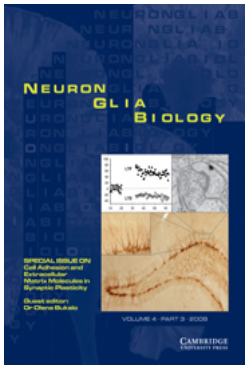

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Bidirectional signaling of ErbB and Eph receptors at synapses

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Synapse development and remodeling are regulated by a plethora of molecules such as receptor tyrosine kinases (RTKs), a family of cell surface receptors that play critical roles in neural development. Two families of RTKs implicated in synaptic functions, ErbBs and Ephs, share similar characteristics in terms of exhibiting forward and reverse signaling. In this review, we will discuss the latest advances in the functions of ErbBs and Ephs at the synapse, including dendritic spine morphogenesis, synapse formation and maturation, and synaptic transmission and plasticity. In addition to signaling at interneuronal synapses, communication between neuron and glia is increasingly implicated in the control of synaptic functions. Studies on RTKs and their cognate ligands in glial cells enhance our understanding on the nature of 'tripartite synapse'. Implications of these signaling events in human diseases will be discussed.

Keywords: Neuregulin, ephrin, glia, dendritic spine, synaptic plasticity

INTRODUCTION

Neurons communicate through specialized contact sites called synapses, the precise formation of which is vital for neuronal functions. Emerging evidence reveals that deregulation of synaptic functions is associated with various neurodegenerative diseases (e.g. Alzheimer's disease) and psychiatric disorders (e.g. schizophrenia) (Fiala *et al.*, 2002). Dendritic spines, small protrusions on neuronal dendrites, serve as the primary sites of excitatory postsynaptic compartments. Changes in synaptic functions are often accompanied by dendritic spine morphogenesis and reorganization of protein networks, including cytoskeletal and scaffold proteins, as well as receptors and ion channels clustered on the synaptic membranes (Bourne and Harris, 2008). Receptor tyrosine kinases (RTKs) are cell surface molecules implicated in a variety of neuronal functions, including neuronal survival, axon and dendrite outgrowth, and synapse development. A typical RTK consists of an extracellular ligand-binding domain, a transmembrane region and an intracellular region containing the kinase domain. Upon binding to their cognate ligands, RTKs dimerize and autophosphorylate at multiple tyrosine residues located in their intracellular domains, thereby triggering a series of signaling cascades. Other than this classical ligand-to-receptor signaling pathway, two families of RTKs, ErbBs and Ephs, exhibit capacities to transduce signals in a reverse direction such that membrane-tethered ligands elicit signaling events in ligand-expressing cells upon binding to their receptors. The forward and reverse signaling of ErbBs and Ephs have been implicated in various aspects of synapse development, including dendritic spine morphogenesis, synapse formation, maintenance and plasticity (Pasquale, 2005; Mei and Xiong, 2008; Klein, 2009). Here we review the emerging functions of ErbB and Eph receptors at synapses in the central nervous system.

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ErbBs AND NEUREGULINS ARE ESSENTIAL PLAYERS AT SYNAPSES

ErbB receptors and their ligands, neuregulins

ErbB is a family of RTKs that play active roles in various aspects of neuronal development, such as neuronal migration, axon guidance and synaptic plasticity. To date, four receptors have been identified: ErbB1, ErbB2, ErbB3 and ErbB4. ErbB1, also known as epidermal growth factor (EGF) receptor, is activated by specific ligands, including EGF and transforming growth factor α. Since the high-affinity ligand for ErbB2 has not yet been identified, ErbB2 is believed to function as a co-receptor for other ligand-bound ErbBs. Lacking the kinase domain, ErbB3 elicits functional responses only when it dimerizes with other ErbBs (Guy *et al.*, 1994; Tzahar *et al.*, 1996). The ligands that bind to ErbB3 and ErbB4 receptors are called neuregulins (NRGs), which are encoded by four genes: *NRG1*, *NRG2*, *NRG3* and *NRG4*. A single *NRG1* gene gives rise to six types of NRGs and over 30 isoforms by utilizing different 5′ regulatory elements and alternative splicing. While the functional roles of NRG1 are well characterized, our knowledge on NRG2–4 is very limited (Falls, 2003; Linggi and Carpenter, 2006; Mei and Xiong, 2008).

A number of molecules have been identified to be the downstream targets of ErbB activation, including Src family kinases (SFKs), phosphoinositide 3-kinase (PI3K) and cyclin-dependent kinase 5 (Cdk5) (Fu *et al.*, 2001; Li *et al.*, 2001; Bjarnadottir *et al.*, 2007). Apart from recruiting downstream signaling proteins, NRG/ErbB forward signaling can be transduced through an alternative pathway involving the intracellular domain of ErbB (ErbB-ICD). Generated via sequential cleavage of ErbB by metalloprotease and γ-secretase, the ErbB-ICD translocates into nucleus and controls gene transcription (Vecchi and Carpenter, 1997; Rio *et al.*, 2000; Ni *et al.*, 2001; Lee *et al.*, 2002; Sardi *et al.*, 2006).

