Published in final edited form as:
Brain Res. 2007 December 12; 1184: 72–80.

Bidirectional ephrin/Eph Signaling in Synaptic Functions

Jason Aoto[1] and **Lu Chen**[1,2,*]

1 *Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3200*

2 *Helen Wills Neuroscience Institute, University of California, Berkeley, California 94720-3200*

Abstract

Eph receptors, the largest family of receptor tyrosine kinases, and their membrane bound ligands, the ephrins, are involved in multiple developmental and adult processes within and outside of the nervous system. Bi-directional signaling from both the receptor and the ligand is initiated by ephrin-Eph binding upon cell-cell contact, and involves interactions with distinct subsets of downstream signaling molecules related to specific functions. In the CNS, Ephs and ephrins act as attractive/repulsive, migratory, and cell adhesive cues during development and participate in synaptic functions in adult animals. In this review, we will focus on recent findings highlighting the functions of ephrin/Eph signaling in dendritic spine morphogenesis, synapse formation, and synaptic plasticity.

Introduction

Erythropoietin-producing hepatocellular carcinoma (Eph) receptors form the largest known family of receptor tyrosine kinases [10]. Currently, 16 genes (EphA1–10, EphB1–6) have been identified in the vertebrate genome [66] and 14 of them are present in mammals [58]. All Eph receptors are transmembrane proteins with highly conserved extra- and intracellular domains. The extracellular part of the Eph receptors includes a N-terminal ligand binding domain, a cysteine-rich region and two fibronectin type III repeats [85]. Following the juxtamembrane region is a tyrosine kinase domain, followed by a sterile-α-motif (SAM), and a type-II PSD-95/Disc large/ZO-1 (PDZ) binding motif at the carboxyl terminus [49] (Fig. 1). Eph receptors can undergo homo- as well as heterodimerization [26], which is mediated directly by the extracellular cysteine-rich region, the fibronectin type III repeats [50] and the SAM motif [73,78] or indirectly through PDZ protein interactions [25]. The Eph family receptors are divided into two groups based on the similarity of their extracellular domain sequences [10], which coincidently corresponds to their binding affinities for their respective ligands, the ephrins (Table 1).

In the vertebrate genome, nine Eph Receptor Interacting Proteins (ephrin) ligands have been identified and classified based on their attachment to the cell membrane (ephrinA1–6 and ephrinB1–3). The A-ephrin ligands are bound to the membrane via a glycosylphosphoinositol (GPI) anchor while B-ephrins are type-I transmembrane proteins. Both classes of ligands contain a 20-kD receptor-binding domain, which consists of approximately 180 amino acids [60]. The cytoplasmic tail of the B-ligands is short and consists of approximately 80 amino acids. The last 33 amino acids of the ephrinB ligands display 95% sequence homology (100% conserved between ephrinB1 and B2) with a conserved type-II PDZ binding motif at the c-

* Address Correspondence to: Lu Chen, Department of Molecular and Cell Biology, University of California, 201 LSA, MC 3200, Berkeley, CA 94720-3200, Phone: (510) 643-8163, Fax: (510) 643-6791, email: luchen@berkeley.edu.

terminus [49,54]. Phosphorylation of several tyrosine residues in the ephrinB intracellular domain upon receptor binding enables their direct interactions with SH2/SH3 adaptor proteins, which play important roles in ephrinB reverse signaling [59,69,76] (Fig. 1).

The receptor-ligand interactions between Ephs and ephrins follow a general rule that A-ligands interact preferentially with A-receptors and B-ligands with B-receptors. The only exceptions found so far are that EphA4 and EphB2 interact with ephrinB2/3 and ephrinA5, respectively [31,35] (Table 1). Within each subfamily, the receptor-ligand interaction is believed to be rather promiscuous. High affinity heterodimers are formed between Ephs and ephrins upon cell-cell contact. The receptor-ligand heterodimers, in a 2:2 ratio, form tetramers [36–38,60]. The extracellular domains on both receptors and ligands mediate tetramerization and may even enhance subfamily specificity [35,38,60]. Tetramers may then form higher order aggregates at higher concentrations [38], and may cluster into lipid raft microdomains on the cell membrane when interact with cytoplasmic PDZ proteins such as GRIP [4]. High-density clusters of Ephephrin complexes are believed to serve as signaling centers for the localization, concentration and activation of intracellular signaling molecules [3,4,58]. An interesting characteristic of Eph receptors and ephrin ligands is that they are capable of bidirectional signaling. Signaling pathways directly associated with Eph receptor activation are termed “forward” and those with ephrin activations, “reverse” signaling [41,58] (Fig. 1).

