The EMBO Journal Vol.18 No.19 pp.5159–5165, 1999

EMBO MEMBER'S REVIEW

Ephrins and their Eph receptors: multitalented directors of embryonic development

Jonas Frisén[1], Johan Holmberg and Mariano Barbacid[1,2]

Department of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institute, S-171 77 Stockholm, Sweden and [2]Centro Nacional de Investigaciones Oncológicas Carlos III, 28220 Majadahonda, Madrid, Spain

[1]Corresponding authors

Keywords: angiogenesis/axon guidance/nervous system development/topography/tyrosine protein kinase

Introduction

Ephrins are membrane-bound ligands for the Eph family of protein tyrosine kinase receptors. Recent genetic studies have indicated that these molecules play key roles in diverse biological processes such as the development of the nervous system and angiogenesis. In the nervous system, they provide positional information by employing mechanisms that involve repulsion of migrating cells and growing axons. Understanding the mechanisms that mediate these biological responses will help to establish the molecular basis of topographic positioning within the developing embryo.

The molecules

Eph receptors have been conserved in a variety of eukaryotic species from *Caenorhabditis elegans* to man. They constitute the largest subgroup within the tyrosine protein kinase receptor family, with 14 receptors in mammals known to date. These receptors interact with cell-surface-bound ligands known as ephrins. The Eph receptors and their ephrin ligands can be divided into two classes based on structural features and binding affinities (Eph Nomenclature Committee, 1997; Figure 1). Type A ephrins are attached to the outer leaflet of the plasma membrane by a glycosyl phosphatidylinositol (GPI) moiety and they bind to the type A class of structurally related Eph receptors (Eph Nomenclature Committee, 1997). Type B ephrins have, in addition to their extracellular domain, a single transmembrane domain and a cytoplasmic tail. They bind to type B Eph receptors (Eph Nomenclature Committee, 1997). With the exception of EphA4, which can bind members of class A and class B ephrins, there appears to be very limited cross-talk between the A and B classes (Gale *et al*., 1996). Although there is a high degree of promiscuity between ephrins and Eph receptors of the same class, they may not be functionally interchangeable. For instance, there are considerable differences in binding affinities between different ligand–receptor pairs within the same class, suggesting that there may be preferred ligands for certain receptors (see e.g. Gale *et al*., 1996; Monschau *et al*., 1997).

All Eph receptors have an N-terminal globular domain which folds into a compact jellyroll β-sandwich (Himanen *et al*., 1998). This domain is necessary and sufficient for ligand binding (Labrador *et al*., 1997). The extracellular domain also contains two fibronectin type III domains, which serve to dimerize receptors (Lackmann *et al*., 1998), and two stretches of cysteine-rich sequence. The intracellular region has a single tyrosine protein kinase domain and a SAM domain. Eph receptors, as well as type B ephrins, have consensus sequences for binding PDZ proteins in the C-termini (Hock *et al*., 1998b; Torres *et al*., 1998; Lin *et al*., 1999).

Signaling pathways

Genes encoding transcription factors with a homeodomain, *Hox* genes, are key regulators in the patterning of the developing organism, but how these transcription factors mediate their effects remains unknown (Krumlauf, 1994). Evidence has started to accumulate suggesting that ephrins and Eph receptors function in the same genetic pathways as *Hox* genes and that, in some situations, they may be effectors of *Hox* genes. For example, the homeobox-containing protein Engrailed regulates the expression of axon guidance cues in the midbrain, and ephrins seem to be the main molecules directing the development of axonal projections in this system (Friedman and O'Leary, 1996; Itasaki and Nakamura, 1996; Logan *et al*., 1996). In line with this evidence, ectopic expression of *Engrailed* results in increased ephrin-A2 and ephrin-A5 expression in the chick midbrain (Logan *et al*., 1996; Shigetani *et al*., 1997). Furthermore, *EphA7* expression is regulated by Hoxa2 as demonstrated by decreased *EphA7* expression in Hoxa2$^{-/-}$ mice (Taneja *et al*., 1996), and Hoxa1 and Hoxb1 positively regulate *EphA2* expression *in vitro* and *in vivo* (Chen and Ruley, 1998; Studer *et al*., 1998). In other situations the ephrin–Eph and Hox pathways may also act in parallel, since Krox-20, a regulator of *Hox* gene expression in the hindbrain, was recently found to directly regulate *EphA4* expression *in vivo* (Theil *et al*., 1998).

The mechanisms by which these molecules mediate their downstream signaling are rapidly being unveiled (Brückner and Klein, 1998). Ephrins, which are monomers, appear to dimerize their receptors by forming aggregates in certain subdomains of the membrane. Soluble ephrins can bind their cognate Eph receptors, but they fail to activate them unless they are clustered (Davis *et al*., 1994). This clustering appears to be facilitated by their interaction with PDZ domain proteins (Hock *et al*., 1998b; Torres *et al*., 1998; Brückner *et al*., 1999; Lin *et al*., 1999). Interestingly, the degree of multimerization of the