Most NRG1 isoforms are synthesized as transmembrane precursor proteins, thus enabling transduction of reverse

signals to NRG1-expressing cells. The reverse signaling triggered by ErbB binding or synaptic activity promotes the proteolytic cleavage of NRG1 at the intracellular region, generating the NRG1-intracellular domain (NRG1-ICD) that regulates gene transcription in nucleus (Bao *et al.*, 2003).

NRG/ErbB forward signaling

REGULATION OF SPINE MORPHOGENESIS AND EXCITATORY SYNAPTIC TRANSMISSION

Since targeted deletion of ErbB receptors or NRG1 leads to early lethality of transgenic mice (Gassmann *et al.*, 1995; Meyer and Birchmeier, 1995; Erickson *et al.*, 1997), investigation on the neuronal functions of NRG/ErbB signaling was primarily conducted in cultured cells or in mice with conditional abrogation of NRG/ErbB functions (Mei and Xiong, 2008). Generation of tissue-specific mutant mice that lack ErbB2 and ErbB4 in the central nervous system (CNS) advances our understanding of NRG/ErbB functions *in vivo* (Barros *et al.*, 2009; Gajendran *et al.*, 2009). In contrast to the previous studies demonstrating crucial roles of NRG/ErbB signaling in neuronal migration and cortical layer organization (Anton *et al.*, 1997; Rio *et al.*, 1997), *ErbB2/ErbB4* double mutant mice develop normal cortical layers, suggesting that NRG/ErbB signaling is not essential for neuronal migration (Barros *et al.*, 2009). Nonetheless, the size and density of dendritic spines in these mutant mice are significantly reduced, while density of the immature filopodia remains unaffected (Anton *et al.*, 1997; Rio *et al.*, 1997; Barros *et al.*, 2009). These findings suggest that NRG/ErbB signaling is important for dendritic spine development.

Dendritic spine morphogenesis is often associated with changes in synaptic transmission, which is regulated by many postsynaptic proteins, e.g. PSD-95, *N*-methyl-D-aspartate (NMDA) receptor and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor (Ethell and Pasquale, 2005; Tada and Sheng, 2006). PSD-95 is a PDZ (PSD-95, Disc-large and ZO-1) domain-containing scaffold protein at the postsynaptic density of glutamatergic synapses that is responsible for organizing postsynaptic protein network and regulation of synaptic transmission (Kim and Sheng, 2004). ErbB2 and ErbB4 interact with PSD-95 either directly through the C-terminal PDZ-binding motif or indirectly through a PDZ protein Erbin (Garcia *et al.*, 2000; Huang *et al.*, 2000, 2001; Ma *et al.*, 2003; Xie *et al.*, 2007). Through this mutual interaction, PSD-95 facilitates the translocation of activated ErbB receptor to a highly ordered microdomain lipid raft, and potentiates ErbB-dependent downstream signaling (Ma *et al.*, 2003). In cultured hippocampal slices, ErbB4 activation promotes spine enlargement and maintains existing spines, which is dependent on its PDZ-binding motif, further emphasizing the critical roles of PDZ protein in regulating ErbB synaptic functions (Li *et al.*, 2007).

As the best-studied PDZ protein, PSD-95 does not only interact with ErbB, but also associates with NMDA receptors. More importantly, the interaction between PSD-95 and NMDA receptors is regulated by NRG/ErbB signaling, as demonstrated by reduced binding of PSD-95 to NMDA receptor subunits in mice lacking ErbB2/ErbB4 in the CNS (Barros *et al.*, 2009). The perturbed interaction between NMDA receptors and PSD-95 in these mutant mice may account for the loss of mature dendritic spines. It has been shown that phosphorylation of NMDA receptor NR2B subunit is closely associated with changes in synaptic strength. Induction of long-term potentiation (LTP) in hippocampal CA1 region is accompanied by enhanced phosphorylation at Tyr^{1472} of NR2B, the major Fyn-mediated phosphorylation site (Nakazawa *et al.*, 2001). NR2B phosphorylation at Tyr^{1472} is attenuated in $NRG1^{+/-}$ or $ErbB4^{+/-}$ mice, which is associated with defective basal synaptic transmission and long-term synaptic plasticity (Bjarnadottir *et al.*, 2007). Activation of ErbB by NRG1 triggers intracellular signaling cascades to promote actin depolymerization, resulting in an enhanced NMDA receptor internalization and decreased NMDA receptor-mediated excitatory postsynaptic current (EPSC) (Gu *et al.*, 2005). In addition to regulating NMDA receptor surface expression, NRG1/ErbB signaling is also implicated in the switching of NMDA receptor subunit expression during neuronal development. While activation of NR2C and downregulation of NR2B expression are induced in granule cells after mossy fiber innervation (Watanabe *et al.*, 1992; Kadotani *et al.*, 1996; Takahashi *et al.*, 1996), treatment of cultured cerebellar slices with NRG1β was reported to induce the expression of NR2C (Ozaki *et al.*, 1997). However, a later study suggests that neuregulin signaling is not essential for the induction of NR2C expression in dissociated cerebellar granule cells (Rieff *et al.*, 1999). This inconsistency may be explained by the difference between these two culture systems. For example, cerebellar slices, when compared with the dissociated neurons, provide a relatively intact cellular context that may be required for the NRG-dependent NR2C regulation. Moreover, NRG may be able to trigger the activation of ErbBs in neighboring neurons or astrocytes, and therefore indirectly regulates the NR2C expression in granule neurons of cerebellar slices (Gajendran *et al.*, 2009). However, the later *in vitro* and *in vivo* loss-of-function studies suggest that ErbB receptors in granule cells are not critical for the regulation of NR2C expression, likely due to redundancy of the signaling pathways (Gajendran *et al.*, 2009). Whether NRG/ErbB signaling regulates NR2C expression requires further investigations.

