Cell-contact-dependent signalling in axon growth and guidance: Eph receptor tyrosine kinases and receptor protein tyrosine phosphatase β

Sacha J Holland*, Elior Peles†, Tony Pawson‡ and Joseph Schlessinger§

The growth and guidance of axons involves the recognition of complex environmental cues by receptor proteins on the surface of the growth cone and their interpretation by cellular machinery, leading to changes in cellular behaviour. Recent advances have demonstrated that the ligands for Eph receptor tyrosine kinases, the ephrins, act as repulsive axon guidance cues, and that Eph receptors are required for correct axonal navigation *in vivo*. Members of the receptor protein tyrosine phosphatase (RPTP) family also play important roles in axon guidance and growth. RPTPβ and Eph receptors interact with cell-surface-bound ligands, and there is increasing evidence that both transmembrane ephrins and contactin, a ligand for RPTPβ, may possess an intrinsic signalling function. Thus, the cell-contact-dependent interactions between these receptors and ligands may lead to initiation of bidirectional signals that regulate axonal growth and migration.

Addresses
*‡Programme in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, M5G 1X5, Canada
*e-mail: holland@mshri.on.ca
‡e-mail: pawson@mshri.on.ca
†Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovet, Israel
§Department of Pharmacology, New York University Medical Center, 550 First Avenue, New York, New York 10016, USA

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Abbreviations

CAH	carbonic anhydrase
Dlg	Discs large
EGFR	epidermal growth factor receptor
GPI	glycosylphosphatidylinositol
LMW-PTP	low molecular weight protein tyrosine phosphatase
Ng-CAM	neuronal–glial cell adhesion molecule
Nr-CAM	Ng-CAM-related cell adhesion molecule
PAK	p21-activated kinase
PDZ	PSD-95, Dlg and ZO1
PI 3′-kinase	phosphatidylinositol 3′-kinase
PSD-95	postsynaptic density of 95 kDa
RasGAP	Ras GTPase-activating protein
RPTP	receptor protein tyrosine phosphatase
RTK	receptor tyrosine kinase
SAM	sterile alpha motif
SH	Src homology
SLAP	Src-like adapter protein
WASP	Wiskott–Aldrich syndrome protein
ZO1	zona occludens 1

Introduction

This review will cover the structure of Eph receptors and ephrin proteins, receptor protein tyrosine phosphatase β and its ligand contactin, and describe recent insights into their biological functions in axon growth and guidance. We will focus on the identification of possible signalling partners for these proteins and discuss evidence for bidirectional cellular signalling in both systems.