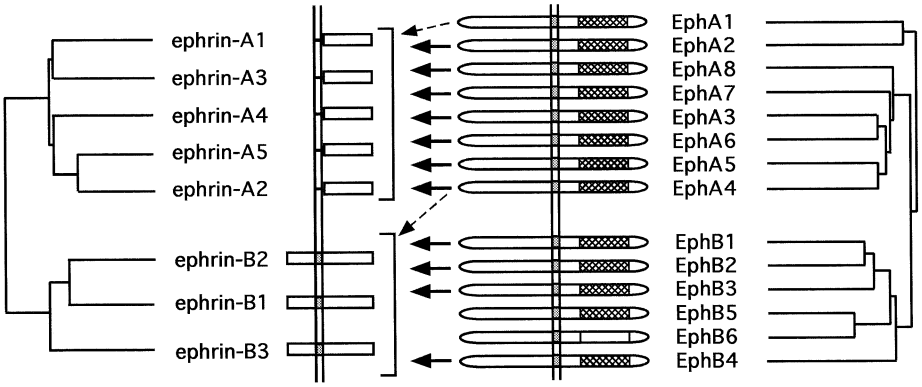

Fig. 1. The ephrins and Eph receptor families. These molecules fall into two classes (A and B) based on sequence homology and binding affinities (Eph Nomenclature Committee, 1997). Type A ephrins are attached to the cell membrane with a GPI linkage. Type B ephrins have transmembrane and cytoplasmic domains. Type A Eph receptors are more structurally related to each other than to type B receptors and preferentially bind type A ephrins. Likewise, type B Eph receptors are more structurally related to each other than to type A receptors and bind only to type B ephrins. The dendrograms were derived using the Clustal program by aligning the extracellular domains of the receptors and the conserved core sequences of the ligands (Eph Nomenclature Committee, 1997).

ephrins affects their biochemical and cellular responses (Gale and Yancopoulos, 1997; Stein *et al*., 1998).

EphB2 receptors have been found to be overexpressed in several different human tumors (Kiyokawa *et al*., 1994), yet there is no evidence that Eph receptors play a role in tumorigenesis. Indeed, Eph receptor activation fails to transform rodent fibroblasts in culture (Lhotak and Pawson, 1993). Thus, it is likely that these receptors may utilize, at least in part, signal transduction pathways distinct from those used by tyrosine kinase receptors involved in mitogenic signaling. Several proteins have been reported to bind to the intracellular domain of Eph receptors. These include p59fyn, PI3-kinase, Grb2, Grb10, RasGap, Nck, Crk and a novel kinase-less Src-like adaptor protein, SLAP (Pandey *et al*., 1994, 1995a; Ellis *et al*., 1996; Stein *et al*., 1996; Holland *et al*., 1997; Hock *et al*., 1998a). Ephrins induce rearrangements of the cytoskeleton in axonal growth cones (Meima *et al*., 1997a,b) and one pathway from the receptor to the cytoskeleton has been suggested to be initiated by the binding of RasGap to activated Eph receptors (Holland *et al*., 1997). The binding of RasGap induces the formation of a ternary complex containing $p62^{dok}$ and Nck, where RasGap and Nck have been implicated in remodeling the cytoskeleton and axonal guidance (Holland *et al*., 1997).

Type B ephrins also signal in response to receptor binding, thus enabling bidirectional signaling. The first indication that transmembrane ephrins may have signaling capabilities came from genetic studies of mice carrying targeted EphB2 alleles. Henkemeyer *et al.* (1996) showed that whereas mice lacking EphB2 receptors had a malformed anterior commissure, similar mice expressing mutant receptors that lacked their kinase domains had no detectable defects. These results suggest that proper formation of the anterior commissure requires interaction between EphB2 receptors and their cognate B ephrins, but not signaling by these receptors. Indeed, binding of type B Eph receptors to transmembrane ephrins induces tyrosine phosphorylation in their cytoplasmic tails (Holland *et al*., 1996; Brückner *et al*., 1997). Since the type B ephrins lack endogenous kinase activity, it is presumed that they are phosphorylated by cytoplasmic kinases. Additional support for the view that Eph receptors may have functions that are independent of their kinase activity has come from genetic studies in *C.elegans* as well as from the identification of EphB6, a kinase-dead Eph receptor (Gurniak and Berg, 1996; George *et al*., 1998). Finally, rapid tyrosine phosphorylation of ephrin-B1 upon exposure of cells to platelet-derived growth factor, and suppression of mitogenic properties mediated by tyrosine kinase receptors by co-expressed ephrin-B1, suggests cross-talk between other tyrosine kinase receptors and class B ephrins (Brambilla *et al*., 1996; Brückner *et al*., 1997).

Cellular repulsion and formation of boundaries

The first experiments that demonstrated the repulsive effect of an ephrin were the result of an ambitious search for repellent molecules in the chick visual system that were known to guide growing axons. In this study, ephrin-A5 was isolated and its repulsive effect demonstrated in *in vitro* assays (Drescher *et al*., 1995). When retinal axons were allowed to choose between growing on ephrin-A5-containing or -depleted substrates in the stripe assay (Walter *et al*., 1987), they avoided ephrin-A5-containing lanes (Drescher *et al*., 1995). Ephrin-A2 was also found to repel retinal axons both *in vitro* and *in vivo* when ectopically expressed with a retroviral vector (Nakamoto *et al*., 1996). These initial experiments have been extended and there are now ample examples of axons from different neuronal types that are repelled by ephrins (Flanagan and Vanderhaegen, 1998).

The repulsion of axons induced by the interaction of ephrins with their cognate Eph receptors is believed to be mediated by rearrangements in the cytoskeleton of the axonal growth cone which result in retraction of the axon in response to a signal transduction event (Meima *et al*., 1997a,b). There is also increasing evidence that ephrins and their receptors may guide migrating cells by mediating a repulsive action and constraining cells to certain migratory routes. For example, migrating neural crest cells express Eph receptors, and ephrins are expressed in territories that the migrating cells normally avoid. Interference with ephrin–Eph interaction or signaling during

neural crest migration disrupts the normal patterning of these cells (Krull *et al*., 1997; Smith *et al*., 1997; Wang and Anderson, 1997). In the branchial neural crest, ephrin-B2 is essential for restricting the intermingling of second- and third-arch neural crests, and for targeting third-arch neural crest cells to the correct destination (Smith *et al*., 1997). In the trunk, B-type ephrins expressed in the caudal half of the sclerotome direct the migration of neural crest cells through the rostral half (Krull *et al*., 1997; Wang and Anderson, 1997).