The forward and reverse signaling

Following ligand binding, Eph signaling is initiated through autophosphorylation. The activation of the kinase domain also results in the phosphorylation of the juxtamembrane domain and downstream target proteins [5,21,40,41]. One of the well studied functions of Ephs is their ability to modulate actin cytoskeletons through activation of Guanine nucleotide exchange factors (GEFs) [62]. GEFs are localized to Eph containing lipid rafts either through recruitment or constitutive binding to the phosphorylated juxtamembrane domain or the kinase domain. Each class of Eph receptors activates a unique subset of GEFs. For example, EphA receptors have been identified as interacting partners for Ephexin, Vav, and Tiam1, the RhoGEFs that are involved in axon guidance [13,44,71,77], although Tiam1 has also been implicated in NMDA receptor-mediated spine morphogenesis [79]. EphB receptors interact with a different subset of RhoGEFs (intersectin and kalirins) [46,58,67,68]. Another actin modulating protein downstream of Eph forward signaling is SHEP1 (SH2 domain-containing Eph receptor-binding protein 1), which binds Ras family GTPases (i.e. R-Ras and Rap1A) and also forms a stable complex with the scaffolding protein Crk-associated substrate (Cas) [14, 20]. Through a direct interaction between the SH2-domain of SHEP1 and a conserved phospho-tyrosine motif the juxtamembrane region, activated Eph receptors can be coupled to R-Ras and Rap1A [20]. Eph receptor activation also regulates membrane ruffling and cell migration by inhibiting the interaction between SHEP1 and Cas [14]. In addition to GEFs, Eph receptors can also associate with the p120-Ras GTPase activating protein (p120-RasGAP) upon ligand binding through SH2 mediated interactions [15,23,47]. This interaction inhibits the Ras-MAPK pathway and plays important roles in growth cone collapse, cell repulsion and morphogenesis of capillary endothelium. The C-terminal PDZ-binding motif of Eph receptors mediates their interactions with many neuronal PDZ scaffolding proteins, such as glutamate receptor interacting protein (GRIP), protein interacting with C-kinase (PICK1) and AF-6 [4,39,42,81]. Forward signaling through Eph receptors induces changes to the local milieu either through modulating actin dynamics or through the localization of scaffolding molecules in lipid rafts.

Reverse signaling of the ephrin ligands is activated by Eph receptor binding. Activated ephrinB ligands are phosphorylated by Src family kinase (SFK) members (Src and Fyn) [28,65], PDGF [5], and FGF receptors [87]. Eph receptor binding leads to a rapid recruitment of SFKs to ephrinB-containing membrane microdomains and transient SFK activation [65]. Tyrosine

phosphorylation of ephrins by SFKs leads to the recruitment of Grb4, a SH2 domain containing scaffolding protein that alters the actin cytoskeleton via the recruitment and activation of FAK [12]. Several mechanisms are known to regulate the tyrosine phosphorylation of ephrinBs. First, EphB receptor stimulates a metalloproteinase cleavage of ephrinB ligands, producing a C-terminal fragment that is further processed by presenilin 1/γ-secretase [28,80]. The final peptide product binds Src and inhibits its association with a negative Src regulator termed c-terminal Src kinase (Csk), allowing further activation of Src [28]. Second, after tyrosine phosphorylation, ephrinB ligands recruit a phosphotyrosine phosphotase PTP-BL through their PDZ-binding domain and are subsequently dephosphorylated [65]. In addition to PTP-BL, a number of other PDZ domain proteins (i.e. GRIP, syntenin, PSD-95, PICK1, and regulator of G-protein signaling (PDZ-RGS-3)) have been found to interact with ephrinB ligands [4,49, 52,55,81]. With the exception of PTP-BL, most PDZ protein interaction with ephrinBs is constitutive and independent of the tyrosine-phosphorylation state of ephrinBs. It remains unknown what prevents PTP-BL binding to the ephrinB PDZ-binding motif prior to receptor stimulation. One possibility is that the hair-pin structure formed by the 22 residues at the N-terminal region of the last 33 residues presents a spatial obstruction for PTP-BL's interaction with the c-terminal PDZ-binding motif, and the conformational change induced by tyrosine phosphorylation relieves this block [72]. This type of phosphorylation-dependent PDZ binding provides a switch mechanism from phosphotyrosine-dependent signaling to PDZ-domain-protein dependent signaling. So far, GRIP1/2, PDZ-RGS3, and PTP-BL are known to interact with the PDZ binding motifs of ephrinBs endogenously [4,52,65]. Beyond the signaling pathways associated with proteins directly interacting with the ephrinB c-terminus, a few novel signaling pathways have been recently discovered, perhaps through indirect interactions mediated by PDZ scaffolds. For example, ephrinB2 is found associated with metabotropic glutamate receptor 1 (mGluR1) and enhances glutamate-induced polyphosphoinositide hydrolysis [6]. EphrinB1 reverse signaling activates a novel JNK (c-Jun N-terminal Kinase) signaling pathway that results in phosphorylation-independent morphological changes in a heterologous HEK293 system [84].