NRG/ErbB signaling is also important for the synaptic functions of AMPA receptors. Overexpression of ErbB4 in hippocampal CA1 neurons significantly enhances the amplitude of AMPA receptor-mediated EPSCs. Consistent with this observation, the potentiation of synaptic transmission is reversed by knockdown of ErbB4 or expression of a kinase-inactive mutant. Interestingly, stabilizing the synaptic localization of AMPA receptor GluR2 subunit with a short peptide overrides the effect of ErbB4 knockdown on EPSC amplitude. These findings indicate a positive role of NRG/ErbB signaling in AMPA receptor-mediated synaptic transmission and synaptic maturation through controlling the stability of AMPA receptor at the synapse (Li *et al.*, 2007).

Consistent with the above findings, NRG/ErbB signaling has been demonstrated to play central roles in long-term synaptic plasticity. Perturbation of NRG/ErbB signaling through overexpression of inactive ErbB4 mutants disrupts both the chemical form and electrically induced LTP (Li *et al.*, 2007). In addition, interference of ErbB signaling by expressing a truncated form of NRG1 in $NRG1(\Delta EGF)^{+/-}$ mice impairs theta burst-induced LTP in hippocampal slices (Bjarnadottir *et al.*, 2007). However, the exact role of ErbB signaling in LTP induction remains controversial, since several studies demonstrate that potentiation of ErbB4 signaling by

NRG1 reverses or suppresses the induction and expression of LTP (Huang *et al.*, 2000; Ma *et al.*, 2003; Kwon *et al.*, 2005, 2008; Pitcher *et al.*, 2008). Interestingly, it has been found that treatment of *NRG1*$(\Delta EGF)^{+/-}$ mouse brain slices with exogenous NRG1 has profound effects on LTP, depending on the concentration of bath applied NRG1 (Bjarnadottir *et al.*, 2007). A recently proposed 'inverted-U model' helps to resolve these apparent discrepancies. This model suggests that the local concentration of NRG1/ErbB4, the expression pattern of NRG isoforms and the nature of incoming stimulation are all critical determinants for the regulation of synaptic strength (Role and Talmage, 2007). Bath application of soluble NRG enhances postsynaptic ErbB signaling but attenuates presynaptic NRG signaling, thus generating an imbalanced NRG/ErbB signaling at synapses that limits synaptic potentiation. An optimal and balanced NRG/ErbB signaling level is therefore essential for achieving maximal synaptic strength.

ROLES IN GABAERGIC TRANSMISSION AND DISEASES

ErbB signaling does not only function at excitatory glutamatergic synapses, but also plays a significant role in modulating inhibitory synaptic transmission. ErbB4 was found to accumulate at GABAergic nerve terminals (Woo *et al.*, 2007). While NRG1 does not affect the basal release of GABA (γ-aminobutyric acid), NRG1 potentiates the release of GABA from a readily releasable pool when the membrane is depolarized. NRG1 has also been shown to downregulate $GABA_A$ receptor α subunit expression specifically in hippocampal CA1 inhibitory synapses (Okada and Corfas, 2004). In contrast, NRG1 increases the mRNA expression of β2 subunit of $GABA_A$ receptor in cultured cerebellar granule neurons, and potentiates the amplitude of cellular response to GABA (Rieff *et al.*, 1999). Pharmacological inhibition of mitogen-activated protein kinase, PI3K or Cdk5 pathway abolishes the action of NRG1 on the regulation of $GABA_A$ receptor expression (Xie *et al.*, 2004). Similar to the case of NRG-dependent regulation of NR2C, the depletion of functional ErbB receptor complexes in mouse cerebellum exerts no observable effect on $GABA_A$ receptor β2 subunit expression (Gajendran *et al.*, 2009). This discrepancy on the results from the gain-of-function studies in dissociated granule cells and in *erbb2/erbb4*-deficient mice further emphasizes the necessity of investigating the functions of NRG/ErbB signaling in an intact cellular context.