Ephrins and Eph receptors are expressed in gradients in some regions of the central nervous system, where they are implicated in the formation of topographic axonal projections (discussed below). In addition, they also appear in complementary and mutually exclusive domains, suggesting that these molecules may underlie boundary formation (Gale *et al*., 1996). Several lines of evidence suggest that axons and migrating cells sense differences in the concentration of ephrins, and that the graded expression or the sharp borders provide positional information. Misexpression of wild-type or dominant-negative forms of ephrins and Eph receptors in zebrafish embryos has proved to be a powerful approach in the study of the role of these genes in boundary formation. In the developing hindbrain, Eph receptors and type B ephrins in complementary segments (rhombomeres) restrict cell intermingling over boundaries (Xu *et al*., 1995, 1999). The sorting of cells to different domains appears to be dependent on bidirectional signaling (Mellitzer *et al*., 1999). However, unidirectional signaling through Eph receptors can restrict cell communication through gap junctions (Mellitzer *et al.*, 1999). In addition to segmentation of the hindbrain, interruption of Eph signaling leads to abnormal somite formation, implicating Eph signaling in boundary formation and patterning of presomitic mesoderm into somites (Durbin *et al*., 1998).

Ephrins and Eph receptors also play an important role in establishing boundaries between arteries and veins during angiogenesis. For instance, in the initial stages of angiogenesis, presumptive arterial and venous endothelial cells can be distinguished by their selective expression of ephrin-B2 or an EphB receptor, respectively (Wang *et al*., 1998; Adams *et al.*, 1999). In *ephrin-B2*-null mice, and in some *EphB2/EphB3* double-deficient mice, angiogenesis is defective and the embryos die in mid-gestation (Wang *et al*., 1998; Adams *et al.*, 1999). These findings indicate a novel role for ephrins and their receptors, and warrant further studies on how these molecules may participate in angiogenesis.

There are several examples in which the role of ephrins cannot be easily explained by repulsive action. For example, ephrin-A1 has chemoattractant effects on endothelial cells (Pandey *et al*., 1995b). Another study demonstrates that whereas axons from cortical neurons normally not projecting to ephrin-A5-containing cortical layers are repelled by ephrin-A5 in *vitro*, ephrin-A5 induces sprouting of axons that normally project to these layers (Castellani *et al*., 1998). Developmental defects in gene-targeted mice, such as the cleft palate observed in *EphB2/EphB3* double-mutant mice (Orioli *et al*., 1996) and the cranial defects observed in *ephrin-A5*-null mice due to the failure of the neural folds to adhere in the dorsal midline (our unpublished data), are difficult to explain by postulating loss of ephrin-repulsive activity. These genetic studies suggest that ephrins and Eph receptors, either directly or indirectly, may have other biological activities, even cell adhesion in some contexts.

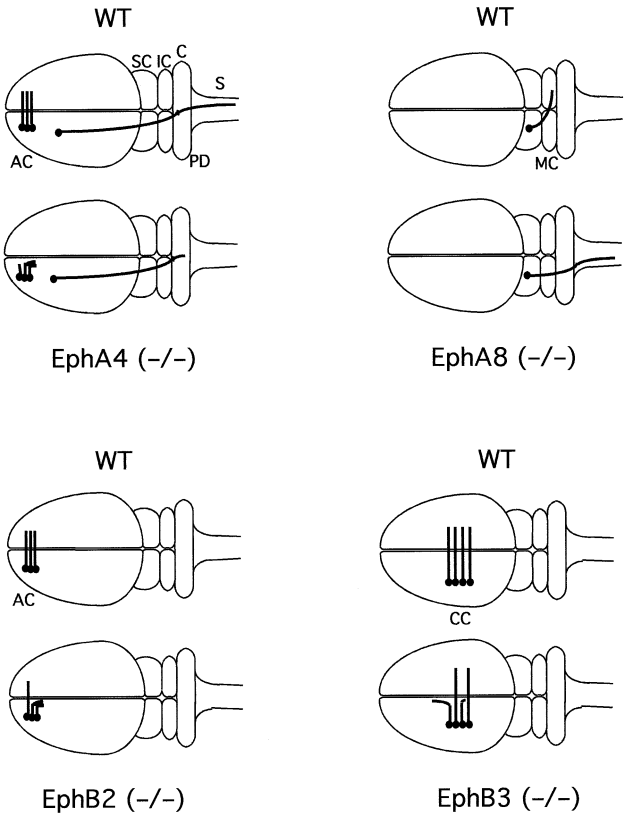

Fig. 2. Mice with targeted disruptions in genes encoding *Eph* receptors have axonal midline crossing defects. Schematic drawings depicting affected neuronal populations and their axonal projections in the central nervous system of wild-type and Eph-mutant mice. The superior colliculus (SC), inferior colliculus (IC), cerebellum (C) and spinal cord (S) are indicated in the top left panel. The labeled commissures are: anterior commissure (AC), pyramidal decussation (PD), midbrain commissure (MC) and corpus callosum (CC). The midline crossing phenotypes are partially penetrant in several of the mutants. In wild-type animals, EphA8 is expressed in the neurons that are affected in the EphA8 mutants and several A-type ephrins are expressed in the midbrain, indicating a cell-autonomous function of the receptor. In contrast, EphA4, EphB2 and EphB3 are not expressed in wild-type animals in the neurons that are affected in the respective mutants. Instead, these neurons express ephrin-B ligands and the receptors are expressed adjacent to the midline at the crossing points, indicating that ligand signaling is essential for the formation of these commissures.