Although ephrinA ligands lack an intracellular domain that could recruit scaffolding molecules, they also participate in reverse signaling by modulating cell adhesion. There is evidence suggesting that ephrinA2 or A5 activation leads to the clustering of β1-integrins in lipid microdomains through a SFK dependent mechanism [18,43]. EphrinAs are also found to interact independent of their ligand-binding domain with EphAs in *cis*, which prevents *trans* interaction and silences EphA forward signaling [8,86].

Most of Eph receptors and their cognate ephrin ligands are found expressed in the mammalian hippocampus (Tables 1 and 2). In this review, we will focus on the role of the ephrin/Eph family in spine morphogenesis, synapse formation, and synaptic plasticity in the hippocampus.

Eph receptors and spine morphogenesis

Dendritic spines are thin, actin rich protrusions that form on the surface of dendrites. Immature spines start out as thin filopodia. Upon axo-dendritic contact, rapid cytoskeletal changes occur concurrently with the recruitment of post-synaptic density proteins [82]. This process of spine formation and retraction is highly dynamic, as a spine may change its shape by as much as 30% within a few minutes [61]. Because spine morphogenesis is usually correlated with synaptogenesis and synaptic plasticity, developmental disorders that result in abnormal synaptic transmission and serious defects in learning and memory, such as Fragile-X mental retardation, Down syndrome, Rett syndrome, and Angleman syndrome, have been attributed to defects in spine formation [16,89].

The roles of Eph receptors in spine morphogenesis have been extensively studied. In the mouse hippocampus, Eph receptors are expressed in distinct regions, as identified by *in situ* hybridization [32]. Immunolabeling of Eph receptors confirmed their localization in dendritic shafts, filopodial protrusions, and spine heads [45,57,68]. Henkemeyer and colleagues observed that in EphB1/B2- and EphB1/B2/B3-deficient mice, dendritic spines failed to develop [34]. F-actin rich spines were replaced by thin filopodia-shaped protrusions that lacked clustered F-actin. However, the neurons were still able to form excitatory synapses. The distribution of excitatory synapses, visualized with immunostaining of pre- and post-synaptic markers, was shifted from spines to shafts in EphB deficient mice. The increase in shaft synapse formation was accompanied by a weakening in postsynaptic PSD95 puncta size. EphB-mediated changes in excitatory synapse distribution were specific for excitatory synapse formation, as inhibitory synapses remained unperturbed. The authors propose that EphB receptors, mainly EphB1/B2, are required for and have redundant functions in spine morphogenesis.

Syndecan-2, a transmembrane heparin sulfate proteoglycan, was among the first molecules identified as major players in EphB-mediated spine formation [24]. EphB2 kinase activation upon ligand binding induces syndecan-2 clustering. Pathways downstream of syndecan-2 that ultimately leads to cytoskeletal rearrangement of the spine have yet to be elucidated. It was hypothesized that EphB2, through phosphorylation and possible direct interaction, localizes syndecan-2 to sites of nascent spines. Subsequent recruitment of syntenin and CASK by syndecan-2 via PDZ interactions may promote spinogenesis.

