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Current Opinion in Neurobiology

Synapse development and plasticity: roles of ephrin/Eph receptor signaling

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The receptor tyrosine kinase Eph and its membrane-bound ligand ephrin are emerging key players in synapse formation and plasticity in the central nervous system. Understanding how ephrin/Eph regulate synapse formation and functions is often complicated by the fact that both ligands and receptors are expressed in the pre-synaptic and post-synaptic neurons and upon their interaction, bi-directional signaling cascades can be triggered. By elucidating the respective downstream targets and generating signaling-deficient mutants, the specific roles of forward (Eph receptor) and reverse (ephrin) signaling are beginning to be unraveled. In this review, we summarize recent advances in our understanding of how ephrin and Eph differentially participate in specific aspects of synapse formation in developing neurons, and activity-dependent plasticity in the adult brain.

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Current Opinion in Neurobiology 2009, **19**:275–283

This review comes from a themed issue on
Signalling mechanisms
Edited by Michael Ehlers and Gina Turrigiano

Available online 3rd June 2009

0959-4388/$ – see front matter

DOI 10.1016/j.conb.2009.04.009

Introduction

Neurons communicate with each other via chemical neurotransmission at synapses, morphologically and functionally specialized structures where synaptic vesicles are concentrated at the active zone of the pre-synaptic axon terminal and neurotransmitter receptors are clustered at the post-synaptic density (PSD) on the dendrites. The formation and maturation of synapses in the central nervous system (CNS) is a dynamic and coordinated process, in which the initial axon–dendrite contact triggers the formation of nascent pre-synaptic and post-synaptic specializations by recruiting multiple protein complexes [1]. For excitatory glutamatergic synapses, synapse maturation involves the growth of dendritic spines. Synapse formation and spine morphogenesis continue in the adult brain during activity-dependent changes in neuronal connections, which underlies long-lasting changes in synaptic strength during the formation of long-term memory.

It is well established that trans-synaptic interaction of adhesion molecules is crucial for synaptogenesis. The homophilic or heterophilic interaction between adhesion molecules, such as neurexin/neuroligin, the immunoglobulin-containing membrane protein SynCAM, and cadherin/catenin, stabilizes the initial axon–dendrite contact and allows simultaneous bi-directional differentiation of the pre-synaptic and post-synaptic specialization [2]. Many of these adhesion molecules possess PDZ-binding motifs at the intracellular tails, which mediate direct interaction with synaptic scaffold proteins such as PSD-95, thereby recruiting and clustering synaptic proteins at the nascent synapses. In addition to these adhesion molecules, emerging studies reveal an important role of the receptor tyrosine kinase (RTK) Eph and its ligand ephrin in synaptogenesis and spine morphogenesis. Originally identified as major players in axon guidance and topographic mapping, this unusual class of RTK has been extensively studied in recent years, which provides significant insights into the molecular mechanisms of synapse formation and maturation. Moreover, several ephrins/Eph receptors are abundant in the adult CNS, and recent studies demonstrate their significance in modulating synaptic plasticity in the adult CNS [3,4]. In this review, we highlight recent advances in our understanding of how ephrin/Eph signaling participates in synapse formation and synaptic plasticity.

Properties of ephrin/Eph underlying their role in synaptogenesis

Eph receptors represent the largest family of RTK, with 14 homologous members being identified in the mammalian genome [3]. Unlike most of the conventional RTKs, the ligands of Eph receptors are membrane-bound proteins, which are classified into A-subclass and B-subclass on the basis of their modes of membrane-anchorage. EphrinAs (ephrinA1 to A5) anchor to the plasma membrane via a GPI moiety, whereas ephrinBs (ephrinB1 to B3) contain a transmembrane domain. In general, ephrinAs bind to EphA receptors (EphA1 to A8, and A10), while EphB receptors (EphB1 to B4, and B6) are preferentially activated by ephrinBs. The notable exception is EphA4, which binds to both the A-class and B-class ephrins with high affinity. Many of the Eph receptors, as well as the three transmembrane ephrinB ligands, contain PDZ-binding motif at their C-termini, which is crucial for

interaction with post-synaptic PDZ domain-containing proteins during synaptogenesis (see below). In addition, all three ephrinB ligands contain tyrosine residues in their intracellular domain, which are phosphorylated upon interaction with Eph receptors and thereby trigger signaling events downstream of the ligands. The bi-directional signaling of both the ephrin-expressing cells and Eph receptor-expressing cells is important for the simultaneous differentiation of the pre-synaptic and post-synaptic specialization during synapse formation (see below).