Axon guidance

Several studies in mutant mice lacking Eph receptors have illustrated the requirement for these molecules for correct axonal path finding in certain projections (summarized in Figure 2). *EphA8* expression in the nervous system is restricted to small subpopulations of neurons in the superior colliculus, hindbrain and spinal cord (Park *et al*., 1997). In the superior colliculus, *EphA8 is* expressed in a gradient with the highest levels rostrally (Park *et al*., 1997). In EphA8-deficient mice, axons from a group of neurons in the superior colliculus (which normally express *EphA8*) fail to reach their normal target in the contralateral inferior colliculus, and instead extend into the ipsilateral

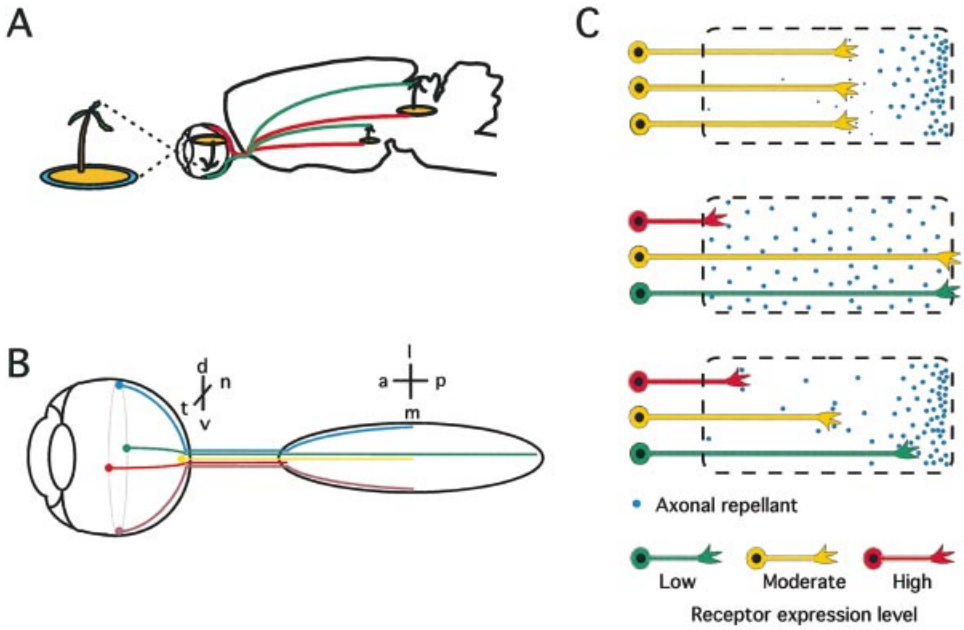

Fig. 3. Topographic organization of retinal projections. (**A**) The retina projects in a topographic manner to the lateral geniculate nucleus in the forebrain and to the superior colliculus in the midbrain, allowing ordered transfer of information. (**B**) Depending on the location of a retinal ganglion cell in the retina on the dorso-ventral and naso-temporal axes, its axon will terminate at a distinct position along the antero-posterior and medio-lateral axes, respectively, of the superior colliculus. (**C**) Three scenarios where neurons project to a target area indicated by the broken line. In the upper panel, all neurons are equally sensitive to the graded ligand and will reach the threshold where the ligand induces them to terminate at the same point. In the middle panel, the neurons are differentially sensitive to the ligand, but since the ligand is present in a uniform concentration, an axon will either terminate upon reaching the target or grow through it if the concentration is too low to induce the axon to stop. In the lower panel, both receptor and ligand are present in gradients, allowing the establishment of a topographic map in which individual axons terminate at distinct positions within the target.

cervical spinal cord (Park *et al*., 1997). *EphA4*-null mice exhibit motor dysfunction, which is probably caused by disruption of the corticospinal tract (Dottori *et al*., 1998). In addition, in the majority of these mice, the anterior commissure is missing (Dottori *et al*., 1998).

EphB3-null mice display a partially penetrant defect in the formation of the corpus callosum, where in some animals the axons fail to cross the midline and instead form bundles of axons at the midline or grow along the antero-posterior axis instead of crossing the midline (Orioli *et al*., 1996). In mice lacking *EphB2* receptors, the posterior part of the anterior commissure is malformed with many of the axons that normally form this tract projecting aberrantly to the floor of the brain (Henkemeyer *et al*., 1996). Interestingly, the axons of the anterior commissure do not express EphB2 receptors, but express the EphB2 ligand ephrin-B1, whereas EphB2 is expressed in cells surrounding the growing axons (Henkemeyer *et al*., 1996). This lends strong support to the concept that B ephrins may signal upon Eph receptor binding. Finally, *EphB2* and *EphB3* double-mutant mice show higher penetrance of the partial defects seen in mice in which only one gene has been deleted (Orioli *et al*., 1996), demonstrating limited functional redundancy between different Eph receptors.

These double-mutant mice also show defective fasciculation of axons in the habenular–interpeduncular tract, although these axons reach their target (Orioli *et al*., 1996). These results suggest a role for ephrins and Eph receptors in axon fasciculation independent of axon guidance. Previous *in vitro* studies have demonstrated that blocking ephrin–Eph-receptor interactions in co-cultures of cortical neurons and astrocytes with soluble chimeric ligand or receptor molecules results in defasciculated axon growth (Winslow *et al*., 1995). How ephrins and Eph receptors might participate in axonal fasciculation is not clear. They may mediate cell adhesion or induce the synthesis of cell adhesion molecules. Alternatively, Eph-receptor-expressing neurons may favor growing on each other rather than on astrocytes expressing ephrins, resulting in fascicle formation (Tessier-Lavigne, 1995).

Formation of topographic axonal projections

In 1963, Sperry suggested that gradients of a few molecules may guide growing axons to distinct locations in a topographic projection. This theory requires that neurons are differentially sensitive to such molecules, a situation that might be achieved by graded expression of their receptors (Figure 3). The identification of graded expression of ephrins, along with their repulsive activity, has lent support to the hypothesis that these molecules may play a role in the formation of topographic maps. The pre-eminent model system for studies on topographic projections has been the visual system, where retinal ganglion cells project in a topographic manner to several targets, mainly the superior colliculus (tectum in birds) in the midbrain and the lateral geniculate nucleus in the forebrain. The projection of retinal axons is topographically ordered along two axes. Depending on the location of a given neuron on the dorso-ventral and naso-temporal axes of the retina, its axon will terminate at distinct positions along the medio-lateral and antero-posterior axes

of the superior colliculus (Figure 3B) and the lateral geniculate nucleus, respectively.

