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Masaru Nakamoto, *Valparaiso University*

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Molecules in focus

Eph receptors and ephrins

Masaru Nakamoto*

Department of Neurosciences/NC30, Lerner Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA

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Abstract

The Eph receptors are the largest known family of receptor tyrosine kinases. The Eph receptors and their membrane-attached ligands, ephrins, show diverse expression patterns during development. Recent studies have demonstrated that Eph receptors and ephrins play important roles in many developmental processes, including neuronal network formation, the patterning of the neural tube and the paraxial mesoderm, the guidance of cell migration, and vascular formation. In the nervous system, Eph receptors and ephrins have been shown to act as positional labels to establish topographic projections. They also play a key role in pathway finding by axons and neural crest cells. The crucial roles of Eph receptors and ephrins during development suggest involvement of these genes in congenital disorders affecting the nervous system and other tissues. It has also been suggested that Eph receptors and ephrins may be involved in carcinogenesis. It is therefore of clinical importance to further analyse the function of these molecules, as manipulation of their function may have therapeutic applications. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Receptor tyrosine kinases (RTKs) play crucial roles in cell proliferation, differentiation, and embryonic development. RTKs can be divided into distinct subfamilies based on their structural similarities. In 1987, the first member of the Eph subfamily, EphA1, was cloned by Hirai et al. from an erythropoietin producing hepatocellular carcinoma cell line [1]. To date, the Eph subfamily contains 14 members in vertebrates, and is by

far the largest known subfamily of RTKs. Most of the members are predominantly expressed in the nervous system, with distinct and overlapping patterns [1]. Recently, a family of ligands for the Eph receptors, named ephrins, has been identified, with eight members thus far cloned in vertebrates [1]. The ephrins are all membrane-attached molecules, and this attachment appears to be crucial for function. Interactions between Eph receptors and ephrins have been implicated in many processes of embryonic patterning. Here, I discuss Eph receptors and ephrins in terms of structural features and signaling, biological functions focused on neural development, and potential clinical implications.

* Tel.: +1-216-444-9513; fax: +1-216-444-7927.

E-mail address: nakamom@ccf.org (M. Nakamoto).

Fig. 1. Structure and signaling of the Eph receptors and ephrins. (Left) The Eph receptors: the extracellular region of the Eph receptors consists of a globular domain (Glb), a unique cysteine-rich motif (Cys), and two fibronectin type III motifs (FN III). The intracellular region contains a tyrosine kinase domain (TK), SAM domain (SAM), and a carboxyl-terminal PDZ-domain-binding motif. Two tyrosine residues in a highly conserved juxtamembrane motif are the major autophosphorylation sites. Nck, RasGAP, and Src family tyrosine kinases (Src, Fyn) interact with these tyrosine residues. SLAP has also been predicted to bind to these tyrosine residues. Grb2 and the p85 subunit of PI3 kinase have been suggested to bind within the tyrosine kinase domain. A conserved tyrosine residue within the SAM domain serves as an association site for Grb10 and low molecular weight phosphatase (LMW-PTP). The carboxyl terminus constitutes a PDZ-domain-binding motif. The solid arrows indicate demonstrated interaction sites, and the broken arrows indicate predicted interaction sites. Y-P, phosphotyrosine, V, carboxyl-terminal valine. (Right) Ephrins: ephrins are attached to the membrane either via a GPI-anchor (ephrin-A ligands) or a transmembrane domain (ephrin-B ligands). The cytoplasmic tail of the ephrin-B ligands is highly conserved including five invariant tyrosine residues. The carboxyl terminus forms a PDZ-domain-binding motif.

2. Structure and signaling

2.1. Eph receptors (Fig. 1, left)

The extracellular region of the Eph receptors consists of an N-terminal globular domain followed by a unique cysteine-rich motif and two fibronectin type III motifs. The N-terminal globular domain has been identified to be the principal ligand-binding domain [2,3], and has been recently shown by X-ray crystallography to fold into a compact jellyroll β -sandwich composed of 11 antiparallel β -strands [4].