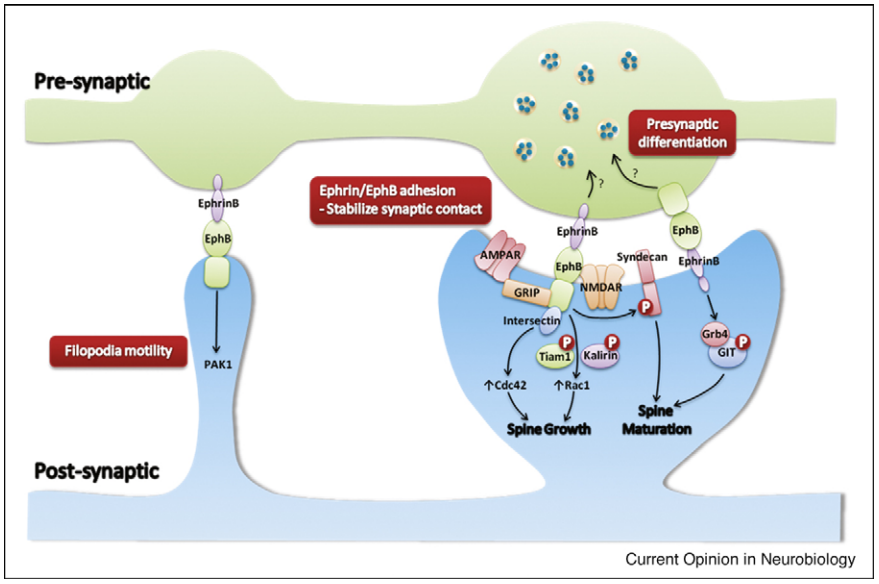
The interaction between ephrins and Eph receptors was initially believed to be repulsive, an essential feature underlying their function in growth cone collapse and segmentation [5,6]. Later studies, however, reveal that the interaction between ephrins and Eph receptors can be adhesive, owing to their high binding affinity. How the switch occurs between repulsion and adhesion is largely unknown. One intriguing possibility involves differential regulation of ephrin/Eph internalization at different developmental stages, such that rapid internalization results in a repulsive response that leads to growth cone collapse during axon guidance and target recognition, whereas enhanced surface expression favors adhesive axon–dendrite contact during synapse formation. This adhesive feature of ephrin/Eph interaction, together with their synaptic localization [7], suggests that ephrin/Eph might play a pivotal role in synapse formation.

Forward signaling of EphB receptor: role in synaptogenesis and spine morphogenesis

Activation of EphB receptor can be achieved by clustering ephrinB-Fc fusion protein with anti-Fc antibody, thereby mimicking the oligomerization of ephrin on the plasma membrane under physiological condition. It has been reported that activation of EphB signaling leads to increased number of glutamatergic synapses in dissociated cultured neurons. The extracellular domain of EphB2 directly interacts with NMDA receptor, which is required for the increased formation of excitatory synapses [8]. The same group further shows that activation of EphB leads to enhanced NMDA receptor-mediated Ca^{2+} influx and gene expression [9]. The importance of EphB2 in synaptogenesis is subsequently verified by transfecting primary cortical neurons with different constructs of EphB2, which induces surface GluR2 clustering and spine formation that depend on the PDZ binding motif and kinase activity of EphB2, respectively [10]. On the contrary, expression of kinase-inactive EphB2 or knockdown EphB2 expression by shRNA in cultured neurons inhibits spine formation and reduces the number of pre-synaptic and post-synaptic specialization [11,12••]. Whereas EphB2 knockout mice display reduced synaptic NMDA receptors *in vivo* [13], somewhat surprisingly, synapse formation and spine morphology appear to be normal in these mice [13,14]. The discrepancy in the synaptogenic function of EphB2 between *in vitro* and *in vivo* studies (i.e. knockdown or expression of dominant-negative EphB2 significantly reduces the density of synapses and spines in dissociated neurons, but normal synapse and spine formation is observed in EphB2 knockout mice) has also been reported for neurexin/neuroligin [15]. It is likely that multiple synaptogenic pathways can compensate for each other during development in knockout mice, whereas compensatory mechanisms do not occur following acute knockdown of individual synaptogenic molecules in cultured neurons [12••]. Nonetheless, triple knockout mice (TKO) lacking EphB1/B2/B3 form fewer synapses and fail to form dendritic spines in the hippocampus [16], therefore supporting the essential role of EphB forward signaling in excitatory synapse formation *in vivo*. Importantly, acute expression of EphB2 in brain slices from EphB TKO mice rescues the defects in synapse formation [10], suggesting that the impairment in synaptogenesis observed in the TKO mice is not attributable indirectly to developmental defects in axon guidance or target recognition.

