and grown in dissociated cell culture for 48 hours on a poly-D-lysine (PL) substrate. RPTPσ immunoreactivity was detected using 17G7.2, a monoclonal antibody against the intracellular domain of RPTPσ and an Alexa 488 coupled secondary antibody (green, panels A and B). F-actin was detected using rhodamine–coupled phalloidin (red, panels A and C, 100x objective, scale bars = 10μm). Panel D is an enlargement of the central domain of the growth cone shown in panel A, illustrating the intermixed, but largely non-overlapping distribution of RPTPσ and F-actin (scale bar = μm).

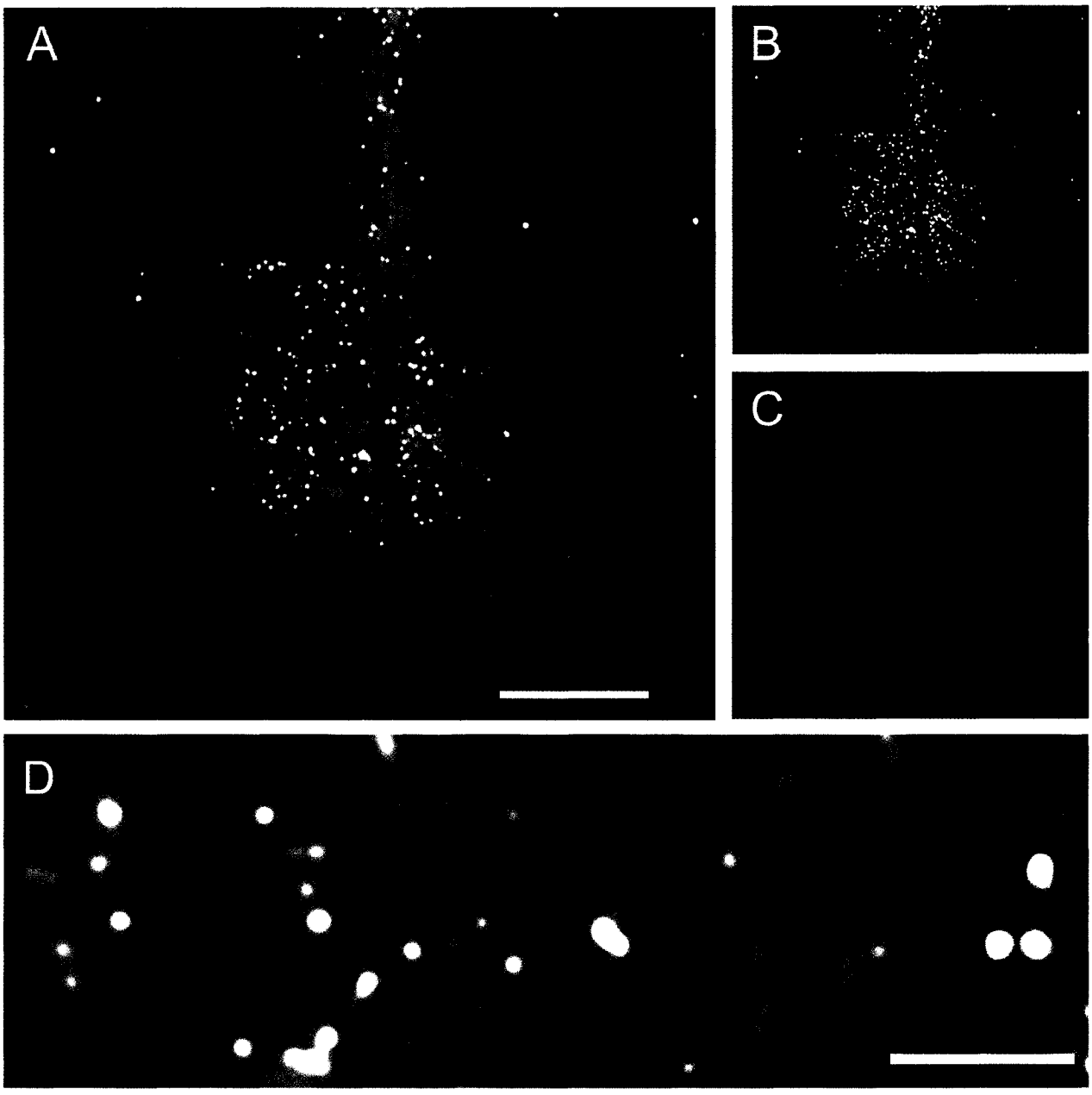

A
B
C
D

Figure 6. Enhanced axon outgrowth in RPTPσ (-/-) embryonic cortical cultures. A) Illustration of the morphology of cortical neurons isolated from E15 RPTPσ (-/-) and wild type littermates dissociated and plated on poly-D-lysine (PL) or poly-D-lysine plus laminin-1 (PLL) coated glass coverslips. After 48 hrs, cultures were fixed and stained for the microtubule associated protein tau. Cell nuclei were identified using Hoescht staining. Panel B shows western blot analysis of total protein homogenates derived from these cultures and probed with a monoclonal antibody against RPTPσ (17G7.2). The antibody reveals an ~80 kDa immunoreactive band in the homogenate of cortical neurons cultured from a wild type embryo. This band is not detected in cortical neurons cultured from a RPTPσ (-/-) embryo. Protein size standards correspond to 116, 97.4, 66.2, 45, and 31 kDa (Bio-Rad). C) The length of the axons of cortical neurons grown on PL and PLL was measured. For each independent experiment, the length of the axons of RPTPσ (-/-) neurons was expressed as percent of the wild type values. The mean values derived from three independent experiments are presented in the histogram. When cultured on PL, the mean axon length of RPTPσ (-/-) neurons was 7% longer than wild type axons (Column 1 and 2). When cultured on a substrate of PLL, the axons of RPTPσ (-/-) neurons were 21% longer than wild type (Columns 2 and 3). The difference in axon length between RPTPσ (-/-) and wild type was significantly on both substrates ($p < 0.005$, PL; $p < 0.01$, PLL; Mann Whitney Rank Sum test).