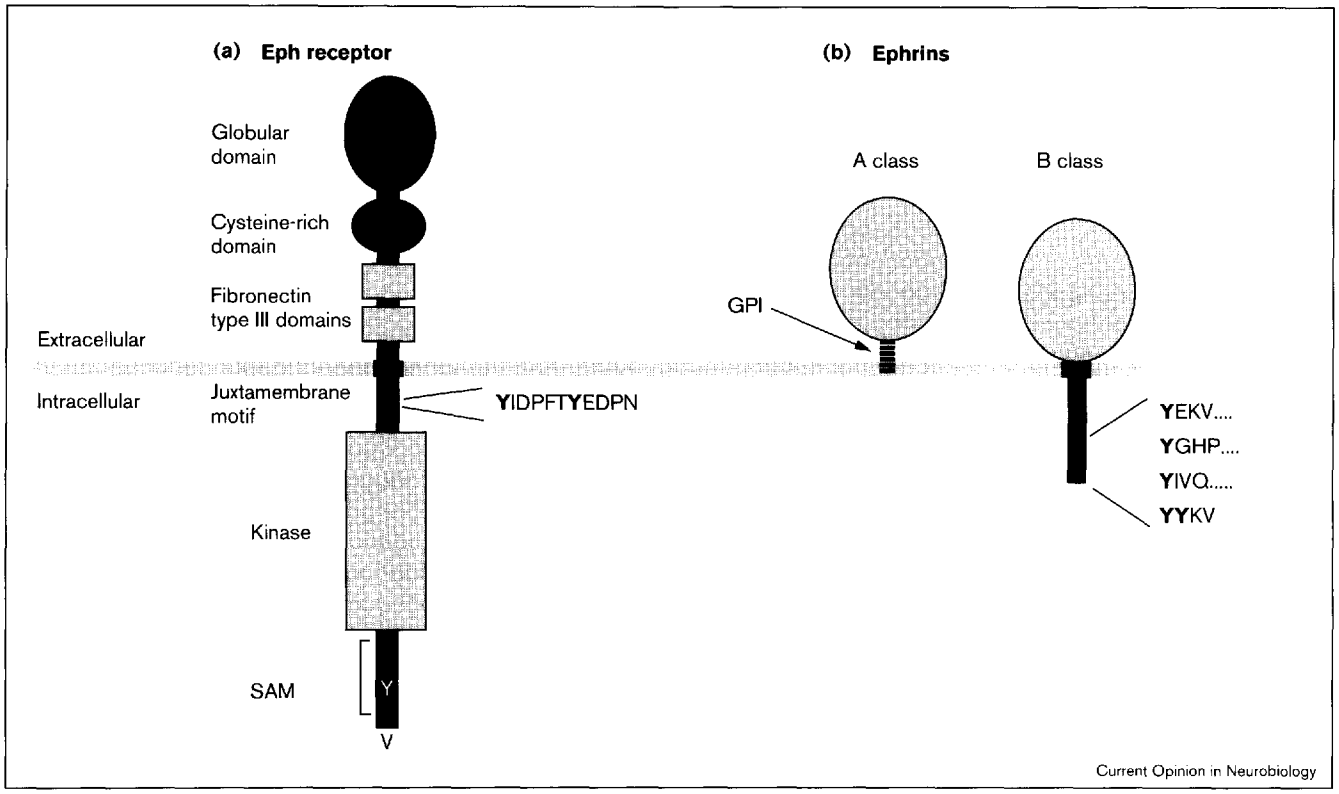
Introduction to Eph receptors

The Eph family of receptor tyrosine kinases (RTKs) includes 14 vertebrate members, which have recently been classified into two groups ('A' and 'B') on the basis of the homology of their extracellular domains [1•,2]. Ligands for these receptors (termed ephrins for *Eph* r*e*ceptor *in*teracting proteins [1•]) are themselves membrane-attached proteins. They fall into two classes (again, 'A' and 'B' in the new nomenclature), relating to sequence conservation and method of membrane attachment. 'A' class ephrins are attached to the membrane via a glycosylphosphatidylinositol (GPI) linkage, whereas those of the 'B' class contain transmembrane and cytoplasmic regions (Figure 1) (see [2]). These groupings also roughly correspond to binding specificities of ligands for the receptors (i.e. A class ephrins bind to A class receptors, and B class ligands bind to the B class receptors); although, within groups, binding interactions are relatively promiscuous and some interactions cross over group boundaries [3,4••]. Expression of Eph family members in the developing embryo is dynamic and is particularly marked in neural structures (see [5] and references therein). Corresponding receptor and ligand classes are often detected in reciprocal and apparently mutually exclusive distributions, suggesting that they may divide the embryo into discrete functional domains [4••]. Receptor–ligand interactions are expected to occur via cell–cell contact, potentially at boundaries at which domains of receptor- and ligand-expressing cells meet [4••,6].

Eph receptor protein structure

The extracellular portion of Eph receptors consists of an amino-terminal domain proposed to have a globular structure followed by a region bearing characteristically spaced cysteine residues and two fibronectin type III domains (Figure 1). Labrador *et al.* [7] have recently demonstrated that the globular domain of EphB2 (formerly known as Nuk) is sufficient to confer ephrin-B1 (formerly known as Elk-L) binding properties upon an

Figure 1



Structure of Eph receptors and ephrins. **(a)** 'A' and 'B' class receptors have similar structures. The extracellular domain contains globular, cysteine-rich and fibronectin type III domains. Inside the membrane, the highly conserved juxtamembrane motif contains two tyrosine residues, which are the major autophosphorylation sites (single-letter amino acid code used). The carboxy-terminal tail contains one conserved tyrosine residue embedded in a SAM motif. Receptors terminate in a hydrophobic residue, usually valine. **(b)** Ligand classes have similar extracellular domains. The 'A' class ephrins are attached to the membrane via a GPI linkage, whereas the 'B' class ephrins possess a cytoplasmic domain containing five conserved tyrosine residues, as indicated.

orphan Eph receptor, and that this region appears to be the principal ligand-binding domain in both A and B class receptors.

A conserved feature of Eph receptors is a ~10 amino acid motif in the intracellular juxtamembrane region that contains two tyrosine residues (Figure 1). *In vitro*, these tyrosines, especially the second of the pair (Tyr602 in EphA4/Sek), are major substrates for the receptor autokinase activity [8•]. Mutation of both these residues in EphB2 (Tyr604 and Tyr610) reduces ligand-induced tyrosine phosphorylation to almost undetectable levels [9•]. However, additional tyrosine residues presumably become phosphorylated as SH2-domain-mediated interactions that do not depend on the integrity of the juxtamembrane region have been demonstrated (Figure 2; Table 1) [10,11].