How does EphB forward signaling cooperate temporally with other synaptogenic pathways to orchestrate synapse formation and maturation? The rate of excitatory synapse formation of cultured neurons is not constant but instead appears to be highest between the first and second week *in vitro*, when the dendritic filopodia are most abundant and motile [17,18]. By exogenously introducing EphB2 into dissociated culture or brain slices prepared from EphB TKO mice, it was found that activation of EphB2 forward signaling rescues the defects in synapse formation only between the first and second week *in vitro* [12••]. The same study further shows that activation of EphB2 increases filopodia motility via activation of the serine/threonine kinase PAK. However, overexpression of constitutively active PAK alone, which increases filopodia motility, is not sufficient to direct synapse formation. Rather, a combination of constitutively active PAK and EphB2, but not EphB2 mutant that lacks the ephrin-binding domain, is able to rescue synaptogenesis in EphB TKO mice. Together, these observations indicate that the synaptogenic function of EphB2 requires its dual ability to activate PAK and promote filopodia motility for the initial axon–dendrite contact, as well as to interact trans-synaptically with axonal ephrinB to stabilize the axon–dendrite contact. This study further raises the interesting idea that EphB2 is specifically involved in synapse formation at a temporally restricted stage when filopodia are most abundant, whereas synapse formation at either earlier (before first week *in vitro*) or later stages (after second week *in vitro*) is independent of EphB2 and may depend on other synaptogenic molecules such as neurexin/neuroligin.

Figure 1



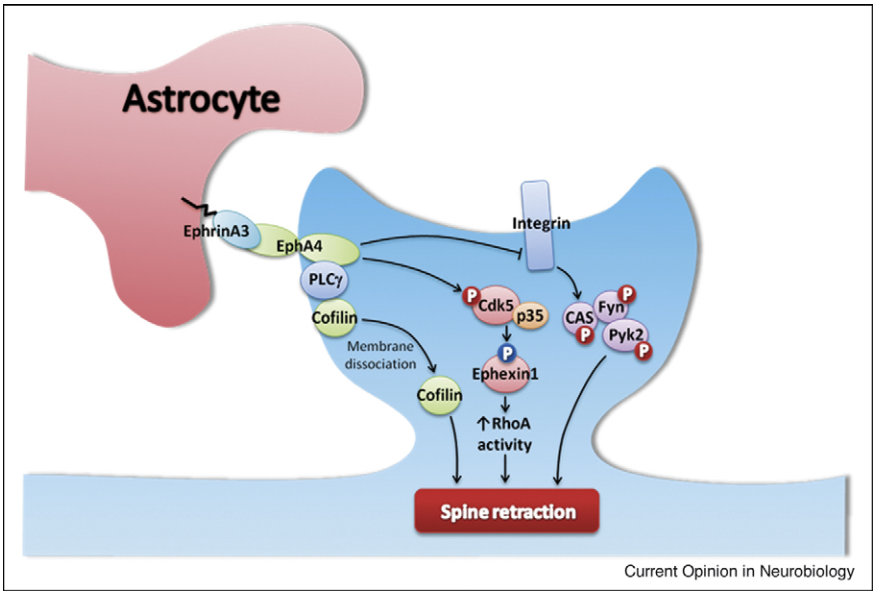
Mechanisms underlying the role of EphB forward and ephrinB reverse signaling in synapse formation and maturation. The motility of immature filopodia, which is crucial during synaptogenesis, is increased upon activation of EphB receptors via the serine/ threonine kinase PAK1. The initial axon–dendrite contact is then stabilized by the adhesive interaction between trans-synaptic ephrinB and EphB receptor. EphB forward signaling also promotes the growth of dendritic spines by increasing the activity of the Rho GTPases Rac1 and Cdc42 through regulation of the GEFs Kalirin, Tiam1, and Intersectin. In addition, the formation of mature mushroom-shaped spines involves tyrosine phosphorylation of the heparan sulphate proteoglycan syndecan. Activation of post-synaptic EphB receptors also leads to clustering of glutamatergic ion channels via direct interaction (NMDA receptor) or indirectly through the PDZ scaffold protein GRIP (AMPA receptor). Besides acting locally at dendritic spines, EphB receptor may have a global effect on synapse formation by facilitating NMDA receptor-mediated Ca^{2+} influx and gene expression. Spine maturation also requires post-synaptic ephrinB reverse signaling, which involves tyrosine phosphorylation of the adaptor protein GIT an its interaction with Grb4. Pre-synaptic EphB/ephrinB signaling also promotes differentiation of axon terminal, but the mechanism remains largely unknown.