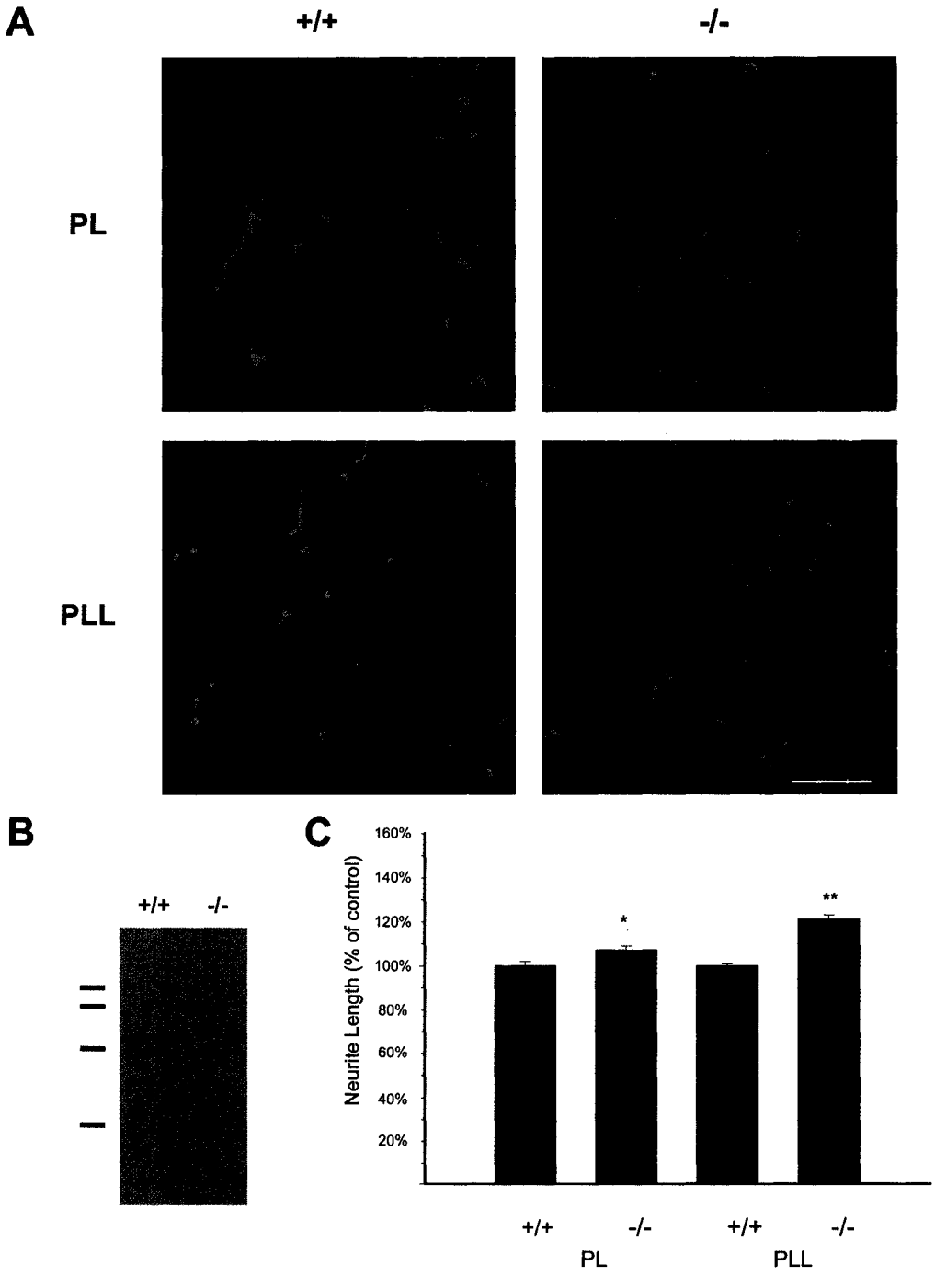

A
+/+
-/-
PL
PLL
B
+/+
-/-
C
Neurite Length (% of control)
160%
140%
120%
100%
80%
60%
40%
20%
*
**
+/+
-/-
+/+
-/-
PL
PLL

Figure 7. Absence of RPTPσ does not influence axon initiation.

Analysis of neurite length in cultures of E15 mouse cortical neurons indicated that RPTPσ does not influence the rate of axon initiation. No significant difference was detected in the percentage of neurons derived from RPTPσ (+/+), (+/-). or (-/-) mice that extended a short neurite, at least 20 μm long. More neurons from RPTPσ (-/-) mice extended at least one tau immuno-positive neurite that was greater than 30 μm long than neurons derived from the RPTPσ (+/+) mice ($p<0.05$, t test). Cells were cultured on glass coverslips coated with PL. (RPPTσ (+/+) 265 cells counted, mean number of cells with neurites (mncn) = 52.75 ± 2.53 SEM, mncn > 30 μm = 26.75 ± 2.29 SEM, n=4; RPTPσ (-/+) 1215 cells counted, mncn > 20 μm = 51.5 ± 2.07 SEM, mncn > 30 μm = 30.3 ± 1.55, n=20; RPTPσ (-/-) 858 cells counted, mncn > 20 μm = 54.25 ± 1.91, mncn > 30 μm = 33.25 ± 1.65 SEM, n =12).

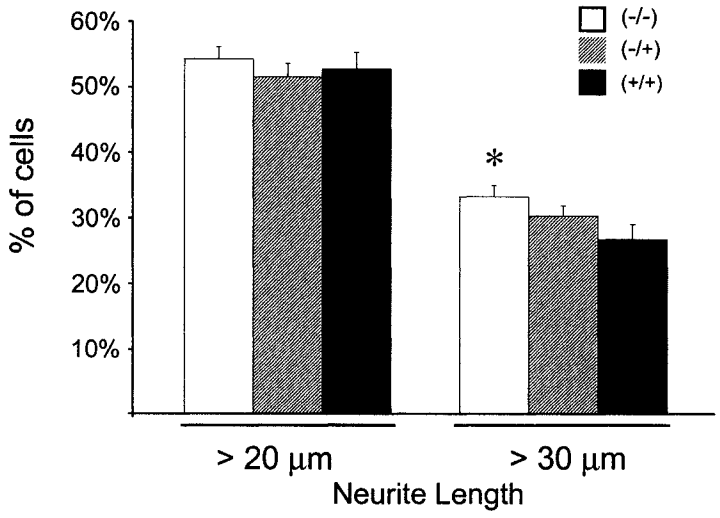

60%
50%
40%
30%
20%
10%
% of cells
(-/-)
(-/+)
(+/+)
*
> 20 µm
> 30 µm
Neurite Length