Aberrant cognitive functions in schizophrenia, a mental disorder affecting large populations, are believed to be associated with the deregulation of glutamate and GABA receptor-mediated transmission (Lewis and Moghaddam, 2006). Intriguingly, *NRG1* and *ErbB4* are identified to be the susceptibility genes for schizophrenia, and a number of mutations in *NRG1* and *ErbB4* genes have been implicated in this mental disorder. The studies on how NRG/ErbB signaling regulates glutamatergic and GABAergic neurotransmission may provide mechanistic insights into the biological basis for the pathogenesis of schizophrenia (Li *et al.*, 2007; Woo *et al.*, 2007). In addition, NRG1 has been reported to regulate dopaminergic transmission through potentiation of dopamine release and activation of dopamine receptors (Kwon *et al.*, 2008). NRG1-dependent signaling also plays important roles in targeting of α7 nicotinic acetylcholine receptor, as well as acetylcholine- or nicotine-regulated synaptic transmission (Liu *et al.*, 2001; Chang and Fischbach, 2006; Zhong *et al.*, 2008). These studies collectively suggest that ErbB and NRG are the key players in various types of neurotransmission implicated in the pathophysiology of schizophrenia.

NRG/ErbB reverse signaling

Although most studies focus on the synaptic roles of NRG1-stimulated ErbB activation, NRG1 also serves as a receptor for the extracellular domain of ErbB (ecto-ErbB4) and plays important roles in synapse development. A yeast two-hybrid screening revealed the interaction between the cytoplasmic tail of NRG and LIM kinase 1 (LIMK1). Colocalization of NRG and LIMK1 at neuromuscular synapses raises an intriguing possibility that a reverse signaling mediated by NRG plays critical roles in synapse development (Wang *et al.*, 1998). On the other hand, overexpression of kinase dead ErbB4 at the postsynaptic neurons of CNS promotes the differentiation of presynaptic terminals, which is blocked by knockdown of ErbB4 or expression of ErbB4 mutant with truncated extracellular domain, indicating an essential role of NRG-mediated reverse signaling in presynaptic maturation (Krivosheya *et al.*, 2008).

Direct evidence supporting the roles of NRG/ErbB reverse signaling in synapse development came from two studies focusing on the transcriptional activity of NRG-ICD. Treatment of neurons with soluble ecto-ErbB2 and ErbB4 or depolarization triggers the cleavage of NRG1. The released NRG1-ICD is then translocated into nucleus, and forms complex with a zinc-finger transcription factor Eos, which binds to PSD-95 promoter and induces the upregulation of PSD-95 mRNA (Bao *et al.*, 2003, 2004). These studies reveal an important role of NRG1-mediated reverse signaling in activity-dependent regulation of PSD-95 expression and synaptic plasticity.

Ephs AND EPHRINS REGULATE SYNAPSE FORMATION, MAINTENANCE AND PLASTICITY

Eph receptors and ephrin ligands

Eph receptors and their cognate ligands ephrin are critical regulators of neuronal development. The best characterized role of ephrin/Eph is their action as repulsive cues in retinotectal topographic mapping and axon guidance (Flanagan and Vanderhaeghen, 1998; Wilkinson, 2001). In recent years, the roles of ephrin/Eph signaling in synapse development are also beginning to be unraveled (Klein, 2009). Based on the extracellular sequence homology and ligand-binding properties, Eph receptors can be classified into two groups: EphA receptors (EphA1–10) preferentially bind to ephrin-A ligands that are anchored to cell membrane via a glycosylphosphatidylinositol linkage, and EphB receptors (EphB1–6) exhibit high affinity to ephrin-B ligands that possess a transmembrane region and a short intracellular domain. There are some exceptions, however, with EphA4 binding to ephrin-B2 and ephrin-B3, and EphB2 associating with ephrin-A5 (Pasquale, 2005).

Upon activation, Eph receptors recruit a number of adaptors and signaling molecules for transduction of downstream signaling, such as SFKs, guanine nucleotide exchange factors (GEFs) and PDZ proteins, which are important players at neuronal synapses (Pasquale, 2005). On the other hand, similar to ErbB receptors, ligand stimulation induces

cleavage of Eph in a presenilin/γ-secretase-dependent manner, generating C-terminal fragments (CTFs) (Litterst *et al.*, 2007; Inoue *et al.*, 2009). Distinct from the action of ErbB-ICD, which enters the nucleus to regulate gene expression, EphB2/CTF was found to be degraded through ubiquitination (Litterst *et al.*, 2007). Although the intracellular fragment of EphA4 (EphA4-ICD) could be detected in nucleus, its role in spine morphogenesis is independent of its nuclear localization (Inoue *et al.*, 2009).