Signal transduction of EphB forward signaling and spine morphogenesis

The signaling pathways downstream of EphB signaling in spine morphogenesis have been well characterized (Figure 1), and involve modulating activities of the Rho family of GTPases, the key regulators of actin dynamics underlying spine morphogenesis [19]. The Rac1-specific guanine nucleotide exchange factors (GEF) Kalirin and Tiam1 promote the exchange of GDP for GTP and thereby activate Rac1 activity. EphrinB stimulation of cultured neurons induces tyrosine phosphorylation and clustering of Kalirin and Tiam1. Moreover, ephrinB-induced spine formation and maturation is blocked by dominant-negative Kalirin, Tiam1, and Rac-1, indicating their functional significance [20,21]. The activated Rac1 acts on PAK, which is required in EphB-mediated spine and synapse formation [12••,20]. EphB2 also interacts with intersectin, a GEF for another Rho GTPase Cdc42. Both intersectin and its binding partner N-WASP, as well as Cdc42, are crucial for spine formation [22]. Finally, EphB forward signaling can lead to increased RhoA activity via focal adhesion kinase (FAK) [23]. It is possible that multiple GEF and Rho GTPases are activated upon EphB activation to serve specific functions. For example, Tiam1 has also been implicated in spine morphogenesis in response to NMDA receptor activation [24], suggesting that Tiam1 may be crucial to integrate the EphB-forward signaling to activity-dependent pathway during synapse maturation and remodeling [9]. On the contrary, activation of RhoA may be specifically required for the shortening of immature filopodia during spine maturation [23].

EphB-dependent formation of dendritic spines also involves phosphorylation of the heparin sulphate proteoglycan syndecan [11], which interacts with the synaptic PDZ domain protein CASK and promotes spine maturation [25]. Interestingly, heparan sulphate proteoglycan was recently shown to form a co-receptor specifically with ephrinA3 but not other ephrins, and regulates ephrinA3-dependent EphA receptor signaling [26]. Since ephrinA3 expressed on glial cells has been implicated to maintain spine morphology in adult brain (see below), it would be interesting to further investigate the role of heparan sulphate proteoglycans in facilitating the ephrinA3-EphA signaling during spine maturation.

Figure 2



Organization of dendritic spines by EphA4 forward signaling. EphrinA3 expressed on astrocytes activates EphA4 on the post-synaptic neuron and restricts the growth of dendritic spines through multiple pathways. It involves tyrosine phosphrylation and subsequent activation of the serine/threonine kinase Cdk5, which then activates the RhoA-specific GEF ephexin1 via serine phosphorylation, leading to increased RhoA activity. EphA4 also inhibits the integrin pathway, and involves reduced tyrosine phosphorylation of downstream targets of integrin signaling such as Cas, Fyn, and Pyk2. Finally, the actin depolymerization factor cofilin is dissociated from the plasma membrane upon activation of EphA4 and PLCγ, leading to actin depolymerization and spine retraction.

Role of EphA forward signaling in spine stabilization

Whereas EphB forward signaling promotes spine growth, activation of EphA4, which is also prominently expressed at both dendritic spines and extrasynaptic regions of hippocampal neurons, produces opposite effects on spine morphology. Treatment of dissociated cultured neurons or adult hippocampal slices with clustered ephrinA1 or A3 leads to spine retraction [27,28••]. On the contrary, inhibiting the interaction between ephrinA and EphA4 by EphA4-Fc fusion protein, or transfecting hippocampal slices with kinase-inactive EphA4, leads to disorganization of dendritic spines, a phenotype that is also observed in EphA4 knockout mice [27]. What is the physiological significance of the observed spine retraction? One plausible hypothesis is that activation of EphA4 restricts the unlimited growth of dendritic spines, therefore retaining the capacity for spine remodeling during plasticity in the mature brain [27]. Interestingly, ephrinA3, the most abundant ephrinA ligand in the adult hippocampus, is mostly expressed in astrocytes processes in close proximity of dendritic spines, suggesting that the astrocytic ephrinA3 activates EphA4 on the dendrite and regulate spine morphology. Recent study shows that astrocytes also express EphA receptors and respond to ephrinA3-Fc stimulation by extending filopodia and reduction in glutamate release [29]. The bi-directional ephrinA3-EphA4 interaction between neurons and glia may represent an example of the emerging regulatory role of glial cells in the plasticity of dendritic spines in adult CNS [30].