Individual retinal ganglion cells express different levels of type A Eph receptors along the naso-temporal axis, creating a smooth gradient of receptor expression and type A ephrin sensitivity (Cheng *et al*., 1995; Drescher *et al*., 1995; Nakamoto *et al*., 1996; Monschau *et al*., 1997; Feldheim *et al*., 1998). Two closely related ephrins, ephrin-A2 and ephrin-A5, are expressed in overlapping gradients in both the superior colliculus and lateral geniculate nucleus (Cheng *et al*., 1995; Drescher *et al*., 1995; Feldheim *et al*., 1998). Altering the smooth gradient of ephrin expression by retroviral misexpression in the chick tectum induces axons to terminate at patches of high ephrin expression and disrupts the normal topography of this projection, providing the first *in vivo* evidence for a role of ephrins in topographic mapping (Nakamoto *et al*., 1996).

Genetic studies have provided additional evidence for the role of ephrins and their cognate Eph receptors in topographic mapping. Analysis of retinal projections in *ephrin-A5*-null mice has demonstrated two distinct functions for ephrin-A5 in the projection of retinal ganglion cell axons to their targets at two different developmental phases (Frisén *et al*., 1998b). In neonatal wild-type and *ephrin-A5*-null mice, most retinal ganglion cell axons have reached the superior colliculus. However, in *ephrin-A5*-null animals, many axons extend beyond the superior colliculus into the inferior colliculus (Frisén *et al*., 1998b). This overshooting suggests that ephrin-A5, which is highly expressed in the inferior colliculus, serves as a stop signal for axons to terminate within its target. Ephrin-A5 plays its second role in the formation of the retino-collicular projection during the phase of establishment of topography. In *ephrin-A5*-null mice, a substantial number of axons terminate at topographically incorrect locations (Frisén *et al*., 1998b). Axons from temporal neurons, which normally project to the anterior superior colliculus, often terminate at more posterior regions.

Ephrin-A5 also serves as a topographic cue for retinal projections to the lateral geniculate nucleus (Feldheim *et al*., 1998). Interestingly, the projection of both temporal and nasal retinal neurons to the lateral geniculate nucleus is affected in the *ephrin-A5*-null mice, lending strong support to the hypothesis that axons compete for space relative to one another, not relative to the target (Feldheim *et al*., 1998). The use of the same set of molecules to establish topography in different targets for retinal neurons bears resemblance to the metameric organization of the body along the anterior–posterior axis and has implications for the emergence of new targets in the central nervous system during evolution (Feldheim *et al*., 1998).

In addition to the expression of Eph receptors in the retina, several ligands are expressed in the retina, and some of them in a gradient. Modulation of type A ephrin levels in retinal neurons *in vitro* and *in vivo* results in altered sensitivity of the axons of these neurons to ephrins (Hornberger *et al.*, 1999). This has led to the suggestion that the responsiveness of a neuron to an ephrin is regulated not only by the level of receptor expression but also by co-expression of ligand, which may render the neuron less sensitive to ephrin (Hornberger *et al.*, 1999).

Although the role of ephrins in directing the development of topographic projections has been tested only in the visual system, there are reasons to believe that they may serve similar roles in other projections. For instance, the expression patterns of ephrins and Eph receptors in the thalamo-cortical projections, septo-hippocampal tract, nigro-striatal pathway and motor neuron projections to muscles along the anterior–posterior axis implicate these molecules in the organization of the topography of these systems (Donoghue *et al*., 1996; Gao *et al*., 1996, 1998a; Yue *et al.*, 1999).

Is there a role for ephrins and Eph receptors beyond embryonic development?

In spite of the large number of studies describing important functions for ephrins and Eph receptors during embryonic development, there are very few that address the role of these molecules in the adult. Most ephrins and Eph receptors are predominantly expressed during development, but several are also expressed in adult tissues. Based on the actions of these genes during development, one may suspect that these molecules could act in plasticity processes. Indeed, in the adult brain, the expression of several ephrins and Eph receptors is most prominent in plastic regions. For example, Eph receptors are found in synapses of the adult hippocampus (Buchert *et al*., 1999). Interfering with Eph signaling by injection of blocking or activating agents has indicated a role for ephrins in synaptic remodeling and long-term potentiation (Gao *et al*., 1998b). Moreover, it is also possible that ephrins and Eph receptors may take part in guiding migration and connectivity of new neurons generated from stem cells in the adult brain (Frisén *et al*., 1998a). Outside the nervous system, it is tempting to speculate that ephrins and their Eph receptors may also play a role in adult angiogenesis.

Acknowledgements

Work in the authors' laboratory described in this review is supported by the Swedish Medical Research Council, the Swedish Cancer Society and the Swedish Foundation for Strategic Research to J.F. M.B. is supported by grants from Pfizer Inc. and the Plan Nacional de I+D from the Ministerio de Educación y Ciencia of Spain.

References

Adams,R.H., Wilkinson,G.A., Weiss,C., Diella,F., Gale,N.W., Deutsch,U., Risau,W. and Klein,R. (1999) Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis and sprouting angiogenesis. *Genes Dev.*, **13**, 295–306.

Brambilla,R., Brückner,K., Orioli,D., Bergemann,A.D., Flanagan,J.G. and Klein,R. (1996) Similarities and differences in the way transmembrane-type ligands interact with the Elk subclass of Eph receptors. *Mol. Cell. Neurosci.*, **8**, 199–209.