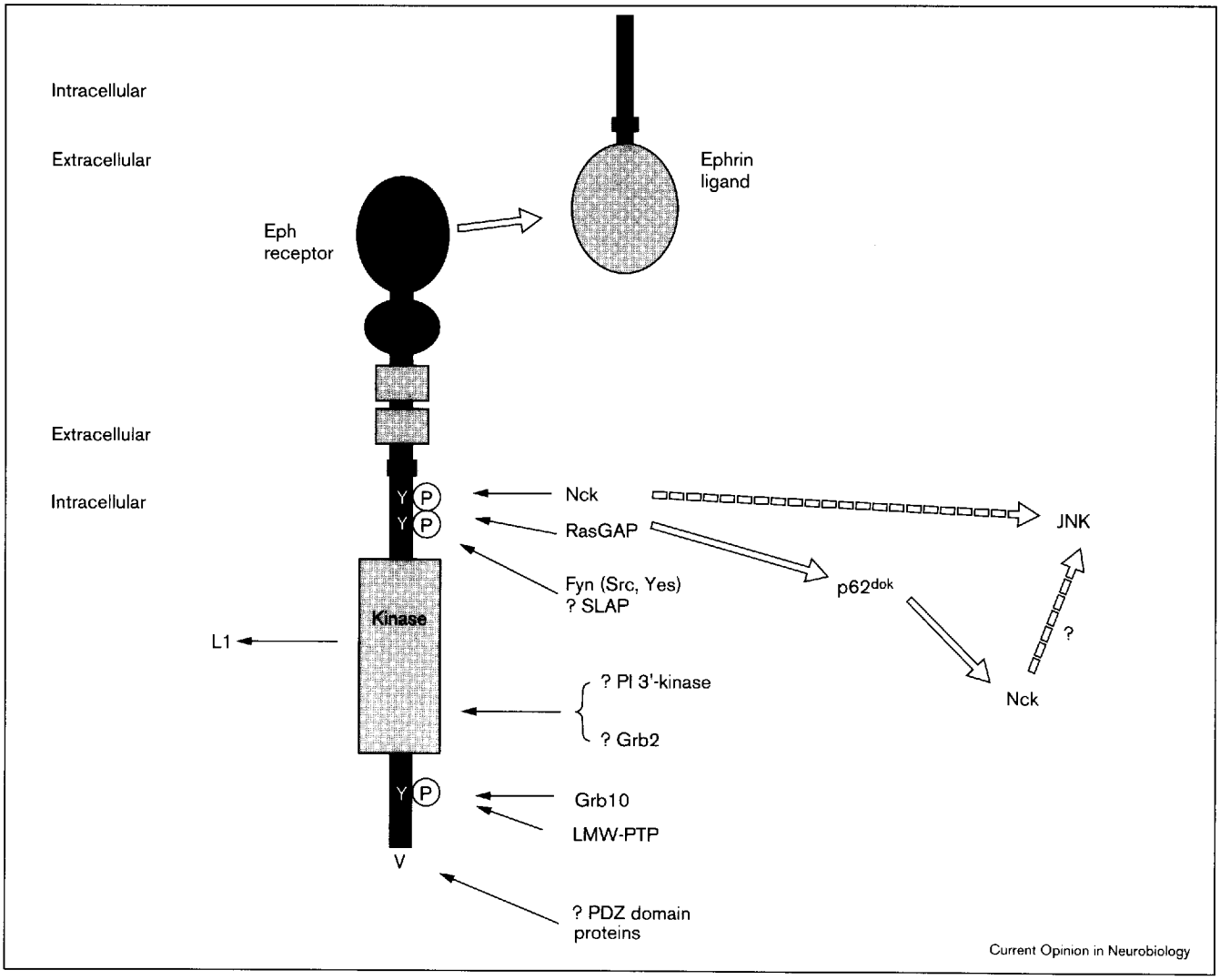
A conserved sterile alpha motif (SAM) domain has recently been identified in the carboxy-terminal tail of Eph receptors [12]. SAM domains were first identified in yeast sexual differentiation proteins, and homology searches have demonstrated their occurrence in a wide variety of proteins, although few functional data are available. A conserved tyrosine residue in the EphB1/Elk SAM domain (Tyr929) appears to mediate the interaction of EphB1 with the SH2-domain-containing protein Grb10 [11] and the low molecular weight protein tyrosine phosphatase (LMW-PTP) (E Stein *et al.*, personal communication; see Note added in proof). Whether the SAM domain has additional functions remains to be determined.

Ephrins

Eph receptor activation can be initiated by contact with ephrin-expressing cells, and, in most cases, attachment of the ligand to the cell surface is crucial for receptor stimulation; soluble ephrin extracellular domains are poorly able to initiate autophosphorylation unless artificially aggregated [6]. Receptors may, therefore, require a high local concentration or clustering of ligands at the cell surface for full activation, although binding affinities measured using soluble receptor ectodomains fall in the nanomolar range (see [13]).

A striking feature of the transmembrane ephrins is the degree of conservation of their carboxy-terminal tails [14••]. The last 33 amino acids of ephrin-B1 and ephrin-B2 are identical, and include five potential tyrosine

Figure 2



Schematic of the protein–protein interactions of a generic Eph receptor. Src family kinases, RasGAP and Nck interact with juxtamembrane tyrosine residues (single-letter amino acid code used). Grb10 and the LMW-PTP engage via the carboxy-terminal conserved tyrosine. PI 3′-kinase and Grb2 have been suggested to bind within the kinase domain. The carboxy-terminal valine residue may be a docking site for PDZ-domain-containing proteins. Downstream intracellular signalling pathways and interaction with membrane-bound ligand are also indicated. These interactions represent the sum of all those shown for both A and B class Eph receptors.

phosphorylation sites (Figure 1). This observation led to the investigation of a possible signalling role of transmembrane ephrins in the ligand-presenting cell.

Biological functions of Eph receptors and ephrins

The first functional evidence of a role for Eph receptors in axon guidance came from the purification and cloning of ephrin-A5 (formerly known as AL-1/RAGS) as a tectal protein with the ability to collapse retinal axon growth cones [15]. Multiple lines of evidence now argue for an important *in vivo* function for Eph receptors and ephrins in directing axonal [16,17••,18•,19•,20••,21•] and neural crest cell [18•,22••,23•] migrations, regulating axonal bundling (fasciculation) [21•,24], and preventing the mixing of discrete cell populations during development ([22••]; see also, in this issue, Holt and Harris, pp 98–105, and Cook, Tannahill and Keynes, pp 64–72). *In vitro* assays have demonstrated that ligand activation of Eph receptors in neuronal cells initiates anti-adhesive responses, characterised by repulsion of axons [15,18•] and neural crest cells [18•,23•], and collapse of neuronal growth cones [15,25•,26•]. Consistent with these observations, patches of ephrin-A2 ectopically expressed in the embryonic chick tectum are avoided by retinal axons, which terminate at abnormally anterior locations [17••].