What are the downstream signaling cascades that are activated by EphA4 in the regulation of spine morphology? Similar to the induction of spine growth, EphA4-mediated spine retraction also involves the Rho family of GTPases (Figure 2). Upon stimulation by ephrinA1, EphA4 recruits the proline-directed serine/threonine kinase Cdk5, which is phosphorylated by EphA4 and becomes activated. The active Cdk5 then phosphorylates the RhoA-specific GEF ephexin1, which promotes RhoA activity and leads to spine retraction via re-organization of actin cytoskeleton. The loss of dendritic spines after ephrinA1 stimulation is abolished in neurons isolated from Cdk5 or ephexin1 knockout mice, indicating their essential roles in mediating EphA4 forward signaling [28••]. Apart from regulating spine morphology, Cdk5 also acts downstream of the neurexin and SynCAM signaling pathways and promotes the formation of pre-synaptic specialization [31]. Since EphA4 and EphB receptors are also expressed in the pre-synaptic neuron [32,33], it will be interesting to determine if EphA or EphB forward signaling also induces pre-synaptic differentiation via Cdk5.

Activation of EphA4 forward signaling also regulates spine morphology through phospholipase Cγ1. Stimulation of hippocampal slices by ephrinA3-Fc activates phospholipase Cγ1 via tyrosine phosphorylation, leading to the reduced membrane association of the actin depolymerization factor cofilin, which is believed to allow actin depolymerization required for ephrinA3-induced spine retraction [34]. Finally, stimulation by ephrinA3-Fc also inhibits integrin signaling and reduces adhesion to the extracellular matrix. This is achieved via decreased tyrosine phosphorylation of Crk-associated substrate (Cas) and the tyrosine kinases FAK and Pyk2, all of which are downstream targets of integrin signaling [35•]. The reduced integrin activity is required for ephrinA3-induced spine retraction, since sustained activation of integrin abrogates the retraction of dendritic spines in response to ephrinA3 (Figure 2).

The significance of ephrinB reverse signaling in synaptogenesis

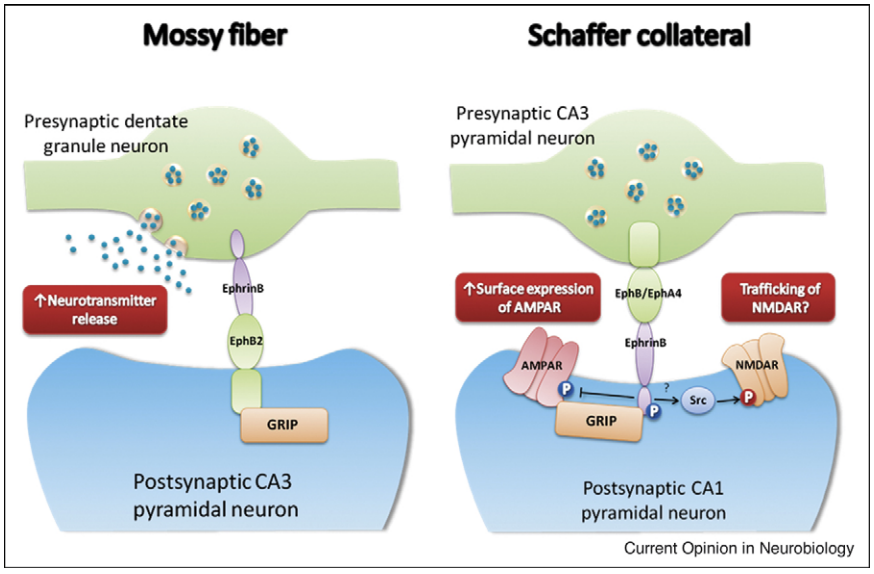
Using ephrinB-Fc fusion protein or EphB mutant constructs lacking cytoplasmic or kinase domain, the importance of EphB forward signaling in synapse and dendritic spine formation has been well established. Similar approach indicates that the reverse signaling on ephrin-B expressing neurons is also crucial to synapse formation, and it functions in the formation of both pre-synaptic and post-synaptic specialization. At the pre-synaptic terminal, the number of synaptic vesicle protein 2 (SV2) puncta and Frie Mao 4-64 (FM4-64) release sites of cultured neurons increases at sites of contact with EphB2-overexpressing 293T cells, whereas post-synaptic neurons that express EphB2 siRNA contain fewer synaptic inputs [10]. The important role of ephrinB reverse signaling in the differentiation of pre-synaptic specialization *in vivo* is further demonstrated by infusion of clustered EphB2-Fc into the optic tectum of developing *Xenopus* embryo, which induces tyrosine phosphorylation of ephrinB and promotes the clustering of pre-synaptic proteins synaptobrevin and SNAP-25 [36••]. EphB2-Fc infusion also results in an early-phase (10 min) increase in mEPSC frequency, followed by a late-phase (after 30 min) elevation in mEPSC amplitude that can be blocked by the NMDA receptor antagonist APV. The interpretation is that EphB2-Fc mainly acts on pre-synaptic terminus to increase neurotransmitter release, which then leads to a delayed post-synaptic enhancement of synaptic transmission that requires NMDA receptor [36••]. The signaling cascade downstream of ephrinB reverse signaling that links to the formation and maturation of pre-synaptic specialization, such as clustering of pre-synaptic vesicles, remains unclear.