As in other RTKs, the intracellular region of the Eph receptors contains domains which are presumed to be involved in signal transduction. In the juxtamembrane region there is a highly conserved motif containing two tyrosine residues, which are the major autophosphorylation sites. Nck, RasGAP, and Src family tyrosine kinases (Src, Fyn) are found to associate with these tyrosine residues. SLAP, a novel SH3-SH2 adaptor protein homologous to Src but lacking a catalytic domain, has also been predicted to bind to these tyrosine residues [5,6]. The tyrosine kinase domain has been suggested to serve as a binding site for Grb2 and the p85 subunit of PI3 kinase [5,6]. A conserved region of 60–70 amino acids in the carboxyl-terminal tail of the Eph receptors forms a sterile alpha motif (SAM) domain [7], which has been implicated in a receptor dimerization [8,9]. Two SH2-containing proteins, Grb10 and low molecular weight phosphatase (LMW-PTP), appear to interact with an invariant tyrosine in this domain [5,6]. Recently, a PDZ (post-synaptic density protein, discs large, zona occludens)-domain-binding motif has been identified in the carboxyl terminus of the Eph receptors [10,11].

Based on homology, and also on preferential ligand binding (see below), the Eph receptors are divided into two groups, EphA receptors and EphB receptors [1].

2.2. Ephrins (Fig. 1, right)

The ephrins fall into two groups based on the mode of membrane attachment [1]. The ephrin-A ligands (A1–A5) have a GPI anchor at their carboxyl termini, and the ephrin-B ligands (B1–B3) have a single transmembrane domain. These groupings roughly correspond to specificity of the receptor–ligand interactions: Ephrin-A ligands interact with EphA receptors, and ephrin-B ligands interact with EphB receptors. However, within each group, binding patterns are complex, with individual ligands binding to multiple receptors with a wide variety of affinities. In addition, some interactions cross over group boundaries [1].

A striking feature of the ephrin-B ligands is the highly conserved carboxyl-terminal tails. In

Fig. 2. Function of Eph receptors and ephrins in axon guidance. (A) Chicken retinotectal projection: (1) Expression pattern of Eph receptors and ephrins. Ephrin-A2 and ephrin-A5 are both expressed in low-anterior, high-posterior gradients across the tectum. The gradient of ephrin-A5 is confined to the posterior part of the tectum. (2) Retinal axon projection in wild type chicken embryos. (3) Aberrant projection caused by ectopic expression of ephrin-A2. When ephrin-A2 is retrovirally overexpressed in the developing tectum, retinal axons from the temporal side of the retina avoid patches of ectopic ephrin-A2 expression (shown as green patches) and project to abnormally anterior positions, while nasal axons are not affected and project to the correct positions. (B) Mouse anterior commissure of the cerebrum: (1) The anterior commissure is comprised of anterior (acA) and posterior part (acP), which project to the contralateral olfactory bulb and temporal cortex, respectively. The EphB2 receptor is expressed in the ventral structure which the anterior commissure axons normally avoid. (2) In EphB2 mutant mice, the acP tract fails to cross the midline and project into the ventral structure (the thalamus).

particular, the last 33 amino acids of ephrin-B1 and ephrin-B2 are identical, and only three amino acids are different in ephrin-B3. This region contains five potential tyrosine phosphorylation sites and a carboxyl-terminal PDZ-domain-binding motif [11,12], suggesting roles in signal transduction. Interestingly, interaction between an EphB receptor and ephrin-B ligands appears to lead to tyrosine phosphorylation of both the receptor and the ligands, and mediates bidirectional signaling [5,6].

3. Biological functions

Eph receptors and ephrins have been impli-

cated in many developmental processes, including neuronal network formation, the regional patterning of the neural tube and the paraxial mesoderm, the guidance of cell migration, and vascular formation [13]. In this review, I focus on functions in neuronal network formation.

Function of the nervous system is critically dependent upon the establishment of appropriate connections between neurons and their target cells. The initial development of these connections typically occurs before neurons become functionally active and is believed to be established by molecular guidance cues. First, axons must follow their correct pathway and reach the target region. Second, within the target region each axon must find and recognize the correct

target cells and form specific connections. In vertebrate nervous systems, this step typically involves topographic mapping, where axons of neighboring neurons project to neighboring areas in the target region so that the spatial order of the projecting neurons is maintained in the target.

3.1. Target selection in topographic maps

The retinotectal projection is a typical example of a topographic projection, in which the temporal-to-nasal axis of the retina maps smoothly along the anterior-to-posterior axis of the tectum. About half a century ago, Sperry proposed the chemoaffinity theory, which postulated the existence of molecular labels on projecting neurons and their target cells. He further suggested that these positional labels take the form of complementary gradients that mark corresponding points in both projecting and target fields, allowing the formation of a precise topographic map.