Regulation of axonal bundling may be an example of repulsion of axons from an ephrin-expressing environment or may reflect modification of cell surface adhesion

Table 1

Cytoplasmic signalling proteins that interact with Eph receptors.

Protein	Receptor	Residue/ region	Domain	Possible function/comment	Reference
PI 3′-kinase p85 subunit	EphA2	ND	Carboxy-terminal SH2 domain	Modest increase in cellular PI 3′-kinase activity upon ephrin-A1 stimulation Role in membrane ruffling	[10]
Fyn	EphA4	Tyr602	SH2 domain	Highly expressed in nervous system	[8•]
SLAP	EphA2	ND	ND	?Competition for Src family kinase binding	[40]
Grb2	EphB1	Kinase domain?	SH2 domain	Regulator of Ras/MAP kinase pathway	[11]
Grb10	EphB1	Tyr929	SH2 domain	Homology to *C. elegans* mig10 Regulation of cell migration?	[11]
RasGAP	EphB2	Tyr604/Tyr610	Amino- and carboxy-terminal SH2 domains	Binds p190 RhoGAP and $p62^{dok}$ Regulation of Nck and cytoskeleton?	[9•]
Nck	EphB1	Tyr594	SH2 domain	Binds PAK, WASP Mediates c-Jun kinase activation Mutations in *Drosophila* homologue result in axon pathfinding defects	[41]
LMW-PTP	EphB1 EphB2	Tyr929	ND (?catalytic domain)	Binding activated by soluble ligand tetramer	(a)

[a]E Stein *et al.*, personal communication; see Note added in proof. ND, not determined.

proteins as a consequence of Eph receptor signalling [24]. Indeed, the L1 neural cell adhesion molecule is a substrate for the EphB2 kinase domain [27]. Regulation of cell–cell junctional complexes involving C-cadherin may also result from Eph receptor activation. Injection of an epidermal growth factor receptor (EGFR)–EphA4 chimera into *Xenopus* blastulas caused a dramatic kinase-dependent loss of cell adhesion when the chimeric receptor was over-expressed or activated by co-injection of the EGFR ligand, tumour necrosis factor α (TNFα), but this phenotype could be rescued by co-injection of C-cadherin [28].

It is important to note that in endothelial cells, different responses to Eph receptor activation are observed. Both transmembrane and GPI-linked ephrins can stimulate cell adhesion and vascular network formation ([29]; E Stein *et al.*, personal communication; see Note added in proof). The difference between these responses may depend on the cell type or alterations in ligand clustering.

Mutations in mouse *Eph* genes

Three Eph receptors, EphA8 (Eek) [19•], EphB2 [20••] and EphB3 (Sek4) [21•] have been inactivated by gene targeting in the mouse. Despite the widespread expression of Eph family members in the developing embryo, defects in the mutant animals are limited to one or two distinct structures (Table 2). Reassuringly, these include several axon tracts. Homozygous mutation of *EphA8* and *EphB2* genes allows specific axons to project into areas avoided in wild-type animals, in accordance with a repulsive function for Eph receptors. Redundancy in signalling due to the large number of receptors with overlapping expression and ligand-binding specificity may explain the rather limited phenotypes of *EphA8*, *EphB2* and *EphB3* homozygous null mice. Indeed when *EphB2* and *EphB3* mutations were combined, the defects in the double homozygotes were more severe than in either single mutant [21•], and affected axonal pathfinding and fasciculation in the brain, as well as closure of the palate.

Signalling pathways controlling axon guidance

In contrast to most growth factor RTKs, activation of Eph receptors does not cause marked mitogenesis [30]. Stimulation of rat cortical neurons with soluble ephrin-A5 leads to growth cone collapse preceded by redistribution of F-actin from the distal to central part of the growth cone, and eventual net loss of F-actin [25•]. Interestingly, the morphological features of ephrin-B2-induced collapse in this system are slightly different, with no F-actin depletion but additional disruption of microtubule organisation [26•]. Possibly, therefore, GPI-linked and transmembrane ephrins exert collapsing effects by different mechanisms, which may be attributable to differing signalling capabilities of their cognate receptors. The signalling pathways activated by Eph receptors probably culminate in the regulation of cytoskeletal architecture and cellular adhesive properties. Whilst total growth cone collapse probably results from destabilisation of actin structures and/or loss of adhesion from the substrate across the whole

growth cone, turning could be achieved by a local loss of actin polymerisation initiated, for example, when filopodia contact a ligand-expressing cell (see [31]).