Table 1. Enhanced axonal extension of RPTPσ (-/-) E15 cortical neurons

Mouse	Genotype	Substrate	Neurite Length	n
2028-5	WT	PL	101.8 +/- 2.7	129
2028-7	KO	PL	110.6 +/- 3.9	86
2028-5	WT	PLL	114.6 +/- 2.5	183
2028-7	KO	PLL	135.9 +/- 3.1	166
887-8	WT	PL	103.5 +/- 2.7	127
887-7	KO	PL	100.4 +/- 2.9	111
887-8	WT	PLL	107.2 +/- 2.3	120
887-7	KO	PLL	131.6 +/- 3.3	145
901-6	WT	PL	123.0 +/- 3.4	142
901-5	KO	PL	143.7 +/- 4.6	106
901-6	WT	PLL	129.0 +/- 3.4	126
901-5	KO	PLL	158.0 +/- 4.5	110

Neurite lengths of cortical neurons cultured on substrates of poly-D-lysine (PL) and poly-D-lysine plus laminin-1 (PLL) were measured 48 hours after plating. Neurite length is the mean neurite length, measured in microns, +/- the standard error of the mean (SEM). n is the number of cells measured for mean neurite length.

Chapter 3. Summary and Conclusions:

Extracellular cues that influence growth cone motility act by regulating the organization of the cytoskeleton. The integrated response of the growth cone to multiple extracellular signals underlies axon growth and guidance. A variety of factors responsible for mediating axon growth and guidance during development have been identified. Many of these factors are also expressed following injury in the adult CNS and play a role in regenerative growth. Additional factors remain unidentified. This thesis describes a role for RPTPσ in axonal regeneration following peripheral nerve injury.

RPTPσ is a type IIa receptor protein tyrosine phosphatase that is widely expressed in the mammalian nervous system. Transgenic mice lacking RPTP exhibit severe neural defects demonstrating that RPTPσ is required for the nervous system to develop properly. Other type IIa phosphatases have been demonstrated to play a role in axon growth and guidance. RPTPσ continues to be expressed in the adult, suggesting an additional role in the mature nervous system.

The generation of transgenic mice lacking RPTPσ provided the opportunity to investigate if this type IIa phosphatase might play a role in axon extension during development and regeneration in the adult. We used facial nerve crush as a model to examine nerve regeneration *in vivo*. This model is advantageous for several reasons. The facial nerve, at the point at which it is injured, is composed solely of motor neurons and thus allows us to examine a uniform population of axons. Functional recovery following facial nerve crush can easily be assessed using a well-established behavioural score. Finally, the contralateral uninjured facial nerve serves as a control

for each experiment. We demonstrate that RPTPσ (-/-) mice exhibit enhanced functional recovery following facial nerve crush. The more rapid functional recovery that we observed raised the possibility that the absence of RPTPσ might increase the rate of axon growth. We tested this directly using cultures of dissociated cortical neurons derived from wild type or RPTPσ (-/-) littermates. These studies demonstrated that RPTPσ slows the rate of axon extension. Our results suggested that RPTPσ might act in the neuronal growth cone to directly mediate growth. We demonstrated the presence of RPTPσ in neuronal growth cones immunohistochemically. Furthermore, using subcellular fractionation of embryonic brain, we show that RPTPσ protein is enriched in growth cones. Our findings suggest that enhanced recovery observed in RPTPσ (-/-) mice results from an enhanced rate of axon extension by injured neurons, and allows us to propose a model in which RPTPσ acts within the growth cone to slow axon extension.

In conclusion, our results demonstrate that RPTPσ is an important mediator of axonal regeneration and provide a basis for investigating the role of RPTPσ in axon regeneration in the CNS. Our findings suggest that manipulating the activity of RPTPσ *in vivo* following injury may promote neural axonal regeneration. As such, molecules or compounds that modulate RPTPσ activity may be useful as therapeutic agents that modulate the regenerative capacity of the nervous system. These agents may be useful not only following traumatic injuries such as spinal cord lesions, but also in other neurodegenerative diseases.

References

Aicher, B., Lerch, M.M, Muller, T., Schilling, J., and Ullrich, A. (1997) Cellular redistribution of protein tyrosine phosphatases LAR and PTPσ by inducible proteolytic processing. J. Cell Biol. 138(3): 681 – 696.

Angers-Loustau, A., Cote, J.F., Tremblay, M.L. (1999). Roles of protein tyrosine phosphatases in cell migration and adhesion. Biochem. Cell Biol. 77: 493-505.

Aricescu AR, McKinnell IW, Halfter W, and Stoker AW. (2002) Heparan sulfate proteoglycans are ligands for receptor protein tyrosine phosphatase sigma. Mol. Cell Biol. 22(6):1881-92

Awasaki, T., Saitoh, M., Sone, M., Suzuki, E., Sakai, R., Ito, K., and Hama, C. (2000) The *Drosophila* Trio plays an essential role in the patterning of axons by regulating their directional extension. Neuron 26: 119 – 131.

Baker, M.W., and Macagno, E.R. (2000) RNAi of the receptor tyrosine phosphatase HmLAR2 in a single cell of an intact leech embryo leads to growth cone collapse. Curr. Biol. 19(17): 1071 – 1074.

Baker, M.W., Rauth, S.J., and Macagno, E.R. (2000) Possible role of the receptor protein tyrosine phosphatase HmLAR2 in the interbranch repulsion in a leech embryonic cell. J. Neurobiol. 45(1): 47 – 60.

Barford, D., Flint, A.J., and Tonks, N.K. (1994) Crystal structure of human protein tyrosine phosphatase 1B. Science 263: 1397 – 1404.

Bartsch, U., Bandtlow, C.E., Schnell, L., Bartsch, S., Spillmann, A.A., Rubin, B.P., Hillenbrand, R., Montag, D., Schwab, M.E., and Schachner, M. (1995) Lack of evidence that myelin-associated glycoprotein is a major inhibitor of axonal regeneration in the CNS. Neuron 15(6):1375-81

Bateman, J., and Van Vactor, D. (2001) The Trio family of guanine-nucleotide-exchange factors: regulators of axon guidance. J.Cell Sci. 114: 1973 – 1980.