Recent evidence indicates Eph receptors and ligands can act as positional labels in the retinotectal system. In chicken, two GPI-anchored ligands, ephrin-A2 and ephrin-A5, are expressed in high-posterior, low-anterior gradients across the tectum [14,15]. Correspondingly, the EphA3 receptor is expressed in the retina in a high-temporal, low-nasal gradient [15]. Ephrin-A2 shows topographically specific repulsive activity to retinal axons both *in vitro* and *in vivo*: Ephrin-A2 repels temporal, but not nasal retinal axons [16] (Fig. 2A), suggesting ephrin-A2 can guide the development of this topographic projection.

In mammals, a major portion of the retinal axons project to several targets in the forebrain, including the dorsal lateral geniculate nucleus (dLGN) in the thalamus, in addition to projecting to the superior colliculus (SC, the mammalian equivalent of the optic tectum) in the midbrain. Interestingly, all the major targets of retinal output share a common expression pattern of ephrin-A2 and -A5 [17], and targeted disruption of ephrin-A5 results in aberrant retinal axon projections in the SC and dLGN [17,18]. These results indicate that the same set of ephrins are

used repeatedly as positional labels to map a sensory input onto multiple targets.

Topographic maps are not limited to the retinotectal projection, but are found throughout the vertebrate nervous system. It is therefore an intriguing question whether gradients of Eph receptors and ephrins serve as positional labels in other mappings. The hippocamposeptal projection is another example of a topographic projection in which Eph signaling provides positional information [19]. Considering the large number and diversity of expression patterns, the Eph receptors and ephrins are likely to play a general role in topographic map formation.

3.2. Motor axon guidance

During development, motor neurons in the spinal cord are divided into subclasses and segregate into longitudinally aligned columns. Motor neurons in different columns express different sets of nuclear factors, select specific paths, and innervate distinct muscle targets. For example, motor neurons in the medial motor column (MMC) innervate axial muscles, while motoneurons in the lateral motor column (LMC) in the brachial and lumbar segments innervate limb muscles. In chicken spinal cord, the EphA3 receptor is expressed in a subset of the MMC neurons [20], and the EphA4 receptor is expressed in the LMC [21]. Since at least two ligands (ephrin-A2 and -A5) which have high affinity to EphA3 and low affinity to EphA4 are expressed in the limb bud, it has been suggested that these ligands repel MMC axons expressing high affinity EphA3 receptor and prevent them from entering the limb, while LMC axons with low affinity EphA4 receptor can innervate limb muscles.

In early embryos, outgrowth of spinal motor axons and migration of trunk neural crest cells are restricted to the anterior halves of somites by repulsive factors located in the posterior halves. EphB2 and EphB3 receptors are expressed in spinal motor axons and migrating neural crest cells, while interacting ephrin-B ligands are found in posterior halves of somites [1]. *In vitro* assays indicate that the repulsive interaction between

the ligands and the receptors underlie the segmental pathway selection by motor axons and trunk neural crest cells [22].

Ephrin-A5 has been shown to be expressed to a greater extent in anterior (head and neck) than in posterior (trunk and limb) muscles, and to inhibit axon outgrowth from the spinal cord and dorsal root ganglia, with stronger inhibition of posterior over anterior explants [23]. These results suggest that specificity in connections of spinal motor and sensory neurons to periphery may rely on Eph receptors and ephrins.

3.3. Axon pathfinding in the central nervous system

The analysis of targeted gene disruption provided strong evidence for functions of Eph receptors in pathway selection in the central nervous system. Mice deficient in EphB2 have defects in axon pathfinding in the posterior part of the anterior commissure of the cerebrum [24] (Fig. 2B), while EphB3 mutant mice failed to form the corpus callosum [25]. The phenotype in both axon tracts is more severe in EphB2/B3 double mutants, indicating that the two receptors have partially redundant functions [25]. Interestingly, axons affected in these mutants do not express the receptors, but instead express ephrin-B ligands. The areas of the receptor expression are adjacent to the pathways, suggesting that the receptors provide guidance cues that are transduced through the transmembrane ephrin-B ligands [24,25]. The biochemical finding that tyrosine phosphorylation of ephrin-B ligands is induced by interaction with EphB receptors strongly supports this model [5,6].