EphB receptors also promote spine formation through activating Rho family small GTPases. For example, two Rac1GEFs, kalirin-5 and –7, are coupled to EphB receptors and involved in regulating actin dynamics related to spine formation [67,68]. In cultured hippocampal neurons, activation of EphB forward signaling by pre-clustered ephrinB1 leads to the activation of kalirin-5 and -7 and, consequently, to an increase in dendritic spine density. In ephrinB1 treated HEK293 cells expressing both EphB2 and kalirin, EphB2 kinase activity was required for kalirin recruitment to the plasma membrane, which leads to the activation of Rac1 and its downstream effector, p21-activated protein kinase (PAK) [67]. Dominant negative forms of Rac1 and PAK inhibit EphB-mediated spine morphogenesis. Intersectin, another Rho specific for Cdc42, has also been implicated in spine formation downstream of EphB activation [46]. Intersectin is activated by ligand binding to EphB receptors. Activation of Cdc42 by intersectin promotes N-WASP-Arp2/3 complex-mediated actin polymerization. In addition, N-WASP also binds to intersectin and further upregulates its GEF activity. Linked together by intersectin, EphB and N-WASP promotes spine formation synergistically. Although some RhoGEFs are predicted to bind to EphB receptors directly, others are recruited indirectly through signaling complexes assembly upon receptor activation. For example, EphB activation leads to the assembly of a focal adhesion complex-like structure at its cytoplasmic tail, which, through a RhoGEF (possibly p190RhoGEF) activates RhoA to mediate changes to the cytoskeletal architecture. The components found in this signaling complex include FAK (focal adhesion kinase), Src, Grb2, and paxillin [56]. In the FAK knockout mouse or cultured neurons transfected with FAK-siRNA, EphB-mediated spine formation is greatly impaired. Dominant negative forms of RhoA phenocopies FAK-siRNA's effects, while constitutively active RhoA mimics the effects of activated EphB in dendritic spine formation. Taken together, these studies demonstrate the involvement of multiple Rho family GTPases in EphB-mediated forward signaling and spine morphogenesis.

Although EphA receptors do not seem to directly promote spine formation, recent studies suggest that they may regulate spine stability. Activation of EphA4 in spines by clustered ephrinA3-Fc leads to the retraction and pruning of dendritic spines [57]. It was proposed that ephrinA3 expressed by glia cells activates EphA4 forward signaling in spine heads, possibly

through the recruitment of SHEP1/Ras GTPases complexe or the RhoGEF ephexin (i.e. ephexin-5). The subsequent activation of Ras or other still unidentified Rho family GTPases achieves spine retraction and may counterbalance the pro-spinogenic effect of the EphB receptors.

Eph receptor forward signaling in synapse formation and plasticity

Eph receptors have also been studied for their roles in synapse formation. Dalva et al. [17] demonstrated that the extracellular juxtamembrane region of EphB directly interacts with the NMDA receptor subunit NR1. The EphB-NR1 interaction promotes clustering of Ca^{2+} permeable glutamatergic receptors at synaptic locations. EphB activation with ephrinB1-Fc clustered NMDA receptors, and unexpectedly, also led to the formation of functional presynaptic release sites, identified by FM1–43 dye labeling. The synaptogenic effect of activated EphB is dependent on the intrinsic receptor tyrosine kinase activity. Another study found that ephrinB-EphB interaction leads to the phosphorylation of key cytoplasmic residues on NR2B by Src family tyrosine kinases, which potentiates the NMDA receptor-dependent Ca^{2+} influx and enhances Ca-dependent gene expression [75]. The capability of postsynaptic Eph receptors to rapidly alter actin dynamics, induce synapse formation, and modulate Ca^{2+} influx strongly suggests a role for Ephs in mediating synaptic plasticity. In addition, Eph receptors and ephrin ligands are expressed in distinct regions of the hippocampus, and may participate in pathway-specific synaptic functions such as induction and expression of different forms of synaptic plasticity [9,31,32,48,64].