It is well documented that regulation of the cytoskeleton and adhesion can be controlled by small GTPases of the Rho/Rac/Cdc42 family [32]. In fibroblasts, activation of Rho family GTPases controls formation of actin structures: Cdc42–GTP induces filopodia or actin microspikes — structures found predominantly in motile cells and neuronal growth cones [33]; Rac–GTP induces web-like lamellipodia [34]; and Rho–GTP induces stress-fibre formation and substrate adhesion due to assembly of focal complexes [35]. Inactivation of Rho family members may therefore be required to allow disassembly of such structures. Rac-1 appears to mediate collapsin-induced growth cone collapse in chick dorsal root ganglion neurons [36], whereas Rho seems to be involved in lysophosphatidate-mediated neurite retraction in cultured neuroblastoma cells [37]. Mutations in Rho family members lead to defects in cell migration and axon outgrowth *in vivo*. Expression of dominant-negative or constitutively active *Drac1* in the *Drosophila* nervous system truncates axon growth [38], as do some mutant alleles of *Caenorhabditis elegans mig-2* (a Rho family member) [39••]. However, several *mig-2* mutant alleles lead to misguided axon trajectories, suggesting that Rho can function to couple guidance cues to process outgrowth, at least in *C. elegans* [39••]. Investigation of Eph receptor signalling may help to link Rho/Rac/Cdc42 family GTPases to activation of axon guidance cue receptors.

Intracellular targets of Eph receptors

A number of SH2-domain-containing signalling proteins able to interact with EphA2 and EphB1 have been identified using the yeast two-hybrid system (Table 1; Figure 2) [10,11,40,41]. Whilst several of these proteins have been shown to bind to ligand-activated receptors in cells, their physiological functions in Eph receptor signalling are unclear. Nevertheless, it is encouraging that several of these proteins are implicated in the regulation of the cytoskeleton and cell migrations.

The p85 subunit of PI 3′-kinase and a novel adapter protein SLAP (which is homologous to the Src tyrosine kinase but lacks a catalytic domain) were identified in a screen using EphA2 [10,40]. A separate screen using EphB1 as bait pulled out the SH2 domains of adapter proteins Grb2 and Grb10 as well as Nck [11,41]. Grb10 is of particular interest because it shares a central ~300 amino acid region of homology, including a pleckstrin homology (PH) domain, with the *C. elegans* protein mig-10, which is involved in axonal and cellular migrations [42].

The Src family kinase Fyn interacts with EphA4 *in vitro* via a juxtamembrane tyrosine residue (Tyr602) [8•]. There are reports that Src and Yes similarly interact with Eph receptors [5], and it is possible that engagement of Src family kinases may be regulated by competition with the catalytically inactive SLAP. Src family kinases are highly expressed in the developing nervous system and are concentrated in axons and growth cones [43]. Functions of Src family kinases include regulation of phosphorylation of cytoskeletal proteins and assembly of focal adhesions [44,45].

We have found that the Ras GTPase-activating protein (RasGAP) associates with EphB2 [9•]. In the NG108 neuronal cell line, activation of EphB2 also leads to the phosphorylation of the docking protein p62^{dok}, which

Table 2

Phenotypes of targetted mutations in Eph receptor genes.

Receptor	Defect	Comment	Reference
EphA8$^{-/-}$	Abnormal ipsilateral projection of some superior colliculus axons into spinal cord		[19•]
EphB2$^{-/-}$	Abnormal ventral projection of posterior anterior commissure axons	EphB2 expression ventral to commissure Commissure axons express B class ephrins	[20••]
EphB2$^{lacz/lacz}$	Normal anterior commissure (in 129 and CD1 genetic backgrounds)	Replaces kinase domain with β-galactosidase Retains extracellular transmembrane and juxtamembrane sequences Suggests possible signalling function for ligand in axons	[20••]
EphB3$^{-/-}$	Failure of axons of corpus callosum to cross the midline	Partially penetrant	[21•]
EphB2$^{-/-}$; *EphB3*$^{-/-}$	More severe anterior commissure and corpus callosum defects Additional defect in fasciculation of axons of habenular–interpenduncular tract Cleft palate	Indicates functional redundancy of EphB2 and EphB3	[21•]

subsequently binds both RasGAP and the SH2/SH3 domain adapter protein Nck. These events appear to be coupled to receptor activation through juxtamembrane tyrosine residues Tyr604 and Tyr610 (of EphB2). An independent report suggests that the SH2 domain of Nck is able to bind directly to EphB1 via juxtamembrane tyrosine Tyr594, and that this engagement leads to activation of c-Jun kinase [41]. While cell-type differences may determine whether Nck interacts directly or via $p62^{dok}$ with activated Eph receptors, this protein is now strongly implicated in Eph receptor signal transduction. These findings are of great interest considering the known role of the *Drosophila* homologue of Nck (Dock) in the pathfinding of photoreceptor axons [46•]. Mammalian Nck is known to interact with two Cdc42/Rac-binding proteins, WASP (Wiskott–Aldrich syndrome protein) [47] and the serine/threonine kinase mPAK-3 [48]. Thus, Nck may be an important regulator of cellular function downstream of Eph receptor activation. In addition to mediating interactions with $p62^{dok}$ and Nck, the amino-terminal region of RasGAP affects the actin cytoskeleton, causing cell rounding and dissolution of actin stress fibres, possibly as a consequence of its association with p190, a GTPase-activating protein for Rho [49,50].

Recent evidence suggests that the state of ligand clustering is also exquisitely important in determining the cellular response to receptor engagement (E Stein *et al.*, personal communication; see Note added in proof). Soluble aggregated ephrin-B1 presented in tetrameric form (in contrast to dimeric or higher-order forms) was able to initiate vascular network formation in EphB1-expressing vascular endothelial cells, and engagement of the LMW-PTP at Tyr929 of EphB1. Conversely, all multimeric forms of the ligand could cause receptor tyrosine phosphorylation and binding of SH2-domain-containing proteins.