Bateman, J., Shu H., and Van Vactor, D. (2000) The guanine nucleotide exchange factor Trio mediates axonal development in the *Drosophila* embryo. Neuron 26:93 - 106

Bilwes, A.M., den Hertog, J., Hunter, T., and Noel, J.P (1996) Structural basis for inhibition of receptor protein-tyrosine-α phosphatase by dimerization. Nature 382: 555 – 559.

Bixby, J.L. (2000) Receptor tyrosine phosphatases in axon growth and guidance. Neuroreport 11(10): R5 – R10

Brady-Kalney, S.M., and Tonks, N.K. (1995) Protein tyrosine phosphatases as adhesion receptors. Curr. Opin. Cell Biol. 7: 650 – 657.

Braissant, O. and Wahli, W. (1998). A simplified *in situ* hybridization protocol using non-radioactively labeled probes to detect abundant and rare mRNAs on tissue sections. Biochemica 1: 10-16.

Bregmann, B.S., Kunkel-Bagden, E., Schnell, L., Dai, H.N., McAtee, M., and Gao, D. (1995) Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. Nature 378: 498 – 501.

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(13): 4731 – 4739.

Cai, D., Shen, Y., DeBellard, M., Tang, S., and Filbin, M.T. (1999) Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism. Neuron 22: 89-101.

Canning, D.R., Hoke, A., Malemud, C.J., and Silver, J. (1996) A potent inhibitor of neurite outgrowth that predominates in the extracellular matrix of reactive astrocytes. Int J. Dev. Neurosci. 14(3):153-75

Chen, S., and Bisby, M.A. (1993). Impaired motor axon regeneration in the C57BL/Ola mouse. J. Comp. Neurol. 333: 449–454.

Chien, C.B., Rosenthal, D.E., Harris, W.A., and Holt, C.E. (1993) Navigational errors made by growth cones without filopodia in the embryonic *Xenopus* brain. Neuron. 11: 237 – 251.

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.

Debant, A., Serra-Pages, C., Seipel, K., O'Brien, S., Tang, M., Park, S., and Streuli, M. (1996) The multidomain protein Trio binds the LAR transmembrane tyrosine phosphatase, contains a protein kinase domain, and has separate rac-specific and rho-specific guanine nucleotide exchange factor domains. Proc of the Natl. Acad. of Sci. USA 93: 5466-5471.

Desai, C.J., Krueger, N.X., Saito, H., and Zinn, K. (1997a) Competition and cooperation among receptor tyrosine phosphatases control motoneuron growth cone guidance in *Drosophila.* Development. 124: 1941 – 1952.

Desai, C.J., Sun, Q., and Zinn, K. (1997b). Tyrosine phosphorylation and axon guidance: of mice and flies. *C*urr. Opin. Neurobiol. 7: 70–74.

Desai, C. J., Gindhart, J.G., Goldstein, L.S.B., and Zinn, K. (1996) Receptor tyrosine phosphatases are required for motor axon guidance in the *Drosophila* embryo. Cell 84: 599 – 609.

Dickson, B.J. (2001) Rho GTPases in growth cone guidance. Curr. Opin. Neurobiol. 11: 103 – 110.

Elchebly, M., Wagner, J., Kennedy, T.E., Lancot, C., Michaliszyn, E., Itie, A., Drouin, J., and Tremblay, M.L. (1999) Neuroendocrine dysplasia in mice lacking protein tyrosine phosphatase σ. Nature Genetics. 21: 330 – 333.

Faissner, A. (1997) The tenascin gene family in axon growth and guidance. Cell. Tissue Res. 290(2):331-41

Fawcett, J.W. and Asher, R.A. (1999) The glial scar and central nervous system repair. Brain Res. Bulletin 49(6): 377 – 391.

Fawcett, J.W. and Keynes, R.J. (1990). Peripheral nerve regeneration. Annu Rev Neurosci. 13: 43-60.

Ferri, C.G., Moore, F.A., and Bisby, M.A. (1998). Effects of facial nerve injury on mouse motoneurons lacking the p75 low-affinity neurotrophin receptor. J. Neurobiol. 34: 1–9.

Garrity, P.A., Lee, C.H., Salecker, I., Robertson, H.C., Desai, C.J., Zinn, K., and Zipursky, S.L. (1999) Retinal axon target selection in *Drosophila* is regulated by receptor protein tyrosine phosphatase. Neuron 22:707-717.

Gershon, T.R., Baker, M.W., Nitabach, M., Wu, P., and Macagno, E.R. (1998a) Two receptor tyrosine phosphatases of the LAR family are expressed in the developing leech by specific central neurons as well as select peripheral neurons, muscles, and other cells. J. Neurosci..18(8):2991-3002

Gershon, T.R., Baker, M.W., Nitach, M., and Macagno, E.R. (1998b) The leech receptor protein tyrosine phosphatase HmLAR2 is concentrated in growth cones and is involved in process outgrowth. Development 125(7): 1183 – 1190.

Gordon-Weeks, P.R. (1987). The cytoskeletons of isolated, neuronal growth cones. Neuroscience 21: 977 – 989.

Haj, F., McKinnell, I., and Stoker, A. (1999) Retinotectal Ligands for the receptor tyrosine phosphatase CRYPα. Mol. Cell. Neurosci. 14:225 – 240.

Hall, A. (1998) Rho GTPases and the actin cytoskeleton. Science 279: 509 – 514.

Haworth, K., Shu, K.K., Stokes, A., and Morris, R. (1998) The expression of receptor tyrosine phosphatases is responsive to sciatic nerve crush. Mol. Cell. Neuroscience. 12: 93–104.

Hoffmann, K.M.V., Tonks, N.K., and Barford, D. (1997) The crystal structure of domain 1 of receptor protein-tyrosine phosphatase μ. J. Biol. Chem. 272(44): 275-5 – 27508.

Horner, P.J. and Gage, F.H. (2000) Regenerating the damaged central nervous system. 407:963970.

Huang, D.W., McKerracher, L., Braun, P.E., and David, S. (1999) A therapeutic vaccine approach to stimulate axon regeneration in the adult mammalian spinal cord. Neuron 24: 639 – 647.

Huber, A.B., and Schwab, M.E. (2000) Nogo-A, a potent inhibitor of neurite outgrowth and regeneration. Biol. Chem. 381: 407 – 419.