Indeed, both Eph A and B receptor families are required for synaptic plasticity. Perturbing endogenous EphA/ephrinA interaction by infusing recombinant immunoadhesins that specifically bind to the receptor binding site of the ephrin-A ligand *in vivo* impaired hippocampal-dependent learning [30]. Similarly, application of soluble EphA5-IgG in hippocampal slices impairs the induction of LTP [27]. Conversely, activation of endogenous EphAs by clustered ephrinA5-IgG increased synaptic transmission and improved learning [27,29]. These results indicate that the EphA/ephrinA interaction may be required for all phases of LTP at the CA3-CA1 synapse.

The functions of EphB receptors in synaptic plasticity are starting to be delineated. Impaired LTP at the Schaffer collateral-CA1 synapse and the perforant/dentate gyrus synapse, a complete absence of LTD and depotentiation at the CA1 synapse, as well as defects in hippocampus-dependent learning tasks in the EphB2 knockout mouse were reported [32,33]. Interestingly, impaired CA1 LTP, LTD and learning in the $EphB2^{-/-}$ mouse can be completely rescued by an $EphB2^{LacZ}$ knock-in allele where most of the cytoplasmic domain of EphB2 except the juxtamembrane region was replaced by β-galactosidase [32]. This suggests that EphB2 mediates synaptic plasticity in a kinase-independent manner. In CA1 pyramidal neurons, EphB2 is expressed in both pre- and post-synaptic neurons [32] (Fig. 2). It could act at the postsynaptic side by clustering NMDA receptors at the synapse [17], or at the presynaptic side by activating postsynaptic ephrinB reverse signaling, which is implicated in this form of LTP [31,70]. A regional-specific (CA1 or CA3) knock-in of $EphB2^{LacZ}$ in the EphB2 null mouse would directly address this issue.

The involvement of EphB2 in LTP at the mossy fiber-CA3 synapse was first reported by Contractor et al. [11]. Mossy fiber LTP was completely blocked when EphB2 c-terminal peptides or antibodies directed against the EphB2 c-terminus were introduced via a recording electrode, suggesting that the c-terminus of the postsynaptic EphB2 is necessary for trans-synaptic induction of mossy fiber-CA3 LTP, which is expressed pre-synaptically. It was also shown that a postsynaptic PDZ protein, the glutamate receptor interacting protein 1 (GRIP1), is required for this trans-synaptic action, probably by enhancing EphB2 clustering through their

direct interaction. Moreover, extracellular application of a blocking peptide, ephrinB1-Fc, blocked mossy fiber-CA3 LTP, while direct activation of presynaptic ephrinB reverse signaling by EphB2-Fc enhanced basal mossy fiber synaptic transmission and occluded LTP. This suggests that postsynaptic GRIP1-dependent EphB activation is necessary for presynaptic ephrinB reverse signaling, which directly mediates the presynaptic expression of this form of synaptic plasticity. The involvement of presynaptic ephrinBs in the mossy fiber LTP was also demonstrated with the ephrinB3 signaling-deficient mouse [2] (discussed below).

EphrinB ligand reverse signaling and synapse formation

Until recently, research investigating the roles of ephrinB/EphB in synaptic function has focused mainly on ephrinB-induced forward signaling through postsynaptic EphB receptors. EphrinB ligands are expressed in both pre- and post-synaptic neurons in the hippocampus [32] and are capable of reverse signaling [41]. Although ephrinB reverse signaling is not implicated in spine formation, recent evidence suggests that postsynaptic ephrinB3 may regulate synapse formation in CA1 neurons [70]. In the ephrinB3 knockout mouse, the number of excitatory CA1 synapses was increased while the size of the postsynaptic-density (PSD) was decreased, which may explain their normal basal synaptic transmission [31,70]. In the signaling-deficient ephrinB3^{lacZ} knock-in mouse, in which the ephrinB3 cytoplasmic domain was replaced by β-galactosidase, the PSD size but not synaptic density is restored to the wild-type level [70]. Furthermore, relative to wild type animals, hippocampal synaptosomal preparations from ephrinB3 null mice showed altered levels of various synaptic proteins, most of which can be restored to normal levels in the ephrinB3^{lacZ} knock-in mouse [70]. Although developmental compensation for the loss of synaptic function cannot be formally excluded, the observed phenotypes nonetheless provide important insights in signaling-dependent and signaling-independent functions of postsynaptic ephrinB3 in synapse formation.