An intrinsic signalling function for ephrins?

The striking degree of conservation between the cytoplasmic domains of the three transmembrane ephrins initially hinted that they may possess an intrinsic signalling function [14••]. Genetic evidence for transmembrane ephrin signalling came unexpectedly from analysis of mice homozygous for *EphB2* mutant alleles [20••]. The misrouting of anterior commissure axons in *EphB2*$^{-/-}$ animals appears to be a non-cell-autonomous effect of EphB2 as it is cells underlying the anterior commissure that express the receptor, whereas the axons themselves express B class ephrins (Table 2; Figure 3). In some genetic backgrounds, however, the anterior commissure forms normally when the kinase domain of EphB2 is replaced with β-galactosidase. One possible interpretation of these results is that the EphB2-expressing cells can guide the migration of transmembrane-ephrin-expressing axons by a process requiring the extracellular but not the kinase domain of the receptor, implying a signal may be relayed into the axons through the ligand. It is attractive to imagine that such a bidirectional signal could be important in setting up boundaries where receptor- and ligand-expressing cells are not allowed to mix, such as in specification of rhombomeres.

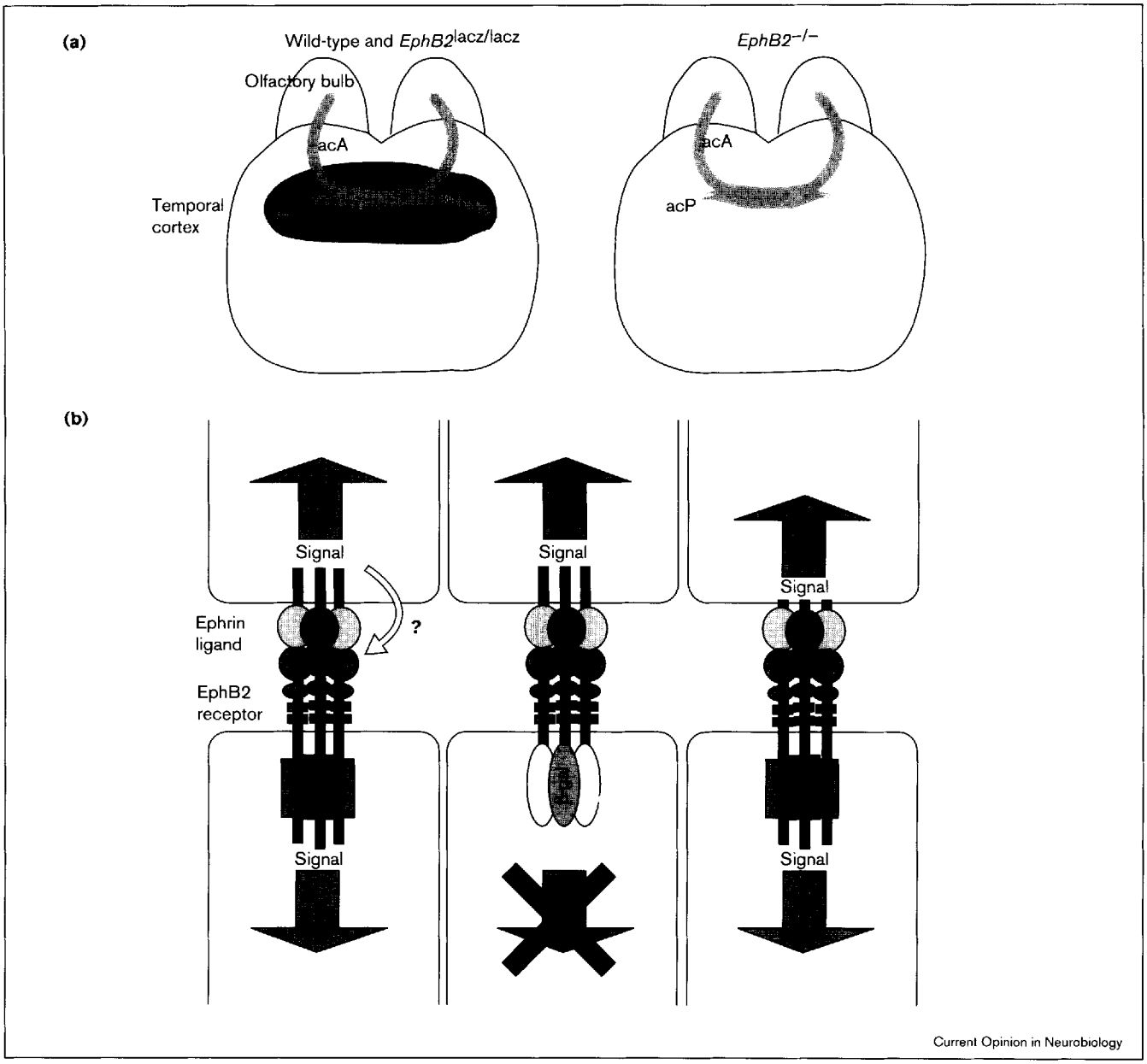
Biochemical evidence to suggest that the cytoplasmic tails of B class ephrins might have a dynamic function in signalling came with the demonstration that the cytoplasmic region of ephrin-B1 was a good *in vitro* and *in vivo* substrate for the activated Src tyrosine kinase [14••,51••]. In addition, treatment of B class ephrin-expressing cells with the soluble extracellular domain of EphB2 or co-culture with EphB2-expressing cells stimulated tyrosine phosphorylation of the ligand cytoplasmic domain [14••]. In a similar study, Bruckner *et al.* [51••] demonstrated that tyrosine phosphorylation of transmembrane ephrins could also be achieved by stimulation of the cells with platelet-derived growth factor (PDGF). Intriguingly, the cytoplasmic domain of ephrin-B1 was able to suppress the transforming ability of activated tyrosine kinases when expressed in the same cell, suggesting a possible two-way crosstalk between transmembrane ephrins and RTKs [3,51••].