EphrinB reverse signaling also takes place on the post-synaptic neurons during synapse maturation (Figure 1). Treatment of cultured hippocampal neurons with clustered EphB2-Fc activates ephrinB reverse signaling and promotes spine maturation, leading to higher proportion of spines with larger spine heads. In contrast, expression of ephrinB1 mutants that lack the cytoplasmic domain or contain mutation of the six intracellular tyrosine residues leads to formation of immature filopodia, indicating the requirement of reverse signaling and tyrosine phosphorylation [37•]. Upon activation of ephrinB reverse signaling, the G-protein-coupled receptor kinase-interacting protein (GIT) 1, a signaling adaptor that has been shown to regulate synapse formation [38], is recruited to ephrinB clusters at the synapses via the SH2-SH3 adaptor protein Grb4. Phosphorylation of GIT1 is induced by ephrinB activation and is required for the recruitment of GIT1 to Grb4/ephrinB complex. Notably, the interaction between ephrin-B and Grb4/GIT1 is necessary for the observed spine morphogenesis and synapse formation [37•]. EphrinB3 reverse signaling also promotes the formation of synapses on the dendritic shaft, which depends on interaction between the C-terminal PDZ-binding motif of ephrinB and the scaffold protein GRIP [32]. Consistent with the *in vitro* data, ultrastructural analysis of CA1 area of the hippocampus reveals that the number of shaft synapses *in vivo* is reduced in ephrinB3 null mice, but the total density of excitatory synapses is not affected [32]. This finding differs from the earlier study showing an increase in the number of excitatory synapses in the hippocampal CA1 region of ephrinB3$^{-/-}$ or mutant mice lacking the cytoplasmic domain of ephrinB3 [39]. Nonetheless, both studies did not report impaired spine morphogenesis in ephrinB3 knockout mice, suggesting that the effect of ephrinB reverse signaling on spine maturation observed by Segura *et al.* [37•] may be attributed to other ephrinBs.

Regulating synaptic functions by ephrin/Eph receptor in the mature CNS: role in synaptic plasticity

Several ephrins and Eph receptors are abundant in certain areas of the adult brain, including hippocampus, the synaptic plasticity of which has been well studied and is closely associated with spatial learning in rodent animals. Given their signaling capability to regulate ion channels clustering, synapse formation and spine morphology, it is perhaps not surprising that ephrins and Eph receptors are also important players in modulating synaptic plasticity in mature neurons. Although the precise mechanisms underlying such plasticity might be different in distinct populations of hippocampal neurons, one emerging notion is that the expression of activity-dependent plasticity such as long-term potentiation (LTP) and long-term depression (LTD) require Eph receptors (especially EphB2 and EphA4) in a kinase-independent manner [3]. This suggests that forward signaling-dependent spine morphogenesis, which depends on kinase activity of the receptors [10,27,28••], is not a major mechanism underlying Eph receptor-mediated regulation of synaptic plasticity. The