Ephrin ligands and synaptic plasticity

The involvement of B-ephrin reverse signaling in synaptic plasticity has been extensively explored [2,31,70]. In agreement with the results from Contractor et al. [11], mossy fiber-CA3 LTP is completely blocked in the ephrinB^{LacZ} mouse, the ephrinB3 signaling-deficient mutant [2], suggesting that presynaptic ephrinB3 reverse signaling is required for this pre-synaptically expressed plasticity. However, mossy fiber-CA3 LTP is normal in ephrinB3 null mutants, implying that ephrinB^{LacZ} has a dominant negative effect and presynaptic ephrinB1 and ephrinB2, although expressed at lower levels, compensate for the loss of ephrinB3 function. Mossy fiber synapses can still be potentiated in ephrinB3^{LacZ} mutants through forskolin-induced PKA activation. It is possible that PKA and ephrinB3 signaling are two independent pathways leading to increased presynaptic efficacy. An alternative explanation is that PKA acts downstream of presynaptic ephrinB3 signaling although the molecular mechanism connecting PKA signaling and ephrinB3 has yet to be identified.

The orientation of ephrinBs/EphBs is reversed at the CA3-CA1 synapse relative to the mossy fiber-CA3 synapse [31] (Fig. 2). Although similar genetic knockout/knock-in methods were used, the role of postsynaptic ephrins in CA1 LTP remains highly controversial [2,31,70]. Grunwald et al. [31] suggest that postsynaptic ephrinB2 and ephrinB3 are not functionally redundant in mediating CA1 LTP because ephrinB2 and ephrinB3 knockout mice exhibit similar degrees of impairment. Although multiple presynaptic Eph receptors may serve as binding partners for ephrinBs, only EphB2 and EphA4 have been studied. It was proposed that EphB2 may be required for late phase LTP [32] (but see [33]) while EphA4 serves as a presynaptic receptor for ephrinBs and functions in a signaling-independent manner in the early phase LTP [31]. By contrast, Armstrong et al. [2] did not observe any defect in the CA1 LTP in either the ephrinB3 null mouse or the ephrinB3 signaling-deficient mutant, while Rodenas-

Ruano et al. [70] only observed LTP defects in the ephrinB3 null mouse but not in the signaling-deficient mouse. Mice deficient in ephrinB3 reverse signaling also performed normally in learning and memory tasks, suggesting that classical ephrinB reverse signaling is not required for mediating LTP or learning and memory. These discrepancies may be due to different lines of transgenic mice as well as varying degrees of functional compensation occurred during development. Further studies are required to reconcile the controversies and delineate the functional significance of postsynaptic ephrinBs in plasticity.

Conclusions and perspectives

Eph receptors and their ephrin ligands have long been studied for their roles in diverse aspects of development, such as tissue patterning, angiogenesis, and axon guidance [19,49,90]. The rich diversity of ephrin/Eph functions suggests that while the receptor-ligand interaction is conserved, distinct downstream signaling pathways must exist in different cell types to achieve diverse functions. In addition, the multiple combinations of receptor-ligand interactions within the large family of Eph RTKs and ephrin ligands may also serve to accomplish downstream signaling specificity.

In the nervous system, ephrin/Eph signaling mediates axon pathfinding and topographic organization of various brain regions. The roles of trans-synaptic ephrin/Eph signaling in dendritic spine morphogenesis, synapse formation, and plasticity represent exciting new additions to their known functions. The molecular mechanisms of many ephrin/Eph-related synaptic functions are still largely unknown. For example, while multiple pathways between Eph receptor activation and changes in actin dynamics have been revealed, it remains to be determined how Eph mediated spine morphogenesis is coupled to glutamatergic synapse formation, which occurs mostly on the spine structure. How does ephrinB reverse signaling contribute to synaptogenesis? What are the interactions between Eph- and ephrin- regulated synapse formation when both are present in the same neurons? Similarly, what are the interplay between ephrin/Eph and other synaptic adhesion molecules in inducing pre- and post-synaptic differentiation and maturation? In addition, both Eph receptors and ephrinB ligands are required for several forms of long-term synaptic plasticity. What are the downstream signaling mechanisms, and how do they tie in with the known classical mechanisms for LTP and LTD? Future research in synaptic ephrin/Eph signaling is expected to be exciting because understanding the fundamental mechanisms of various synaptic functions has important therapeutic implications in human mental health.