Hunter, T. (1989). Protein-tyrosine phosphatases: the other side of the coin. Cell 58: 1013–1016.

Johnson, K.G., McKinnell., I.W., Stoker, A.W., and Holt, C.E. (2001) Receptor protein tyrosine phosphatases regulate retinal ganglion cell axon outgrowth in the developing *Xenopus* visual system. J. Neurobiol. 49: 99 – 117.

Johnson, K.G, and Holt, C.E. (2000) Expression of CRYPα, LAR, PTPδ and PTPρ in the developing Xenopus visual system. Mech. Devel. 92:291 – 294.

Juliano, R.L. (2002) Signal transduction by cell adhesion receptors and the cytoskeleton: Function of integrins, cadherins, selectins, and immunoglobulin-superfamily members. Annu. Rev. Pharmacol.Toxicol. 42: 283 – 323.

Keirstead, H.S., Hasan, S.J., Muir, G.D., and Steeves, J.D. (1992) Suppression of the onset of myelination extends the permissive period for the functional repair of embryonic spinal cord. Proc. Natl. Acad. Sci. U. S.A. 89(24):11664-8

Krueger, N. X., Van Vector, D., Wan, H.I., Gelbart, W.M., Goodman, C.S., and Saito, H. (1996) The transmembrane tyrosine phosphatase DLAR controls motor axon guidance in *Drosophila*. Cell. 84:611 – 622.

Kuhn, T.B., Brown, M.D., and Bamburg, J.R. (1998). Rac1-dependent actin filament organization in growth cones is necessary for beta1-integrin-mediated advance but not for growth on poly-D-lysine. J. Neurobiol. 37: 524-540.

Ledig, M.M., Haj, F., Bixby, J.L., Stoker, A.W., and Mueller, B.K. (1999) The receptor tyrosine phosphatase CRYPα promotes intraretinal axon growth. J. Cell Biol. 147(2): 375-388

Li, M., Shibata, A., Li, C., Braun, P.E., McKerracher, L., Roder, J., Kater, S.B., and David, S. (1996) Myelin-associated glycoprotein inhibits neurite/axon growth and causes growth cone collapse. J. Neurosci. Res. 46(4):404-14

Liebl, E.C., Forsthoefel, D.J., Franco, L.S., Sample, S.H., Hess, J.E., Cowger, J.A., Chandler, M.P., Jackson, A.M., and Seeger, M.A. (2000) Dosage-sensitive, reciprocal genetic interactions between the Abl tyrosine kinase and the putative GEF trio reveal trio's role in axon pathfinding. Neuron 25: 107 – 118.

Lin, M.Z., and Greenber, M.E. (2000) Orchestral maneuvers in the axon: Trio and the control of axon guidance. Cell. 101:239 – 242.

Lin, C.H., Thompson, C.A., and Forscher, P. (1994) Cytoskeletal reorganization underlying growth cone motility. Curr. Opin. Neurobiol. 4: 640 – 647.

Liu, B.P., and Strittmatter, S.M. (2001) Semaphorin-mediated axonal guidance via Rho-related G proteins. Curr. Opin. Cell Biol. 13: 619 – 626.

Longo, F.M., Martignetti, J.A., LeBeau, J.M., Zhang, J.S., Barnes, J.P., and Brosius, J. (1993) Leukocyte common antigen-related receptor-linked tyrosine phosphatase. Regulation of mRNA expression. J.Cell Biol. 268(35): 25403 – 26511.

Luo, L., Jan, L.Y., and Jan, Y.N. (1997) Rho family GTP-binding proteins in growth cone signaling. Curr. Opin. Neurobiol. 7:81 – 86.

Manitt, C., Colicos, M.A., Thompson, K.M., Rousselle, E., Peterson, A.C., and Kennedy, T.E. (2001) Widespread expression of netrin-1 by neurons and oligodendrocytes in the adult mammalian spinal cord. J. Neurosci. 21(11):3911-22

Meiri, K.F., Pfenninger, K.H., and Willard, M.B. (1986). Growth-associated protein, GAP-43, a polypeptide that is induced when neurons extend axons, is a component of growth cones and corresponds to pp46, a major polypeptide of a subcellular fraction enriched in growth cones. Proc. Natl. Acad. Sci. USA 83: 3537-3541

Meng, K., Rodriguez-Pena, A., Dimitrov, T., Chen, W., Yamin, M., Noda, M., and Duel, T.F. (2000) Pleiotrophin signals increased tyrosine phosphorylation of β-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase β/ζ. PNAS. 97(6): 2603 – 2608.

Ming, G-l., Song, H-j., Berninger, B., Holt, C.E., Tessier-Lavigne, M., and Poo, M-m. (1997) cAMP-dependent growth cone guidance by netrin-1. Neuron 19: 1225 – 1235.

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(1):218-22

Mitchison, T., and Kirschner, M. (1988) Cytoskeletal dynamics and nerve growth. Neuron 1: 761 – 772.

Mizuno, K., Hasegawa, K., Ogimoto, M., Katagiri, T., and Yakura, H. (1994) Developmental regulation of gene expression for the MPTP delta isoforms in the central nervous system and the immune system. FEBS Lett. 355(3):223-8

Mizuno, K., Hasegawa, K., Katagiri, T., Ogimoto, M., Ichikawa, T., and Yakura, H. (1993) MPTP delta, a putative murine homologue of HPTP delta, is expressed in specialized regions of the brain and in the B-cell lineage. Mol. Cell. Biol. 13(9):5513-23

McKerracher, L., David, S., Jackson, D.L., Kottis, V., Dunn, R.J., and Braun, P.E. (1994) Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron 13: 805-811.

Mukhopadhyay, G., Doherty, P., Walsh, F., Crocker, P.R., and Filbin, M.T. (1994) A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron 13: 757-767.

Niederöst, B.P., Zimmermann, D.R., Schwab, M.E., and Bandtlow, C.E. (1999) Bovine CNS myelin contains neurite growth-inhibitory activity associated with chondroitin sulfate proteoglycans. J. Neurosci. 19: 8979 – 8989.

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(1):83-91

Newsome, T.P., Asling, B., and Dickson, B.J. (2000a) Analysis of Drosphila photoreceptor axon guidance in eye-specific mosaics. Development 127: 851-860.