Figure 3



EphrinB reverse signaling is required for the expression of LTP in hippocampus. In mossy fiber in which LTP is expressed on the pre-synaptic neurons, EphB receptors are clustered at the post-synaptic apparatus by interacting with GRIP, and the activation of pre-synaptic ephrinB reverse signaling increases neurotransmitter release through unknown mechanism. By contrast, LTP in the Schaffer collateral is expressed on the post-synaptic neurons and involves increased surface expression of AMPA receptors. Activation of post-synaptic ephrinB reverse signaling induces its own serine phosphorylation and in turn increases the interaction between GRIP and AMPA receptor. Stimulation by EphB-Fc fusion protein also reduces PKC-mediated serine phosphorylation of AMPA receptors and prevents their internalization. Tyrosine phosphorylation of NMDA receptor by the tyrosine kinase Src, which has been demonstrated to be crucial for CA1 LTP, is also induced upon activation of ephrinB reverse signaling. The outcome of the ephrinB-induced NMDA receptor phosphorylation and its role in CA1 LTP require further investigation.

kinase-independent role of Eph receptors in activity-dependent plasticity can be explained either by *cis*-interaction of Eph receptors with synaptic proteins such as NMDA receptors [8,14], or the induction of ephrinB reverse signaling. Indeed, recent studies provide evidence that ephrinB reverse signaling in the pre-synaptic or post-synaptic neurons are crucial for the expression of mossy fiber and Schaffer collateral LTP in the hippocampus, respectively (Figure 3).

EphrinB reverse signaling in the pre-synaptic neurons during LTP

The expression of LTP in mossy fiber, in which granule neurons of the dentate gyrus connect with pyramidal neurons in area CA3 of the hippocampus, is dependent on increased neurotransmitter release and is not mediated by NMDA receptor. Perfusion of peptides that disrupts the interaction between EphB2 and GRIP specifically in the post-synaptic neurons, or extracellular application of ephrinB fusion protein that blocks the endogenous ephrin/EphB interaction, reduce mossy fiber LTP [40]. This suggests that mossy fiber LTP can be induced at the post-synaptic side, where EphB receptors are clustered by GRIP and trans-synaptically activates ephrinsB on the pre-synaptic axon terminal. The requirement of ephrinB reverse signaling is further demonstrated by the impaired mossy fiber LTP observed in mice in which the cytoplasmic domain of ephrinB3 is replaced by β-galactosidase [41]. However, the same study shows that mossy fiber LTP is normal in ephrinB3 null mice, indicating that the deficit due to lack of ephrinB3 can be compensated by ephrinB1 or B2. Enhanced neurotransmitter release is also observed by infusion of EphB-Fc into *Xenopus* optic tectum, which activates the pre-synaptic ephrinB1, or after electroporation of embryos with full-length EphB2, but not truncated EphB2 lacking ephrin-binding domain. Furthermore, the increased transmitter release after activation of pre-synaptic ephrinB facilitates theta-burst stimulation (TBS)-induced LTP [36••], providing additional evidence for the pre-synaptic function of ephrinB reverse signaling during synaptic plasticity. The molecular mechanisms underlying the facilitated function of ephrinB in mossy fiber or optic tectum LTP remain unclear.

EphrinB reverse signaling in the post-synaptic neurons during LTP

Unlike mossy fiber LTP, the activity-dependent plasticity in the Schaffer collateral (CA3-CA1) depends on NMDA receptor on the post-synaptic neurons. LTP in CA1 hippocampal neurons is impaired in EphB2 or EphA4 knockout mice, but appears to be normal in knock-in mice lacking the catalytic cytoplasmic domain of EphB2 and EphA4, indicating that the kinase domain

and PDZ-binding motif of Eph receptors are dispensable [13,14,33]. One potential complication from the use of EphB2 knock-in mice is that the juxtamembrane region of EphB2, which contains phosphorylated tyrosine residues, is retained in the mutant mice, raising the possibility that there is still active forward signaling of the truncated EphB2 construct [14]. Nonetheless, defective LTP is observed in ephrinB2 mutant mice lacking intracellular tyrosine residues or PDZ-binding motif [33,42•], suggesting that the impairment in Schaffer collateral LTP observed in EphA4 and EphB2 mutant mice could be explained, at least partly, by defects in the trans-synaptic activation of ephrinB2 on the post-synaptic CA1 neurons. Consistent with this notion, ephrinB2 is abundantly expressed in the post-synaptic CA1 neurons, while EphB2 and EphA4 are expressed in both CA3 and CA1 neurons. The role of ephrinB3, which is also highly expressed in the post-synaptic CA1 neurons, is more ambiguous: whereas ephrinB3 null mice exhibit impaired LTP in CA3-CA1 and spatial learning, ephrinB3 mutant mice lacking the entire catalytic cytoplasmic domain show normal LTP and spatial memory [39]. Further study is needed to delineate why Schaffer collateral LTP specifically requires the reverse signaling of ephrinB2 but not ephrinB3, and how ephrinB3 is involved in the process.