Brückner,K. and Klein,R. (1998) Signaling by Eph receptors and their ephrin ligands. *Curr. Opin. Cell Biol.*, **8**, 375–382.

Brückner,K., Pasquale,E.B. and Klein,R. (1997) Tyrosine phosphorylation of transmembrane ligands for eph receptors. *Science*, **275**, 1640–1643.

Brückner,K., Labrador,J.P., Scheiffele,P., Herb,A., Seeburg,P. and Klein,R. (1999) EphrinB ligands recruit GRIP family PDZ adaptor proteins into raft membrane microdomains. *Neuron*, **22**, 511–524.

Buchert,M., Schneider,S., Meskenaite,V., Adams,M.T., Canaani,E., Baechi,T., Moelling,K. and Hovens,C.M. (1999) The junction-associated protein AF-6 interacts and clusters with specific eph receptor tyrosine kinases at specialized sites of cell-cell contact in the brain. *J. Cell Biol.*, **25**, 361–371.

Castellani,V., Yue,Y., Gao,P.-P., Zhou,R. and Bolz,J. (1998) Dual action of a ligand for Eph receptor tyrosine kinases on specific populations of axons during the development of cortical circuits. *J. Neurosci.*, **18**, 4663–4672.

Chen,J. and Ruley,H.E. (1998) An enhancer element in the EphA2 (Eck) gene sufficient for rhombomere-specific expression is activated by HOXA1 and HOXB1 homeobox proteins. *J. Biol. Chem.*, **273**, 24670–24675.

Cheng,H.-J., Nakamoto,M., Bergemann,A.D. and Flanagan,J.G. (1995) Complementary gradients in expression and binding of Elf-1 and Mek4 in development of the topographic retinotectal projection map. *Cell*, **82**, 371–381.

Davis,S., Gale,N.W., Aldrich,T.H., Maisonpierre,P.C., Lhotak,V., Pawson,T., Goldfarb,M. and Yancopoulos,G.D. (1994) Ligands for EPH-related receptor tyrosine kinases that require membrane attachment or clustering for activity. *Science*, **266**, 816–819.

Donoghue,M.J., Lewis,R.M., Merlie,J.P. and Sanes,J.R. (1996) The Eph kinase ligand AL-1 is expressed by rostral muscles and inhibits outgrowth from caudal neurons. *Mol. Cell. Neurosci.*, **8**, 185–198.

Dottori,M. *et al*. (1998) EphA4 (Sek1) receptor tyrosine kinase is required for the development of the corticospinal tract. *Proc. Natl Acad. Sci. USA*, **95**, 13248–13253.

Drescher,U., Kremoser,C., Handwerker,C., Löschinger,J., Noda,M. and Bonhoeffer,F. (1995) *In vitro* guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal protein related to ligands for Eph receptor tyrosine kinases. *Cell*, **82**, 359–370.

Durbin,L., Brennan,C., Shiomi,K., Cooke,J., Barrios,A., Shanmugalingam,S., Guthrie,B., Lindberg,R. and Holder,N. (1998) Eph signalling is required for segmentation and differentiation of the somites. *Genes Dev.*, **12**, 3096–3109.

Ellis,C., Kasmi,F., Ganju,P., Walls,E., Panayotou,G. and Reith,A.D. (1996) A juxtamembrane autophosphorylation site in the Eph family receptor tyrosine kinase, Sek, mediates high affinity interaction with p59fyn. *Oncogene*, **12**, 1727–1736.

Eph Nomenclature Committee (1997) Unified nomenclature for Eph family receptors and their ligands, the ephrins. *Cell*, **90**, 403–404.

Feldheim,D.A., Vanderhaeghen,P., Hansen,M.J., Frisén,J., Lu,Q., Barbacid,M. and Flanagan,J.G. (1998) Topographic guidance labels in a sensory projection to the forebrain. *Neuron*, **21**, 1303–1313.

Flanagan,J.G. and Vanderhaegen,P. (1998) The ephrins and Eph receptors in neural development. *Annu. Rev. Neurosci.*, **21**, 309–345.

Friedman,G.C. and O'Leary,D.D.M. (1996) Retroviral missexpression of engrailed genes in the chick optic tectum perturbs the topographic targeting of retinal axons. *J. Neurosci*., **16**, 5498–5509.

Frisén,J., Johansson,C.B., Lothian,C. and Lendahl,U. (1998a) Central nervous system stem cells in the embryo and adult. *Cell. Mol. Life Sci.*, **54**, 935–945.

Frisén,J., Yates,P.A., McLaughlin,T., Friedman,G.C., O'Leary,D.D.M. and Barbacid,M. (1998b) Ephrin-A5 (AL-1/RAGS) is essential for proper retinal axon guidance and topographic mapping in the mammalian visual system. *Neuron*, **20**, 235–243.

Gale,N.W. and Yancopoulos,G.D. (1997) Ephrins and their receptors: a repulsive topic. *Cell Tissue Res.*, **290**, 227–241.

Gale,N.W. *et al*. (1996) Eph receptors and ligands comprise two major specificity subclasses and are reciprocally compartmentalized during embryogenesis. *Neuron*, **17**, 9–19.

Gao,P.-P., Zhang,J.-H., Yokoyama,M., Racey,B., Dreyfus,C.F., Black,I.B. and Zhou,R. (1996) Regulation of topographic projection in the brain: Elf-1 in the hippocamposeptal system. *Proc. Natl Acad. Sci. USA*, **93**, 11161–11166.

Gao,P.-P., Yue,Y., Zhang,J.-H., Ceretti,D.P., Levitt,P. and Zhou,R. (1998a) Regulaton of thalamic neurite outgrowth by the Eph ligand ephrin-A5: implications in the development of thalamocortical projections. *Proc. Natl Acad. Sci. USA*, **95**, 5329–5334.