The function of tyrosine phosphorylation of B-type ephrins remains to be established. It might induce or inhibit interactions with cytoplasmic signalling proteins or cytoskeletal components, producing a response in ligand-expressing cells. Phosphorylation might also modify ligand clustering, and hence could have an inside-out effect on receptor activation (Figure 3b). Whilst there are no data to suggest that A class ephrins function other than as classical surface bound tyrosine kinase ligands, it is interesting to speculate that they may also relay cellular signals, as Src family kinases can be co-precipitated with other GPI-linked proteins [52,53].

Bidirectional signalling mediated by receptor protein tyrosine phosphatase β

Although much has been learned about the action of RTKs in the response of cells to extracellular signals, less is known about the regulation and function of receptor-type protein tyrosine phosphatases (RPTPs) in these processes. Genetic studies in *Drosophila* have demonstrated that receptor protein phosphatases play an important role in the guidance of several motor neurons to their target muscles [54•,55•]. Their function appears to be similar to that of cell adhesion molecules in sensing environmental cues during the process of cell guidance. All RPTPs are composed of an extracellular domain, a single transmembrane domain and a cytoplasmic portion, which usually contains two tandem protein tyrosine phosphatase domains. The extracellular domain of many receptor-like tyrosine phosphatases shares structural similarities with cell adhesion molecules, suggesting that they play a role in cell–cell communication by directly coupling cell recognition events to signal transduction pathways within the cell.

Figure 3



Transmembrane ephrins may relay cellular signals. **(a)** Mice homozygous for the *EphB2* null allele exhibit a defect in the anterior commissure, where transmembrane-ephrin-expressing axons (light grey) originating in the temporal cortex plunge ventrally into a territory that expresses EphB2 (dark grey) in wild-type mice. In *EphB2*$^{lacz/lacz}$ mice (in 129 and CD1 backgrounds), axons are correctly guided by EphB2 protein lacking the kinase domain. **(b)** Model for bidirectional signalling: EphB2 receptor interaction with transmembrane ephrins causes mutual clustering of proteins, activating both receptor and ephrin tyrosine phosphorylation, and initiating putative signals in both cells (left). When the EphB2 kinase domain is replaced with β-galactosidase (β-gal), no signal is relayed into the receptor-expressing cell, but ephrin signalling is still initiated (centre). This signal may guide anterior commissure axons – see (a). It is also tempting to speculate that GPI-linked ephrins may mediate such a bidirectional signal (right). acA, anterior commissure pars anterior tract; acP, anterior commissure pars posterior tract.

RPTPβ (also known as RPTPζ) is expressed on the surface of glial cells and may function to regulate the growth of axons via reverse signalling through a protein complex on an adjacent cell, analogous to the Eph receptor–ephrin system. RPTPβ contains in its extracellular portion a carbonic anhydrase (CAH) domain, a fibronectin type III repeat and a large cysteine-rich region [56,57]. It exists in three forms that are generated by alternative RNA splicing: one form is a secreted protein composed of the entire extracellular domain, whereas the two other forms are transmembrane receptors that differ by the absence of 860 amino acids from the cysteine-rich region of the extracellular domain of the longer form. Both the secreted form and the long receptor

form were identified as chondroitin sulfate proteoglycans [58]. It was demonstrated that the proteoglycan forms of RPTPβ bind *in vitro* to the extracellular matrix protein tenascin, as well as to the adhesion molecules neuronal cell adhesion molecule (N-CAM) and neuronal–glial cell adhesion molecule (Ng-CAM), suggesting that these proteins may function as ligands of RPTPβ [59,60]. However, it was impossible to detect any effect on the intrinsic protein tyrosine phosphatase activity upon binding of these proteins to the extracellular domain of RPTPβ.