Similar to NRG, membrane-anchored ephrins are activated by Eph receptors to trigger reverse signaling in the ephrin-expressing cells. Ephrin-As and ephrin-Bs utilize different pathways to elicit signaling events. Ephrin-As are able to elicit localized signaling in a Fyn-dependent manner (Davy *et al.*, 1999). However, since ephrin-As do not possess intracellular domains, how the reverse signals are transduced into cells remains unclear. A recent study suggested that there is *cis* interaction between ephrin-A5 and Trk neurotrophin receptor, implicating that ephrin-As trigger intracellular signaling through its association with other transmembrane receptor kinases or adaptor proteins (Marler *et al.*, 2008). Unlike ephrin-As, ephrin-Bs bear a short intracellular sequence containing several tyrosine residues and a PDZ-binding motif that is capable of recruiting various signaling molecules. Moreover, ephrin-B/CTF generated by presenilin/γ-secretase-mediated cleavage is implicated in Src kinase activation (Georgakopoulos *et al.*, 2006; Tomita *et al.*, 2006).

Ephrin/Eph forward signaling

Various Eph receptors such as EphA4, EphB1–B3 are found to be concentrated at synaptic regions, supporting their involvement in synapse development (Henkemeyer *et al.*, 2003; Murai *et al.*, 2003; Fu *et al.*, 2007). Accumulating evidence suggests that ephrin/Eph signaling is indispensable for various aspects of synapse development, including synaptic differentiation, neurotransmitter receptor clustering and long-term synaptic plasticity (Klein, 2009).

REGULATION OF DENDRITIC SPINE MATURATION AND MAINTENANCE

Both EphA and EphB receptors regulate dendritic spine morphogenesis. In EphA4 knockout neurons, the morphology of dendritic spines is aberrant. Activation of EphA4 signaling triggers the retraction of dendritic spines and reduction of spine density in mature hippocampal neurons (Murai *et al.*, 2003; Fu *et al.*, 2007; Carmona *et al.*, 2009). In contrast, potentiation of EphB receptor-mediated signaling enhances spine maturation (Henkemeyer *et al.*, 2003). Hippocampal neurons in EphB1/B2/B3 triple-mutant mice fail to develop mature dendritic spines. Nonetheless, no obvious defect in spine formation was observed in single-mutant mice, suggesting a redundant role of EphB receptors in regulating dendritic spine morphology (Grunwald *et al.*, 2001; Henkemeyer *et al.*, 2003).

Changes in dendritic spine morphology require the reorganization of actin cytoskeleton. Eph receptors have been demonstrated to regulate a couple of proteins that are implicated in the control of actin dynamics, including Rho GTPases and actin-binding proteins. The activity of Rho GTPases (RhoA, Rac1 and Cdc42) is induced by GEFs and negatively regulated by GAPs (GTPase-activating proteins) (Govek *et al.*, 2005). Eph receptors regulate Rho GTPase activity via distinct GEFs, such as ephexin1, kalirin, intersectin and Tiam1 (T lymphoma invasion and metastasis 1) (Shamah *et al.*, 2001; Irie and Yamaguchi, 2002; Penzes *et al.*, 2003; Fu *et al.*, 2007; Tolias *et al.*, 2007). For example, our laboratory has previously demonstrated that ephexin1 is a substrate of Cdk5. Activation of EphA4 receptor increases Cdk5-dependent phosphorylation of ephexin1, which is an indispensable step in ephrin-A-triggered RhoA activation and dendritic spine retraction (Fu *et al.*, 2007). In contrast, EphB2 regulates Rac1 activity through Tiam1 and kalirin-7, and Cdc42 activity through intersectin, leading to the formation of dendritic spines (Irie and Yamaguchi, 2002; Penzes *et al.*, 2003; Tolias *et al.*, 2007).

In addition to modulating Rho GTPase activity, Eph receptors are capable of regulating actin-binding proteins during dendritic spine morphogenesis. EphA4 interacts with and phosphorylates an actin-binding protein cortactin, which has been shown to associate with NMDA receptor/PSD-95 complex. This observation suggests that a potential mechanism by which ephrin/Eph signaling regulates spine morphology and synaptic plasticity involves a direct interaction of Eph and actin-binding proteins at spines (Naisbitt *et al.*, 1999; Lai *et al.*, 2001). On the other hand, actin depolymerizing factor (ADF)/cofilin is a family of actin-binding proteins that destabilize actin cytoskeleton (Bamburg, 1999). EphA4 interacts with PLC-γ1 (phospholipase C-γ1) and potentiates PLC-γ1 activity, which facilitates the detachment of cofilin from membrane and subsequent actin depolymerization, leading to the retraction of dendritic spines (Zhou *et al.*, 2007). Interestingly, EphB signaling was recently found to induce phosphorylation and inactivation of cofilin, contributing to the maintenance of mature dendritic spine morphology (Shi *et al.*, 2009).