Acknowledgements

The work of the authors is supported by Mabel and Arnold Beckman Foundation, David and Lucile Packard Foundation, W. M. Keck Foundation, and NIMH.

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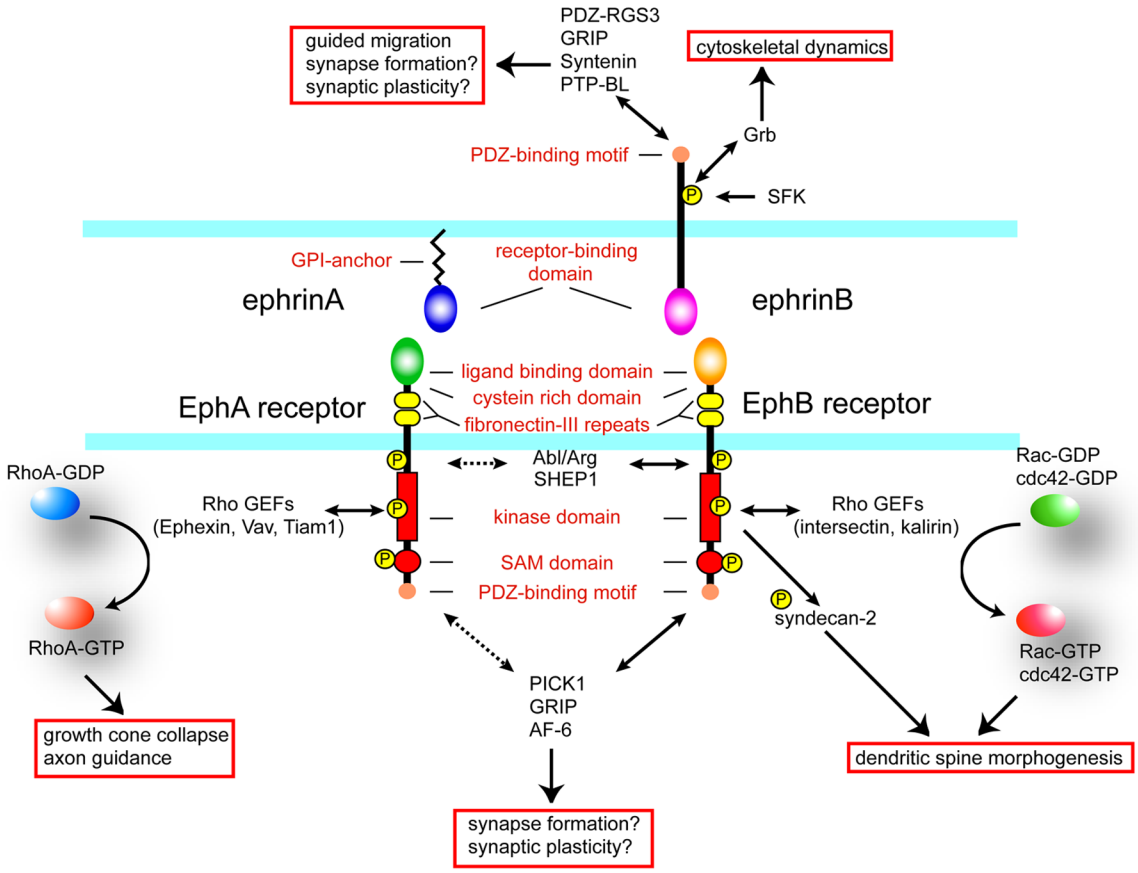

Figure 1.

Schematic drawing of the ephrin and Eph domain structures and summary of protein interactions involved in forward and reverse signaling. Discrete functional domains are labeled in red and interacting proteins are labeled in black. ↔ with solid line depicts established interactions while ↔ with dashed line depicts predicted interactions.