Targeted disruption of the EphA8 receptor gene affects a population of neurons in the superior colliculus, whose axons fail to reach the correct target in the contralateral inferior colliculus. Instead, mutant mice display an aberrant ipsilateral axonal tract that projects to the cervical spinal cord [26]. Finally, EphA4 null mutant mice exhibit disruptions of the corticospinal tract and show a gross motor dysfunction [27].

4. Clinical implications

Since Eph receptors and ephrins have been found to be key regulators in axonal guidance, it is of great clinical interest to explore links between these genes and congenital diseases affecting neuronal connections, such as agenesis of the corpus callosum. It will also be important to study the regulation of these molecules during regeneration.

A number of Eph receptors have been shown to be overexpressed in tumors, including hepatoma, gastric adenocarcinoma, and malignant melanoma [28]. In vitro, overexpression of the EphA1 receptor in NIH3T3 cells has transforming potential, suggesting that Eph receptors may play roles in carcinogenesis. In addition, Eph receptors may affect cell adhesion and migration, which are involved in tumor metastasis. Indeed, overexpression of EphA2 and EphB4 was found in the undifferentiated and invasive mammary tumors of transgenic mice expressing the Ha-ras oncogene, but not in the well-differentiated and non-metastatic mammary tumors of c-myc transgenic mice [28].

Although functional analysis of the Eph receptors and ephrins has just started, their crucial roles in development suggest clinical importance worthy of future study.

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References

- [1] J.G. Flanagan, P. Vanderhaeghen, The ephrins and Eph receptors in neural development, *Annu. Rev. Neurosci.* 21 (1998) 309–345.
- [2] J.P. Labrador, R. Brambilla, R. Klein, The N-terminal globular domain of Eph receptors is sufficient for ligand binding and receptor signaling, *EMBO J.* 16 (1997) 3889–3897.