Newsome, T.P., Schmidt, S., Dietzl, G., Keleman, K., Asling, B., Debant, A., and Dickson, B.J. (2000b) Trio combines with Dock to regulate Pak activity during photoreceptor axon pathfinding in *Drosophila.* Cell 101:283 – 294.

O'Connor, T.P., Duerr, J.S., and Bentley, D. (1990) Pioneer growth cone steering decisions mediated by single filopodial contacts in situ. J. Neurosci. 10: 3935 – 3946.

Ogata, M., Sawada, M., Kosugi, A., and Hamaoka, T. (1994) Developmentally regulated expression of a murine receptor-type protein tyrosine phosphatase in the thymus. J.Immuno. 153: 4478 – 4487.

O'Grady, P., Thai, T.C. and Saito, H. (1998) The laminin-nidogen complex is a ligand for a specific splice isoform of the transmembrane protein tyrosine phosphatase LAR. J. Cell Biol. 141: 1675-1684.

O'Grady, P., Krueger, N.X., Streuli, M., and Saito, H. (1994) Genomic organization of the human LAR protein tyrosine phosphatase gene and alternative splicing in the extracellular fibronectin type-III domains. J. Biol. Chem. 269(40):25193-9

Pan, M-G., Rim, C., Lu, K.P., Florio, T., and Stork, P.J.S. (1993) Cloning and expression of two receptor-linked protein-tyrosine phosphatases generated by RNA processing from a singly gene. J. Biol. Chem. 268(26): 19284 – 19291.

Pasterkamp, R.J., and Verhaagen, J. (2001) Emerging roles for semaphorins in neural regeneration. Brain Res. Brain Res. Rev. 35(1):36-54

Pasterkamp, R.J., Giger, R.J., Ruitenberg, M.J., Holtmaat, A.J., DeWit, J., DeWinter, F., and Verhaagen, J. (1999) Expression of the gene encoding the chemorepellent semaphoring 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.

Patel, B.N., and Van Vactor, D.L. (2002) Axon guidance: the cytoplasmic tail. Curr. Opin. Cell Biol. 14: 221 – 229.

Powell, S.K., and Kleinman, H.K. (1997). Neuronal laminins and their cellular receptors. Int. J. Biochem. Cell Biol. 29: 401-414.

Pulido, R., Krueger, N.X., Serra-Pages, C., Saito, H., and Streuli, M. (1995a) Molecular characterization of the human transmembrane protein-tyrosine phosphatase delta. Evidence for tissue-specific expression of alternative human transmembrane protein-tyrosine phosphatase delta isoforms. J. Biol. Chem. 270(12):6722-8

Pulido, R., Serra-Pages, C., Tang, M., and Streuli, M. (1995b) The LAR/PTP delta/PTP sigma subfamily of transmembrane protein-tyrosine- phosphatases: multiple human LAR, PTP delta, and PTP sigma isoforms are expressed in a tissue-specific manner and associate with the LAR- interacting protein LIP.1. Proc Natl Acad Sci U S A 92, 11686-11690.

Qiu, J., Cai, D., Dai, H., McAtee, M., Hoffman, P.N., Bregmann, B.S., and Filbin, M.T. (2002) Spinal axon regeneration induced by elevation of cyclic AMP. Neuron 34: 895 – 903.

Sahin, M., Dowling, J.J., and Hockfield, S. (1995) Seven protein tyrosine phosphatases are differentially expressed in the developing rat brain. J. Comp. Neurol. 351: 617 – 631.

Sastry, S.K., and Burridge, K. (2000). Focal adhesions: a nexus for intracellular signaling and cytoskeletal dynamics. Exp. Cell Res. 261: 25-36.

Schaapveld, R.Q.J., Schepens, J.T.G., Bachner, D., Attema, J., Wieringa, B., Jap, P.H.K., and Hendriks, W.J.A.J. (1998) Developmental expression of the cell-adhesion molecule-like protein tyrosine phosphatases LAR, RPTPδ, and RPTPσ in the mouse. Mech. Dev. 77: 59-62

Schlaepfer, D.D., and Hunter, T. (1998). Integrin signaling and tyrosine phosphorylation: just the FAKs? Trends Cell Biol. 8: 151-7.

Schnell, L., and Schwab, M.E. (1993) Sprouting and regeneration of lesioned corticospinal tract fibers in the adult rat spinal cord. Eur. J. Neurosci. 5: 1156 – 1171.

Schoenwaelder, S.M., and Burridge, K. (1999) Bidirectional signaling between the cytoskeleton and the integrins. Curr. Opin. Cell Biol. 11: 274 – 286.

Schwab. M.E. (2002) Repairing the injured spinal cord. Science 295(8): 1029 – 1031.

Schwab, M.E., and Theonen, H. (1985) Dissociated neurons regenerate into sciatic but not optic nerve explants in culture irrespective of neurotrophic factors. J. Neurosci. 5: 2415 – 2423.

Seipel, K., Medley, Q.G., Kedersha, N.L., Zhang, X.A., O'Brien, S.P., Serra-Pages, C., Hemler, M.E., and Streuli, M. (1999) Trio amino-terminal guanine nucleotide exchange factor domain expression promotes cytoskeleton reorganization, cell migration and anchorage-independent cell growth. J.Cell Science 112: 1825 – 1834.

Serra-Pages, C., Medley, Q.G., Tang, M., Hart, A., and Streuli, M. (1998). Liprins, a family of LAR transmembrane protein-tyrosine phosphatase-interacting proteins. J. Biol. Chem. 273: 15611-15620.

Serra-Pages, C., Kedersha, N.L., Fazikas, L., Medley, Q., Debant, A., and Streuli, M. (1995) The LAR transmembrane protein tyrosine phosphatase and a coiled-coil LAR-interacting protein co-localize at focal adhesions. EMBO J. 14(12): 2827-2838.

Serra-Pages, C., Saito, H., and Streuli, M. (1994) Mutational analysis of proprotein processing, subunit association, and shedding of the LAR transmembrane protein tyrosine phosphatase. J. Biol. Chem. 269(38):23632-41

Shekarabi, M. and Kennedy, T.E. (2002) The netrin-1 receptor DCC promotes filopodia formation and cell spreading by activating cdc42 and rac1. Mol. Cell. Neurosci. 19(1): 1-17.