REGULATION OF SYNAPTIC TRANSMISSION AND PLASTICITY

Morphological changes of dendritic spines are often accompanied by the changes of postsynaptic protein composition and synaptic transmission. Our laboratory recently demonstrated that EphA4 activation leads to reduced surface expression of AMPAR and synaptic transmission efficacy (Fu *et al.*, 2008). In EphB-knockout neurons, reduced level of NMDA receptor and AMPA receptor cluster at the postsynaptic sites has also been observed (Henkemeyer *et al.*, 2003). EphB receptor activation stimulates the co-clustering of NMDA receptors and induces phosphorylation of NR2B subunit through Src kinase, enhancing glutamate-induced Ca^{2+} influx through NMDA receptor (Dalva *et al.*, 2000; Takasu *et al.*, 2002). This leads to elevated expression of immediate early genes, such as *c-fos*, *bdnf* and *cpg15* (Takasu *et al.*, 2002). Moreover, AMPA receptor trafficking is regulated by EphB2 through clathrin-mediated endocytosis (Irie *et al.*, 2005). Deletion of C-terminal PDZ-binding motif of EphB2 alters the localization of AMPA receptors, but does not affect the number of dendritic spines, indicating that distinct domains of EphB receptor is responsible for the clustering of postsynaptic proteins or modulation of dendritic spine morphology (Kayser *et al.*, 2006). Interestingly, an AMPA receptor-interacting protein GRIP1 (glutamate receptor-interacting protein 1) binds to EphB2 through a PDZ domain-mediated interaction (Torres *et al.*, 1998). Blocking this interaction by peptides or antibodies reduces mossy fiber

LTP, suggesting a critical involvement of PDZ interaction in Eph receptor-mediated synaptic plasticity (Contractor *et al.*, 2002). Early studies showed that while infusion of an EphA receptor agonist ephrin-A5 potentiates LTP and learning, infusion of EphA receptor antagonist EphA5 impairs LTP (Gao *et al.*, 1998; Gerlai *et al.*, 1999). Although the role of ephrin-mediated reverse signaling could not be excluded, these results indicated that, similar to the 'inverted-U model' in NRG/ErbB system (Role and Talmage, 2007), an optimal level of trans-synaptic ephrin and Eph interaction is crucial for modulating synaptic strength.

TEMPORAL AND SPATIAL CONTROL OF Eph FUNCTION

The timing of Eph receptor-regulated synapse development is tightly controlled. A recent elegant study demonstrated that the action of EphB receptors differs significantly in the early and late phases of synapse development. EphB2 activation apparently does not affect synaptogenesis in neurons before 7 days *in vitro* (DIV), but induces spine and synapse formation in neurons from 7 DIV to 14 DIV. Although EphB receptors do not regulate synapse formation in the late developmental stage after 14 DIV, they do play a role in stabilizing spine morphology (Kayser *et al.*, 2008). In contrast to the studies of EphB receptors in synapse formation, the synaptic roles of EphA receptors were primarily investigated in mature cultured neurons or in hippocampal slices, as EphA4 is believed to regulate dendritic spine morphology in mature neurons (Murai *et al.*, 2003; Bourgin *et al.*, 2007; Fu *et al.*, 2007; Zhou *et al.*, 2007; Inoue *et al.*, 2009). Thus, it is still not clear whether and how EphA receptors play their roles in the early stage of synapse development. Nonetheless, these studies suggest that Eph receptors regulate the formation and maintenance of a subpopulation of spines and synapses (Fu *et al.*, 2007; Kayser *et al.*, 2008), perhaps through restricted expression of Eph receptors or their downstream signaling molecules.

Ephrin/Eph reverse signaling

The short C-terminal domains of ephrin-Bs serve as signaling modules to regulate spine and synapse development. Activation of ephrin-B promotes the shortening of dendritic filopodia and spine formation. This effect is abolished by overexpression of a truncated ephrin-B1 mutant lacking the C-terminal signaling fragment, suggesting that ephrin-B-mediated reverse signaling is critical for spine morphogenesis (Segura *et al.*, 2007). Upon activation, ephrin-B binds to adaptor protein Grb4 (growth-factor-receptor-bound protein 4) (Cowan and Henkemeyer, 2001). Disruption of the interaction between Grb4 and its binding partner GIT1 (G protein-coupled receptor kinase-interacting protein 1) in hippocampal neurons impairs ephrin-B-dependent spine morphogenesis and synapse formation (Segura *et al.*, 2007). Recently, a major serine phosphorylation site was identified at the C-terminal region of ephrin-B2. Phosphorylation of this serine residue is important for the interaction between ephrin-B2 and GRIP, the trafficking of AMPA receptors as well as synaptic transmission in hippocampal neurons (Essmann *et al.*, 2008). Moreover, ephrin-B-mediated reverse signaling is also implicated in presynaptic differentiation. While overexpression of the extracellular domain of EphB2 in postsynaptic neurons is sufficient to trigger the clustering of presynaptic vesicle protein SV2 at the terminals of presynaptic neurons, knockdown of postsynaptic EphB2 expression leads to a significant reduction in the number of presynaptic SV2 puncta, accompanied by a reduced frequency of miniature EPSCs (Kayser *et al.*, 2006). Thus, it is likely that the reverse signaling from EphB2 to ephrin-B regulates presynaptic differentiation. Alternatively, EphB2 at postsynaptic neurons may regulate presynaptic differentiation of neighboring neurons indirectly through modulation of the postsynaptic maturation, since the extracellular domain of EphB2 is capable of interacting with NMDA receptors (Dalva *et al.*, 2000).