Gao,W.-Q., Shinsky,N., Armanini,M.P., Moran,P., Zheng,J.L., Mendoza-Ramirez,J.-L., Phillips,H.S., Winslow,J.W. and Caras,I.W. (1998b) Regulation of hippocampal synaptic plasticity by the tyrosine kinase receptor, REK7/EphA5 and its ligand, AL-1/ephrin-A5. *Mol. Cell. Neurosci.*, **11**, 247–259.

George,S.E., Simokat,K., Hardin,J. and Chisholm,A.D. (1998) The VAB-1 Eph receptor tyrosine kinase functions in neural and epithelial morphogenesis in *C. elegans*. *Cell*, **92**, 633–643.

Gurniak,C.B. and Berg,L.J. (1996) A new member of the Eph family of receptors that lacks protein tyrosine kinase activity. *Oncogene*, **13**, 777–786.

Henkemeyer,M., Orioli,D., Henderson,J.T., Saxton,T.M., Roder,J., Pawson,T. and Klein,R. (1996) Nuk controls pathfinding of commissural axons in the mammalian central nervous system. *Cell*, **86**, 35–46.

Himanen,J.-P., Henkemeyer,M. and Nikolov,D.B. (1998) Crystal structure of the ligand-binding domain of the receptor tyrosine kinase EphB2. *Nature*, **396**, 486–491.

Hock,B., Böhme,B., Karn,T., Feller,S., Rubsamen-Waigmann,H. and Strebhardt,K. (1998a) Tyrosine-614, the major autophosphorylation site of the receptor tyrosine kinase HEK2, functions as multi-docking site for SH′-domain mediated interactions. *Oncogene*, **17**, 255–260.

Hock,B. *et al*. (1998b) PDZ-domain-mediated interaction of the Eph-related receptor tyrosine kinase EphB3 and the ras-binding protein AF6 depends on the kinase activity of the receptor. *Proc. Natl Acad. Sci. USA*, **95**, 9779–9784.

Holland,S.J., Gale,N.W., Mbamalu,G., Yancopoulos,G.D., Henkemeyer, M. and Pawson,T. (1996) Bidirectional signalling through the EPH-family receptor Nuk and its transmembrane ligands. *Nature*, **383**, 722–725.

Holland,S.J., Gale,N.W., Gish,G.D., Roth,R.A., Songyang,Z., Cantley,L.C., Henkemeyer,M., Yancopoulos,G.D. and Pawson,T. (1997) Juxtamembrane tyrosine residues couple the Eph family receptor EphB2/Nuk to specific SH2 domain proteins in neuronal cells. *EMBO J.*, **16**, 3877–3888.

Hornberger,M.R. *et al*. (1999) Modulation of EphA receptor function by coexpressed ephrinA ligands on retinal ganglion cell axons. *Neuron*, **22**, 731–742.

Itasaki,N. and Nakamura,H. (1996) A role for gradient *en* expression in positional specification on the optic tectum. *Neuron*, **16**, 55–62.

Kiyokawa,E. *et al*. (1994) Overexpression of ERK, an EPH family receptor protein tyrosine kinase, in various human tumors. *Cancer Res.*, **54**, 3645–3650.

Krull,C.E., Lansford,R., Gale,N.W., Collazo,A., Marcelle,C., Yancopoulos,G.D., Fraser,S.E. and Bronner-Fraser,M. (1997) Interactions of Eph-related receptors and ligands confer rostrocaudal pattern to trunk neural crest migration. *Curr. Biol.*, **7**, 571–580.

Krumlauf,R. (1994) *Hox* genes in vertbrate development. *Cell*, **78**, 191–201.

Labrador,J.P., Brambilla,R. and Klein,R. (1997) The N-terminal globular domain of Eph receptors is sufficient for ligand binding and receptor signaling. *EMBO J.*, **16**, 3889–3897.

Lackmann,M. *et al*. (1998) Distinct subdomains of the EphA3 receptor mediate ligand binding and receptor dimerization. *J. Biol. Chem.*, **273**, 20228–20237.

Lhotak,V. and Pawson,T. (1993) Biological and biochemical activities of a chimeric epidermal growth factor-elk receptor tyrosine kinase. *Mol. Cell. Biol.*, **13**, 7071–7079.

Lin,D., Gish,G.D., Songyang,Z. and Pawson,T. (1999) The carboxyl terminus of B class ephrins constitutes a PDZ domain binding motif. *J. Biol. Chem.*, **274**, 3726–3733.

Logan,C., Wizenmann,A., Drescher,U., Monschau,B., Bonhoeffer,F. and Lumsden,A. (1996) Rostral optic tectum acquires caudal charactheristics following ectopic *Engrailed* expression. *Curr. Biol.*, **6**, 1006–1014.

Meima,L., Kljavin,I.J., Moran,P., Shih,A., Winslow,J.W. and Caras,I.W. (1997a) AL-1-induced growth cone collapse of rat cortical neurons is correlated with REK7 expression and rearrangement of the actin cytoskeleton. *Eur. J. Neurosci.*, **9**, 177–188.

Meima,L., Moran,P., Matthews,W. and Caras,I.W. (1997b) Lerk2 (ephrin-B1) is a collapsing factor for a subset of cortical growth cones and acts by a mechanism different from AL-1 (ephrin-A5). *Mol. Cell. Neurosci.*, **9**, 314–328.

Mellitzer,G., Xu,Q. and Wilkinson,D.G. (1999) Eph receptors and ephrins restrict cell intermingling and communication. *Nature*, **400**, 77–81.

Monschau,B. *et al*. (1997) Shared and unique functions of RAGS and ELF-1 in guiding retinal axons. *EMBO J.*, **16**, 1258–1267.

Nakamoto,M., Cheng,H.-J., Friedman,G.C., McLaughlin,T., Hansen, M.J., Yoon,C.H., O'Leary,D.M.M. and Flanagan,J.G. (1996) Topographically specific effects of Elf-1 on retinal axon guidance in vitro and retinal mapping *in vivo*. *Cell*, **86**, 755–766.