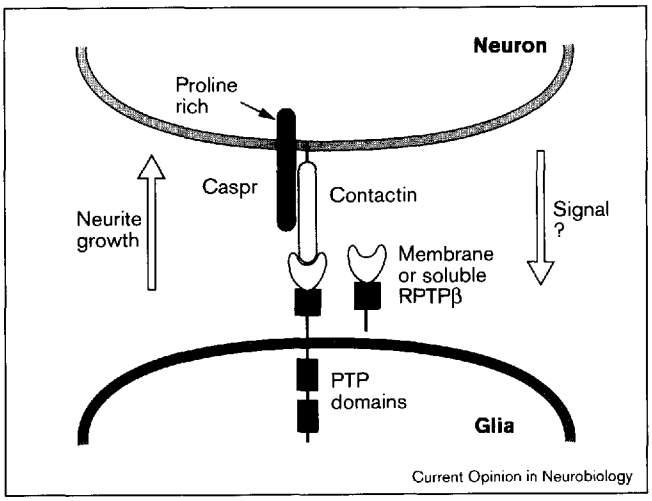
In our search for a physiological ligand of RPTPβ, we found that the CAH domain of this phosphatase binds with high affinity and specificity to a 140 kDa protein that is expressed on the cell surface of neuronal cells [60]. Affinity purification and expression cloning with the CAH domain of RPTPβ as a specific probe demonstrated that the 140 kDa protein is contactin: a GPI-anchored cell recognition molecule that functions as a neuronal receptor. This raised the possibility that the CAH domain of RPTPβ may function as a ligand for contactin. Indeed, the binding of RPTPβ (expressed on glial cells) to contactin (on neuronal cells) leads to cell adhesion and neurite outgrowth, indicating that contactin is a functional neuronal receptor for the CAH domain of RPTPβ [61]. These interactions may lead to bidirectional signalling between neurons and glial cells. In addition to the signal generated in neurons by contactin, RPTPβ may also serve as a receptor that transduces an extracellular signal mediated by its tyrosine phosphatase domains into glial cells (Figure 4). However, so far, we are unable to detect changes in phosphatase activity in response to binding of contactin to the extracellular domain of RPTPβ. In addition, very little is known about the signal generated in glial cells as a result of contactin/RPTPβ complex formation.

RPTPβ as a ligand for contactin in neuronal signalling

RPTPβ expressed on the surface of glial cells binds to a neuronal cell recognition complex that consists of several proteins, including contactin, the neurexin-like protein Caspr and Nr-CAM (Ng-CAM-related cell adhesion molecule) [62•,63]. Analysis of neurite growth induced by different domains of the extracellular region of RPTPβ demonstrated that in addition to contactin, Nr-CAM plays a role in this process [63]. Recent experiments suggest that the cooperation between Nr-CAM and contactin occurs in an ordered manner. The initial association is mediated by interactions between contactin and the CAH of RPTPβ; this interaction may not be sufficient for mediating the full neurite-promoting activity of RPTPβ. It appears that additional interactions between the cysteine-rich region of RPTPβ and Nr-CAM are required for induction of long neurites.

Another protein that is found in a complex with contactin is the transmembrane protein Caspr. The extracellular domain of Caspr contains multiple domains implicated in mediating protein–protein interactions [56,64]. It was demonstrated that Caspr associates with contactin molecules that are present in the same cell membrane, suggesting that Caspr may function as a signalling subunit that mediates the biological effects of contactin. The cytoplasmic domain of Caspr contains a proline-rich sequence capable of binding to a subset of SH3 domains of signalling proteins, which may transduce the biological effects of contactin [62•]. It was demonstrated that the intracellular domain of neurexin, the *Drosophila* homologue of Caspr, is required for the localisation of D4.1-coracle protein, a protein essential for the formation of septate junctions [65]. In vertebrates, Caspr may interact with D4.1/ERM, a protein that could provide a link to the cytoskeletal network [64]. Contactin itself was found to be associated with the protein tyrosine kinase Fyn, raising the possibility that Src family kinases may participate in the control of signalling pathways downstream of contactin [53,66]. As is the case with other GPI-linked proteins, it is not clear how this association occurs. However, it is possible that the interaction between contactin and Src kinases is indirectly mediated by Caspr or by another transmembrane receptor that associates with contactin in the plane of the membrane [53]. In addition, it was demonstrated that Nr-CAM binds to ankyrin, a spectrin-binding protein that links the actin cytoskeleton to the cell membrane [67]. The carboxy-terminal tail of Nr-CAM contains a

Figure 4



A model for bidirectional signals mediated by interactions between RPTPβ and contactin. Soluble and membrane forms of RPTPβ expressed on the surface of glial cells bind to contactin expressed on the surface of neuronal cells. Contactin is a GPI-linked protein, and at least part of the signalling events that are regulated by RPTPβ binding to contactin are probably mediated by the transmembrane receptor Caspr. Both Caspr and contactin are expressed on the cell surface of neuronal cells. A proline-rich sequence in the cytoplasmic domain of Caspr may serve as a binding site for SH3 domains of signalling molecules. The binding of contactin to RPTPβ may lead to a signal in glial cells that is mediated by the protein tyrosine phosphatase domain of RPTPβ.

potential binding site for PDZ-containing proteins. Thus, the cytoplasmic tail of Caspr and Nr-CAM may recruit PDZ and SH3 domain-containing proteins, as well as other signalling molecules, to specific regions of cell–cell contacts, thereby regulating intracellular signalling machinery and cytoskeletal changes that take place during neurite outgrowth.

Conclusions

It appears that in interactions between Eph receptors and transmembrane ephrins as well as between RPTPβ and contactin, it is not only the catalytically active receptors that possess intrinsic signalling activity, but also the cognate ligands. Dissection of signalling downstream of Eph receptors and the contactin/Caspr complex in neurons may enable us to determine the links between axonal pathfinding cue receptors, axonal outgrowth and regulation of the cytoskeleton. The biochemical nature and biological functions of reciprocal ephrin and RPTPβ signalling are at present unclear, but represent interesting and challenging directions for future investigation.

Note added in proof

The work referred to in the text as (E Stein *et al.*, personal communication) is now in press [68].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
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