- [3] M. Lackmann, A.C. Oates, M. Dottori, F.M. Smith, C. Do, M. Power, L. Kravets, A.W. Boyd, Distinct subdomains of the EphA3 receptor mediate ligand binding and receptor dimerization, *J. Biol. Chem.* 273 (1998) 20,228–20,237.
- [4] J.-P. Himanen, M. Henkemeyer, D.B. Nikolov, Crystal structure of the ligand-binding domain of the receptor tyrosine kinase EphB2, *Nature* 396 (1998) 486–491.
- [5] S.J. Holland, E. Peles, T. Pawson, J. Schlessinger, Cell-contact-dependent signalling in axon growth and guidance: Eph receptor tyrosine kinases and receptor protein tyrosine phosphatase β , *Curr. Opin. Neurobiol.* 8 (1998) 117–127.
- [6] K. Brückner, R. Klein, Signaling by Eph receptors and their ephrin ligands, *Curr. Opin. Neurobiol.* 8 (1998) 375–382.
- [7] J. Schultz, C.P. Ponting, K. Hofmann, P. Bork, SAM as a protein interaction domain involved in developmental regulation, *Protein Sci.* 6 (1997) 249–253.
- [8] D. Stapleton, I. Balan, T. Pawson, F. Sicheri, The crystal structure of an Eph receptor SAM domain reveals a mechanism for modular dimerization, *Nat. Struct. Biol.* 6 (1999) 44–49.
- [9] C.D. Thanos, K.E. Goodwill, J.U. Bowie, Oligomeric structure of the human EphB2 receptor SAM domain, *Science* 283 (1999) 833–836.
- [10] B. Hock, B. Böhme, T. Karn, T. Yamamoto, K. Kaibuchi, U. Holtrich, S. Holland, T. Pawson, H. Rübsamen-Waigmann, K. Strebhardt, 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 (1998) 9779–9784.
- [11] R. Torres, B.L. Firestein, H. Dong, J. Staudinger, E.N. Olson, R.L. Haganir, D.S. Brecht, N.W. Gale, G.D. Yancopoulos, PDZ proteins bind, cluster, and synaptically colocalize with Eph receptors and their ephrin ligands, *Neuron* 21 (1998) 1453–1463.
- [12] D. Lin, G.D. Gish, Z. Songyang, T. Pawson, The carboxyl terminus of B class ephrins constitutes a PDZ domain binding motif, *J. Biol. Chem.* 274 (1999) 3726–3733.
- [13] N. Holder, R. Klein, Eph receptors and ephrins: effectors of morphogenesis, *Development* 126 (1999) 2033–2044.
- [14] U. Drescher, C. Kremoser, C. Handwerker, J. Löschinger, M. Noda, F. Bonhoeffer, 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 (1995) 359–370.
- [15] H.-J. Cheng, M. Nakamoto, A.D. Bergemann, J.G. Flanagan, Complementary gradients in expression and binding ELF-1 and Mek4 in development of the topographic retinotectal projection map, *Cell* 82 (1995) 371–381.
- [16] M. Nakamoto, H.-J. Cheng, G.C. Friedman, T. McLaughlin, M.J. Hansen, C.H. Yoon, D.D.M. O'Leary, J.G. Flanagan, Topographically specific effects of ELF-1 on retinal axon guidance in vitro and retinal axon mapping in vivo, *Cell* 86 (1996) 755–766.
- [17] D.A. Feldheim, P. Vanderhaeghen, M.J. Hansen, J. Frisén, Q. Lu, M. Barbacid, J.G. Flanagan, Topographic guidance labels in a sensory projection to the forebrain, *Neuron* 21 (1998) 1303–1313.
- [18] J. Frisén, P.A. Yates, T. McLaughlin, G.C. Friedman, D.D.M. O'Leary, M. Barbacid, Ephrin-A5 (AL-1/RAGS) is essential for proper retinal axon guidance and topographic mapping in the mammalian visual system, *Neuron* 20 (1998) 235–243.
- [19] P.P. Gao, J.-H. Zhang, M. Yokoyama, B. Racey, C.F. Dreyfus, I.B. Black, R. Zhou, Regulation of topographic projection in the brain: Elf-1 in the hippocamposeptal system, *Proc. Natl. Acad. Sci. USA* 93 (1996) 11,161–11,166.
- [20] T.J. Kilpatrick, A. Brown, C. Lai, M. Gassmann, M. Goulding, G. Lemke, Expression of the Tyro4/Mek4/Cek4 gene specifically marks a subset of embryonic motor neurons and their muscle targets, *Mol. Cell. Neurosci.* 7 (1996) 62–74.
- [21] K. Ohta, M. Nakamura, K. Hirokawa, S. Tanaka, A. Iwama, T. Suda, M. Ando, H. Tanaka, The receptor tyrosine kinase, Cek8, is transiently expressed on subtypes of motoneurons in the spinal cord during development, *Mech. Dev.* 54 (1996) 59–69.
- [22] H.U. Wang, D.J. Anderson, Eph family transmembrane ligands can mediate repulsive guidance of trunk neural crest migration and motor axon outgrowth, *Neuron* 18 (1997) 383–396.
- [23] M.J. Donoghue, R.M. Lewis, J.P. Merlie, J.R. Sanes, The Eph kinase ligand AL-1 is expressed by rostral muscles and inhibits outgrowth from caudal neurons, *Mol. Cell. Neurosci.* 8 (1996) 185–198.
- [24] M. Henkemeyer, D. Orioli, J.T. Henderson, T.M. Saxton, J. Roder, T. Pawson, R. Klein, Nuk controls pathfinding of commissural axons in the mammalian central nervous system, *Cell* 86 (1996) 35–46.
- [25] D. Orioli, M. Henkemeyer, G. Lemke, R. Klein, T. Pawson, Sek4 and Nuk receptors cooperate in guidance of commissural axons and in palate formation, *EMBO J.* 15 (1996) 6035–6049.
- [26] S. Park, J. Frisén, M. Barbacid, Aberrant axonal projections in mice lacking EphA8 (Eek) tyrosine protein kinase receptors, *EMBO J.* 16 (1997) 3106–3114.
- [27] M. Dottori, L. Hartley, M. Galea, G. Paxinos, M. Polizzotto, T. Kilpatrick, P.F. Bartlett, M. Murphy, F. Köntgen, A.W. Boyd, EphA4 (Sek1) receptor tyrosine kinase is required for the development of the corticospinal tract, *Proc. Natl. Acad. Sci. USA* 95 (1998) 13,248–13,253.
- [28] Q. Xu, D.G. Wilkinson, Eph-related receptors and their ligands: mediators of contact dependent cell interactions, *J. Mol. Med.* 75 (1997) 576–586.