Orioli,D., Henkemeyer,M., Lemke,G., Klein,R. and Pawson,T. (1996) Sek4 and Nuk receptors cooperate in guidance of commissural axons and in palate formation. *EMBO J.*, **15**, 6035–6049.

Pandey,A., Lazar,D.F., Saltiel,A.R. and Dixit,V.M. (1994) Activation of the Eck receptor protein tyrosine kinase stimulates phosphatidylinositol 3-kinase activity. *J. Biol. Chem.*, **269**, 30154–30157.

Pandey,A., Duan,H. and Dixit,V.M. (1995a) Characterization of a novel Src-like adapter protein that associates with the Eck receptor tyrosine kinase. *J. Biol. Chem.*, **270**, 19201–19204.

Pandey,A., Shao,H., Marks,R.M., Polverini,P.J. and Dixit,V.M. (1995b) Role of B61, the ligand for the Eck receptor tyrosine kinase, in TNF-α-induced angiogenesis. *Science*, **268**, 567–569.

Park,S., Frisén,J. and Barbacid,M. (1997) Aberrant axonal projections in mice lacking EphA8 (Eek) tyrosine protein kinase receptors. *EMBO J.*, **16**, 3106–3114.

Shigetani,Y., Funahashi,J.I. and Nakamura,H. (1997) En-2 regulates the expression of the ligands for Eph type tyrosine kinases in chick embryonic tectum. *Neurosci. Res.*, **27**, 211–217.

Smith,A., Robinson,V., Patel,K. and Wilkinson,D.G. (1997) The EphA4 and EphB1 receptor tyrosine kinases and ephrin-B2 ligand regulate targeted migration of branchial neural crest cells. *Curr. Biol.*, **7**, 561–570.

Sperry,R.W. (1963) Chemoaffinity in the orderly growth of nerve fiber patterns and connections. *Proc. Natl Acad. Sci. USA*, **50**, 703–710.

Stein,E., Ceretti,D.P. and O'Daniel,T.O. (1996) Ligand activation of Elk receptor tyrosine kinase promotes its association with Grb10 and Grb2 in vascular endothelial cells. *J. Biol. Chem.*, **271**, 23588–23593.

Stein,E., Lane,A.A., Cerretti,D.P., Schoecklmann,H.O., Schroff,A.D., Van Etten,R.L. and Daniel,T.O. (1998) Eph receptors discriminate specific ligand oligomers to determine alternative signaling complexes, attachment and assembly responses. *Genes Dev.*, **12**, 667–678.

Studer,M., Gavalas,A., Marshall,H., Ariza-McNaughton,L., Rijli,F.M., Chambon,P. and Krumlauf,R. (1998) Genetic interactions between Hoxa1 and Hoxb1 reveal new roles in regulation of early hindbrain patterning. *Development*, **125**, 1025–1036.

Taneja,R., Thisse,B., Rijli,F.M., Thisse,C., Bouillet,P., Dolle,P. and Chambon,P. (1996) The expression pattern of the mouse receptor tyrosine kinase gene Mdk1 is conserved through evolution and requires Hoxa-2 for rhombomere-specific expression in mouse embryos. *Dev. Biol.*, **177**, 397–412.

Tessier-Lavigne,M. (1995) Eph receptor tyrosine kinases, axon repulsion and the development of topographic maps. *Cell*, **82**, 345–348.

Theil,T., Frain,M., Gilardi-Hebenstreit,P., Flenniken,A., Charnay,P. and Wilkinson,D.G. (1998) Segmental expression of the EphA4 (Sek-1) receptor tyrosine kinase in the hindbrain is under direct transcriptional control of Krox-20. *Development*, **125**, 443–452.

Torres,R., Firestein,B.L., Dong,H., Staudinger,J., Olson,E.N., Huganir,R.L., Bredt,D.S., Gale,N.W. and Yancopoulos,G.D. (1998) PDZ proteins bind, cluster and synaptically colocalize with Eph receptors and their ephrin ligands. *Neuron*, **21**, 1453–1463.

Walter,J., Kern-Veits,B., Huf,J., Stolze,B. and Bonhoeffer,F. (1987) Recognition of position-specific properties of tectal cell membranes by retinal axons *in vitro*. *Development*, **101**, 685–696.

Wang,H.U. and Anderson,D.J. (1997) Eph family transmembrane ligands can mediate repulsive guidance of trunk neural crest migration and motor axon outgrowth. *Neuron*, **18**, 383–396.

Wang,H.U., Chen,Z.-F. and Anderson,D.J. (1998) Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell*, **93**, 741–753.

Winslow,J.W. *et al.* (1995) Cloning of AL-1, a ligand for an eph-related tyrosine kinase receptor involved in axon bundle formation. *Neuron*, **14**, 973–981.

Xu,Q., Alldus,G., Holder,N. and Wilkinson,D.G. (1995) Expression of truncated Sek-1 receptor tyrosine kinase disrupts the segmental restriction of gene expression in the *Xenopus* and zebrafish hindbrain. *Development*, **121**, 4005–4016.

Xu,Q., Mellitzer,G., Robinson,V. and Wilkinson,D.G. (1999) *In vivo* cell sorting in complementary segmental domains mediated by Eph receptors and ephrins. *Nature*, **399**, 267–271.

Yue,Y., Widmer,D.A.J., Halladay,A.K., Cerretti,D.P., Wagner,G.C., Dreyer,J.-L. and Zhou,R. (1999) Specification of distinct dopaminergic neural pathways: roles of the Eph family receptor EphB1 and ligand ephrin-B2. *J. Neurosci.*, **19**, 2090–2101.

Received March 18, 1999; revised and accepted August 6, 1999