Skene, J.H., Jacobson, R.D., Snipes, G.J., McGuire, C.B., Norden, J.J., and Freeman, J.A. (1986). A protein induced during nerve growth (GAP-43) is a major component of growth-cone membranes. Science 233: 783-786.

Skene, J.H., Willard, M. (1981). Characteristics of growth-associated polypeptides in regenerating toad retinal ganglion cell axons. J Neurosci. 1: 419-426.

Sommer, L., Rao, M., and Anderson, D.J. (1997) RPTPδ and the novel protein tyrosine phosphatase RPTPψ are expressed in restricted regions of the developing central nervous system. Dev. Dynamics 208: 48 – 61.

Song, H-j., Ming, G-l., He, Z., Lehmann, M., McKerracher, L., Tessier-Lavigne, M., and Poo, M-m. (1998) Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides. Science 281: 1515-1518.

Song, H-j., Ming, G-l., and Poo, M-m. (1997) cAMP-induced switching in turning direction of nerve growth cones. Nature 388: 275-279.

Sorby, M.J., Sandstrom, J., and Ostman, A. (2001) An extracellular ligand increases the specific activity of the receptor-like protein tyrosine phosphatase DEP-1. Oncogene 20:5219 – 5224.

Stichel, C.C., and Müller, HW. (1998) The CNS lesion scar: new vistas on an old regeneration barrier. Cell Tissue Res. 294:1 – 9.

Steven, R., Kubiseski, T.J., Zheng, H., Kulkarni, S., Mancillas, J., Ruiz Morales, A., Hogue, C.W.V., Pawson, T., and Culotti, J. (1998) UNC-73 activates the rac GTPase and is required for cell growth cone migrations in *C. elegans*. Cell 92: 785 – 795.

Stoker, A.W. (2001) Receptor tyrosine phosphatases in axon growth and guidance. Curr. Opin. Biol. 11:95 – 102

Stoker, A.W. and Dutta, R. (1998) Protein tyrosine phosphatases and neural development. BioEssays 20(6): 463 - 472

Stoker, A.W., Gehrig, B., Haj, F., and Bay, B.H. (1995) Axonal localisation of the CAM-like tyrosine phosphatase CRYPα: a signaling molecule of embryonic growth cones. Development. 121: 1833 – 1844.

Stoker, A.W. (1994) Isoforms of a novel cell adhesion molecule-like protein tyrosine phosphatase are implicated in neural development. Mech. Devel. 46:201 – 217.

Streuli, M., Kreuger, N.X., Ariniello, P.D., Tang, M., Munro, J.M., Battler, W.A., Adler, D.A., Disteche, C.M., and Saito, H. (1992) Expression of the receptor-linked protein tyrosine phosphatase LAR: Proteolytic cleavage and shedding of the CAM-like extracellular region. EMBO J. 11: 897 – 907.

Streuli, M., Kreuger, N.X., Tsai, A.Y.M., and Saito, H. (1989) A family of receptor-linked protein tyrosine phosphatases in humans and Drosophila. Proc. Natl. Acad. Sci. USA 86: 8696 – 8702.

Streuli, M., Kreuger, N.X., Hall, L.R., Schlossman, S.F., and Saito, H. (1998) A new member of the immunoglobulin superfamily that has a cytoplasmic region homologous to the leukocyte common antigen. J.Exp. Med. 168: 1523 – 1530.

Sun, Q.L., Bahri, S., Schmid, A., Chia, W., and Zinn, K. (2000a) Receptor tyrosine phosphatases regulate axon guidance across the midline of the *Drosophila* embryo. Development 127: 801-812.

Sun, Q.L., Wang, J., Bookman, R.J., and Bixby, J.L. (2000b) Growth cone steering by receptor tyrosine phosphatase δ defines a distinct class of guidance cue. Mol. Cell Neurosci. 16: 686 – 695.

Suter, D.M., and Forscher, P. (2000) Substrate-cytoskeletal coupling as a mechanism for the regulation of growth cone motility and guidance. J. Neurobiol. 44: 97 – 113.

Suter, D.M., and Forscher, P. (1998) An emerging link between cytoskeletal dynamics and cell adhesion molecules in growth cone guidance. Curr. Opin. Neurobiol. 8: 106 – 116.

Tanaka, E., and Sabry, J. (1995) Making the connection: cytoskeletal rearrangements during growth cone guidance. Cell. 83: 171 – 176

Tanaka, E., Ho, T., and Kirschner, M.W. (1995) The role of microtubule dynamics in growth cone motility and axonal growth. J.Cell Biol. 128: 139 – 155.

Tessier-Lavigne, M., and Goodman, C.S. (1996) The molecular biology of axon guidance. Science 274: 1123 – 1133.

Tian, S.S., Tsouflas, P, and Zinn, K. (1991) Three receptor-linked protein-tyrosine phosphatases are selectively expressed on central nervous system axons in the *Drosophila* embryo. Cell. 67:675 – 685.

Uetani, N., Kato, K., Ogura, H., Mizuno, K., Kawano, K., Mikoshiba, K., Yakura, H., Asano, M., and Iwakura, Y. (2000) Impaired learning with enhanced hippocampal long term potentiation in PTPdelta-deficient mice. EMBO J. 19: 2275 – 2785.

Van Aelst, L., and D'Souza-Schorey, C. (1997) Rho GTPases and signaling networks. Genes Dev. 11: 2295 – 2322.

Van Lieshout, E.M.M., Van der Heijden, I., Hendriks, W.J.A.J., and Van der Zee, C.E.E.M. (2001) A decrease in size and number of basal forebrain cholinergic neurons is paralleled by diminished hippocampal cholinergic innervation in mice lacking leukocyte common antigen-related protein tyrosine phosphatase activity. Neuroscience. 102(4): 833 – 841.

Wagner, J., Boerboom, D., and Tremblay, M.L. (1994) Molecular cloning and tissue specific RNA processing of a murine receptor like protein tyrosine phosphatase. Eur. J., Biochem. 226: 773 – 782.

Wahl, S., Barth, H., Ciossek, T., Aktories, K., and Mueller, B.K. (2000) Ephrin-A5 induces collapse of growth cones by activating Rho and Rho kinase. J. Cell Biol. 149: 263 – 270.