How does ephrinB reverse signaling act on the post-synaptic neurons to modulate synaptic strength? One plausible mechanism could involve Src mediated tyrosine phosphorylation of NMDA receptors, which is required in LTP of CA1 hippocampal neurons [43]. Stimulation of cultured neurons by EphB4-Fc induces rapid tyrosine phosphorylation of Src and NR2A, which is abrogated in neurons derived from ephrinB2 mutant mice that lack intracellular tyrosine residues, indicating the requirement of reverse signaling [42•]. However, whether ephrinB2 clusters together with NMDA receptors at the membrane thereby directly regulating the phosphorylation of NR2 subunits and the trafficking of NMDA receptors needs to be further investigated. EphrinB2 reverse signaling also regulates AMPA receptor internalization, a well-established mechanism underlying the expression of LTP in CA1 neurons [44,45]. Stimulation of dissociated hippocampal neurons by EphB4-Fc induces the serine phosphorylation of ephrinB ligands, which reduces the internalization of AMPA receptors by increasing their interaction with GRIP [46••]. Activation of ephrinB2 reverse signaling might counteract the internalization of AMPA receptors by reducing the protein kinase C-dependent Ser880 phosphorylation of GluR2 [47]. While it has long been known that reverse signaling involves tyrosine phosphorylation, the crucial role of serine phosphorylation of ephrinB ligands shown by this study raises an interesting notion that ephrinB reverse signaling also requires the activation of serine/threonine kinase(s), the identity of which remains to be determined.

Conclusions and future perspectives

It has been a decade since ephrin/Eph receptor were first described to be synaptically localized [7], and enormous progress has been made in establishing their importance in synapse formation and plasticity, and elucidating the underlying mechanisms. Several molecular properties make them ideal synaptogenic proteins: the high affinity binding between ephrin and Eph receptor enables them to act as adhesion molecules and stabilize the initial axon/dendritc contact during synapse formation; the PDZ-binding motifs present on the C-terminal tails of ephrinB and Eph receptor allow simultaneous clustering of PDZ scaffold proteins at both nascent pre-synaptic and post-synaptic specialization; the ability to trigger bi-directional signaling cascade that regulates actin dynamics enable them to modulate spine growth and motility during synapse formation and maturation. Ephrin and Eph receptor are expressed in the mature CNS, where EphA4 forward signaling is involved to maintain the organization of dendritic spines. Moreover, the localization and function of NMDA and AMPA receptors can be regulated either via direct interaction with EphB2 or ephrinB2, or through phosphorylation by ephrinB reverse signaling, thereby modulating synaptic strength for activity-dependent plasticity.

There are many questions remain to be resolved. For example, very little is known about the molecular events that link either the Eph forward signaling or ephrinB reverse signaling to differentiation of pre-synaptic specialization and regulation of neurotransmitter release. In the post-synaptic neurons, our laboratory has recently demonstrated that stimulation of kinase-dependent EphA4 forward signaling leads to proteasome-dependent reduction in AMPA receptor-mediated current and mEPSC amplitude (Fu and Ip *et al.*, Soc Neurosci Abstr 2008, #325.8, and unpublished observations). It will be important to further investigate how ephrin/Eph is involved in regulating the ubiquitin-proteasome system, which is crucial during synapse formation and different forms of neuronal plasticity [48,49]. Finally, EphA4 and ephrinsA are also localized at synapses outside the CNS, including the neuromuscular junction, where they play an essential role in postsynaptic maturation through regulating the stability of acetylcholine receptor clusters ([50]; Shi and Ip *et al.*, Soc Neurosci Abstr 2008, #325.4). A more elaborate examination of the roles and mechanisms of ephrin/Eph at synapses outside the CNS will further establish them as important synaptogenic molecules in the nervous system.

Acknowledgements

We thank Ka-Chun Lok for preparing the figures in this review. The studies by N.Y. Ip were supported partly by the Research Grants Council of Hong Kong (HKUST 6421/05M, 6431/06M, 6444/06M, 661007 and 1/06C), the Area of Excellence Scheme of the University Grants Committee (AoE/B-15/01) and the Hong Kong Jockey Club. N.Y. Ip and K.O. Lai are Croucher Foundation Senior Research Fellow and Croucher Foundation Fellow, respectively.

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- •• of outstanding interest

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