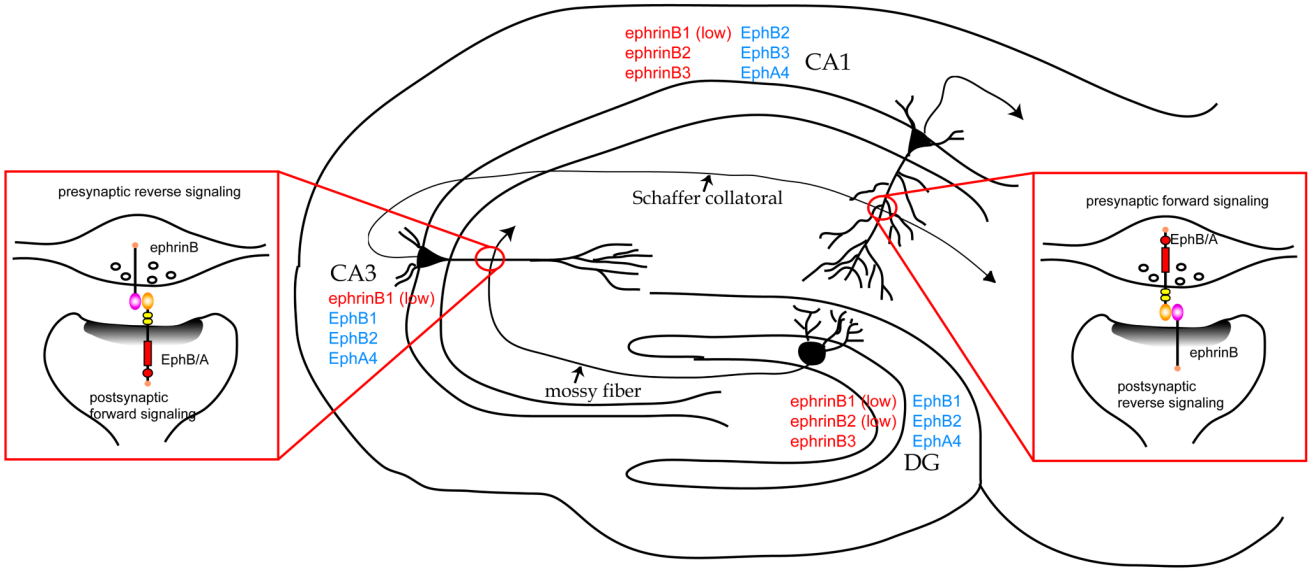

Figure 2.
Localization and signaling directionality of ephrins and Ephs in different regions of the mammalian hippocampus.

Table 1

Ephrin-Eph binding specificity and Eph receptors localization in the hippocampus

Receptors	Ligand Binding	Receptor Localization in the mouse hippocampus *
EphA1	Binds weakly to ephrinA1	N.D. (not detected) [88]
EphA2	Binds to ephrinA1–A5	N.D. [88]
EphA3	Binds to ephrinA1–A5, preferentially ephrinA5	DG [63]
EphA4	Binds to ephrinA1–A5, ephrinB2, -B3	Strong DG, CA1, CA3 [32,51]
EphA5	Binds to ephrinA1–A5	DG, CA1, CA3 [83]
EphA6	Binds to ephrinA1–A5	CA1, CA3 [53]
EphA7	Binds to ephrinA1–A5	DG, CA1, CA3 [22]
EphA8	Binds to ephrinA1–A5	CA1, CA3 [88]
EphA9	Unknown (Chicken)	NA
EphA10	Binds to ephrinA1–A5, kinase defective	N.D.- High in testis [1]
EphB1	Binds to ephrinB1-B3	CA3, DG [32,51]
EphB2	Binds to ephrinB1–B3, ephrinA5	Strong DG, CA1, CA3 [32,33,51]
EphB3	Binds to ephrinB1–B3	Strong CA1, weak CA3 [32,51]
EphB4	Binds to ephrinB1–B3	Non-neuronal
EphB5	Unknown (Chicken)	NA
EphB6	Binds to ephrinB1–B3, kinase defective	NA

* Denotes *in situ* data unless stated otherwise.

Table 2

Ephrin ligands localization in the hippocampus

Ligands	Ligand Localization in the mouse Hippocampus*
ephrinA1	N.D. (Northern Blot) [7]
ephrinA2	N.D. [91]
ephrinA3	CA1–CA3 (FISH + IHC) [57]
ephrinA4	N.D. (Northern Blot) [7]
ephrinA5	DG, CA1, CA3 [27,63,74]
ephrinA6	NA (chicken)
ephrinB1	weak CA1, CA3, DG [32,51]
ephrinB2	Strong CA1, [32,51]
ephrinB3	Strong DG, CA1 [32,51]

* Denotes *in situ* data unless stated otherwise.