Wallace, M.J., Batt, J., Fladd, C.A., Henderson, J.T., Skarnes, W., and Rotin, D. (1999) Neuronal defects and posterior pituitary hypoplasia in mice lacking the receptor tyrosine phosphatase PTPσ. Nature Genetics. 21: 334 – 337.

Wallace, M.J., Fladd, C., Batt, J., and Rotin, D. (1998) The second catalytic domain of protein tyrosine phosphatase δ (PTPδ) binds to and inhibits the first catalytic domain of PTPσ. Mol. Cell. Biol. 18(5): 2608 – 2616.

Walsh, F.S., and Doherty, P. (1997) Neural cell adhesion molecules of the immunoglobulin superfamily: Role in axon growth and guidance. Annu. Rec. Cell Dev. Biol. 13: 425 – 456.

Walton, K.M., Martell, K.J., Kwak, S.P., Dixon, J.E., and Lergent, B.L. (1993) A novel receptor-type tyrosine phosphatase is expressed during neurogenesis in the olfactory neuroepithelium. Neuron. 11: 387 – 400.

Wang, J., and Bixby, J.L. (1999) Receptor tyrosine phosphatase-δ is a homophilic, neurite-promoting cell adhesion molecule for CNS neurons. Mol. Cell Neurosci. 14:370- 384.

Wang, H., Yan, H., Canoll, P.D., Silvennoinen, O., Schlessinger, J., and Musacchio, J.M. (1995) Expression of receptor protein tyrosine phosphatase-σ (RPTP-σ) in the nervous system of the developing and adult rat. J. Neuroscience Research. 41: 297 – 310.

Wills, Z., Bateman, J., Korey, C., Comerm A., and Van Vactor, D. (1999a) The tyrosine kinase Abl and its substrate Enabled collaborate with the receptor phosphatase Dlar to control motor axon guidance. Neuron 22: 301 – 312.

Wills, Z., Marr, L., Zinn, K., Goodman, C.S., and Van Vactor, D. (1999b) Profilin and the Abl tyrosine kinase are required for motor axon outgrowth in the *Drosophila* embryo. Neuron 22: 291 – 299.

Wong, K., Ren, X-R., Huang, Y-Z., Yi, X., Liu, G., Saito, H., Tang, H., Wen, L., Brady-Kalnay, S.M., and Mei, L ETAL (2001) Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit-Robo pathway. Cell 107: 209 – 221.

Xie, Y., Yeo, T.T., Zhang, C., Yang, T., Tisi, M.A., Massa, S.M., and Longo, F.M. (2001) The leukocyte common antigen-related protein tyrosine phosphatase receptor regulates regenerative neurite outgrowth in vivo J. Neurosci. 21(145): 5130 – 5138.

Yan, H., Grossman, A., Wang, H., D'Eustachio, P., Mossie, K., Musacchio, J.M., Silvennoinen, O., and Schlessinger, J. (1993) A novel receptor tyrosine phosphatase-σ that is highly expressed in the nervous system. J. Biol. Chem. 268(33): 24880 – 24886.

Yeo, T.T., Yang, T., Massa, S.M., Zhang, J.S., Honkaniemi, J., Butcher, L.L., and Longo, F.M. (1997) Deficient LAR expression decreases basal forebrain cholinergic neuronal size and hippocampal cholinergic innervation. J. Neurosci. Res. 47: 348 – 360.

Zhang, J.S., Honkaniemi, J., Yang, T., Yeo, T.T., and Longo, F.M. (1998) LAR tyrosine phosphatase receptor: a developmental isoform is present in neurites and growth cones and its expression is regional- and cell-specific. Mol. Cell Neurosci. 10: 271-286.

Zhang, J.S, and Longo, F.M. (1995) LAR tyrosine phosphatase receptor: Alternative splicing is preferential to the nervous system, coordinated with cell growth and generates novel isoforms containing extensive CAG repeats. J.Cell Biol. 128: 415 – 431.

Zhang, W.R., Hashimoto, N., Ahmad, F., Ding, W., and Goldstein, B.J. (1994) Molecular cloning and expression of a unique receptor-like protein-tyrosine-phosphatase in the leukocyte-common-antigen-related phosphatase family. Biochem. J. 302: 39 – 47.

Appendices

Table 1. Behavioural Score for RPTPσ (+/+) mice

Day	9469	9470	9471	9473	9474	9475	9476	AVG	SEM
1	0	0	0	0	0	0	0	0.00	0.00
2	0	0	0	0	0	0	0	0.00	0.00
3	0	0	0	0	0	0	0	0.00	0.00
4	0	0	0	0	0	0	0	0.00	0.00
5	0	0	0	0	0	0	0	0.00	0.00
6	0	0	0	0	0	0	0	0.00	0.00
7	0	0	0	0	0	0	0	0.00	0.00
8	1	0	0	0	0	0	0	0.14	0.14
8.5	1	0	0	0	0	0	0	0.14	0.14
9	1	1	0	1	1	1	1	0.86	0.14
9.5	3	2	1	2	2	2	3	2.14	0.26
10	3	2	1	2	3	2	3	2.29	0.29
10.5	3	2	2	3	3	3	3	2.71	0.18
11	3	3	3	3	3	3	3	3.00	0.00

Table 2. Behavioural Score for RPTPσ (-/-) mice.

Day	9459	9460	9461	9462	9463	9464	9466	9468	AVG	SEM
1	0	0	0	0	0	0	0	0	0.00	0.00
2	0	0	0	0	0	0	0	0	0.00	0.00
3	0	0	0	0	0	0	0	0	0.00	0.00
4	0	0	0	0	0	0	0	0	0.00	0.00
5	0	0	0	0	0	0	0	0	0.00	0.00
6	0	0	0	0	0	0	0	0	0.00	0.00
7	0	0	0	0	0	0	0	0	0.00	0.00
8	1	0	0	1	1	1	1	0	0.63	0.18
8.5	1	1	1	2	2	2	2	0	1.38	0.26
9	1	2	2	3	3	3	2	1	2.13	0.30
9.5	3	3	3	3	3	3	3	2	2.88	0.13
10	3	3	3	3	3	3	3	3	3.00	0.00
10.5	3	3	3	3	3	3	3	3	3.00	0.00
11	3	3	3	3	3	3	3	3	3.00	0.00