Studies with Eph and ephrin transgenic mice reveal critical roles of ephrin reverse signaling in synaptic plasticity *in vivo*. In *EphB2*$^{-/-}$ mice, the late-phase LTP and long-term depression (LTD) in CA3-CA1 synapses are impaired. These defects could be rescued by the expression of a truncated EphB2 lacking the C-terminal domain in mice, suggesting that the kinase activity of EphB2 and the ephrinB/EphB forward signaling is not required for certain forms of synaptic plasticity (Grunwald *et al.*, 2001; Henderson *et al.*, 2001). A later study found that ephrin-B2 and ephrin-B3 are enriched postsynaptically at CA3-CA1 synapses (Grunwald *et al.*, 2004). Deletion of ephrin-B in postnatal forebrain does not affect the number of synapses and spine morphology, while defects in LTP and LTD are obvious when ephrin-B2 is deleted in hippocampal CA1 neurons. Interestingly, knockout of ephrin-B receptor EphA4 results in defective LTP and LTD phenotypes similar to that observed in mice lacking ephrin-B2. This defective phenotype is rescued by knock-in of the extracellular domain of EphA4, supporting the notion that postsynaptic ephrin-B2-mediated reverse signaling is important for the long-term synaptic plasticity at Schaffer collateral CA3-CA1 pathway (Grunwald *et al.*, 2004). Ephrin-B3 knockout mice also exhibit striking defects in CA3-CA1 LTP. However, it is unlikely that ephrin-B3 reverse signaling is involved, as demonstrated by the normal LTP at CA3-CA1 synapses in *ephrin-B3*^{lacZ} mice, in which the reverse signaling is disrupted by replacing ephrin-B3 cytoplasmic domain with β-galactosidase (Grunwald *et al.*, 2004; Rodenas-Ruano *et al.*, 2006). In contrast, LTP in the hippocampal mossy fiber (where ephrin-B3 is highly expressed at presynaptic neurons) is impaired in *ephrin-B3*^{lacZ} mice but not ephrinB3 knockout mice, suggesting that ephrin-B3 reverse signaling is required for mossy fiber LTP, and different ephrin-Bs play redundant roles at the presynaptic nerve terminal (Armstrong *et al.*, 2006). Therefore, ephrin-B-mediated reverse signaling regulates synaptic strength by acting on the pre- or postsynaptic neurons in a cell-type-specific manner.

GLIA-MEDIATED RTK SIGNALING PLAYS ACTIVE ROLES IN SYNAPSE DEVELOPMENT

The communication between presynaptic and postsynaptic components during synapse development has been extensively studied. Nonetheless, emerging evidence suggests that neuron–glia communication also plays indispensable roles in synapse development and plasticity (Stevens, 2008). Various soluble factors secreted by glial cells were found to promote synaptogenesis and regulate synaptic strength. For example, thrombospondins (TSP) secreted from immature

astrocytes induce the formation of structurally normal synapses and glia-derived cholesterol enhances presynaptic activity (Mauch *et al.*, 2001; Christopherson *et al.*, 2005). Tumor necrosis factor α produced by glia is capable of increasing the surface expression of AMPA receptors postsynaptically, potentiating synaptic strength (Beattie *et al.*, 2002). On the other hand, the local contact between neurons and astrocytes triggers integrin signaling and subsequent protein kinase C activation, promoting global synaptogenesis (Hama *et al.*, 2004).

ErbB receptors and various isoforms of NRGs can be detected in glial cells. Activation of NRG/ErbB signaling has been well characterized to have profound effects on glial cell development including survival, differentiation and myelination of oligodendrocytes (Adlkofer and Lai, 2000; Buonanno and Fischbach, 2001; Falls, 2003). It is possible that NRG/ErbB signaling regulates the functions of glial cells, thereby modulating synaptic plasticity. However, direct evidence demonstrating a role of NRG/ErbB-mediated neuron–glia communication in synapse development is still lacking.

It is interesting to note that ephrin-A3, the ligand for EphA4, is expressed in astrocytes and activation of EphA4 in hippocampal neurons induces the retraction of dendritic spines, supporting the idea that contact-mediated signaling between neuron and astrocyte is critical for dendritic spine maintenance (Murai *et al.*, 2003; Thompson, 2003; Carmona *et al.*, 2009). While glia–neuron contact triggers integrin signaling and synaptogenesis, activation of postsynaptic EphA4 by astrocytic ephrin-A3 may counteract this effect by downregulating integrin activity (Hama *et al.*, 2004; Bourgin *et al.*, 2007). In addition, perturbation of neuron–astrocyte contact-mediated ephrin/Eph signaling significantly reduces the lifetime of new dendritic protrusions, but not the preexisting dendritic protrusions. This finding suggests that neuron–astrocyte ephrin/Eph signaling plays a critical role in spine development by stabilizing the newly formed dendritic protrusions (Nishida and Okabe, 2007). Besides ephrin ligands, multiple Eph receptors have been found to be expressed in astrocytes. Activation of astrocytic Eph receptors induces the outgrowth of astrocytic processes surrounding neurons. This effect is abrogated by the expression of a kinase-inactive form of EphA4 in astrocytes, indicating an essential role of Eph receptors in regulating astrocyte morphology and synaptic structure (Nestor *et al.*, 2007). Further evidence demonstrates that activation of astrocytic Eph receptors inhibits the glial release of glutamate, thus interfering the synchronized activation of neurons mediated by extrasynaptic NMDA receptors (Fellin *et al.*, 2004; Nestor *et al.*, 2007). Collectively, these studies emphasize the critical roles of glial cells in RTK-mediated synaptic functions.

SUMMARY AND PERSPECTIVES

Synapse development is central to the establishment of communications between neurons in the nervous system. Two families of RTKs, ErbBs and Ephs, are emerging as key regulators of synapse formation, maintenance and plasticity (Fig. 1).

ErbB and Eph receptors exhibit similar characteristics in term of their bi-directional signaling transduction. Both the receptors and ligands can be processed, generating intracellular CTFs. The intracellular fragments of NRG and ErbB are involved in a translocation pathway targeted to the nucleus, where they regulate gene transcription through binding with specific transcription factors (Vecchi and Carpenter, 1997; Rio *et al.*, 2000; Ni *et al.*, 2001; Lee *et al.*, 2002; Bao *et al.*, 2003, 2004; Sardi *et al.*, 2006). Although the EphA4-ICD and ephrin-B1-ICD are also found in the nucleus, the transcriptional activity of these fragments has not been identified. Nonetheless, EphA4-ICD mutant that cannot be localized to the nucleus exhibits its ability to activate Rac1 signaling and regulate spine formation (Georgakopoulos *et al.*, 2006; Tomita *et al.*, 2006; Litterst *et al.*, 2007; Inoue *et al.*, 2009). The precise functions of these fragments in the nucleus await further characterization.

ErbB and Eph receptors are localized at neuronal synapses. Both *in vitro* and *in vivo* loss-of-function studies demonstrate that ErbB and Eph receptor-mediated signaling is essential for the formation and maintenance of dendritic spines and synapses (Henkemeyer *et al.*, 2003; Murai *et al.*, 2003; Fu *et al.*, 2007; Kayser *et al.*, 2008; Barros *et al.*, 2009; Shi *et al.*, 2009). Regulated trans-synaptic interaction between ligands and receptors is essential for synaptic transmission and plasticity, as shown by defective LTP or LTD in *NRG1*, *ephrin-B*, *EphB2* and *EphA4* transgenic mice (Grunwald *et al.*, 2001, 2004; Henderson *et al.*, 2001; Bjarnadottir *et al.*, 2007). Both ErbB and EphB regulate the phosphorylation of NMDA receptor subunit NR2B and are required for stabilizing AMPA receptors at synapses (Takasu *et al.*, 2002; Henkemeyer *et al.*, 2003; Bjarnadottir *et al.*, 2007; Li *et al.*, 2007). On the contrary, activation of EphA receptors triggers the downregulation of AMPA receptor surface expression and synaptic transmission (Fu *et al.*, 2008). Thus, a balanced EphA and EphB signaling may be required to maintain an optimal number of dendritic spines and effective synaptic transmission.

Deregulation of signaling mediated by these receptors and their ligands may lead to diseases in the CNS. For example, dysfunction of NRG1/ErbB4 signaling has been proposed to be a factor contributing towards the underlying pathology of schizophrenia (Corfas *et al.*, 2004; Mei and Xiong, 2008). Understanding how NRG1/ErbB regulates synapse development and plasticity will therefore provide insights into the pathological basis that links NRG1/ErbB4 polymorphisms to increased risk of schizophrenia. Interestingly, several critical targets downstream of EphA4, including ephexin1, α2-chamearin and Cdk5, have also been implicated in neurological disorders (Shamah *et al.*, 2001; Cheung *et al.*, 2006; Beg *et al.*, 2007; Fu *et al.*, 2007; Iwasato *et al.*, 2007; Shi *et al.*, 2007; Wegmeyer *et al.*, 2007). Reduced expression of EphA4 and EphB2 in hippocampus has been observed in patients with incipient Alzheimer's disease (Simón *et al.*, 2009). In a mouse model of Alzheimer's disease, downregulation of Eph expression and the activity of their signaling targets precedes the occurrence of memory loss, suggesting that Eph receptors may be involved in the deregulation of synaptic function implicated in Alzheimer's disease (Simón *et al.*, 2009).

During the past years, there have been significant advances in our knowledge on the functions of ErbB and Eph receptors. However, a number of questions still await further investigation. For example, although ErbB and Eph signaling can be transduced bidirectionally (Marston *et al.*, 2003; Zimmer *et al.*, 2003), the circumstances during which the signals preferentially go to one direction remain unknown. Ephrins and Ephs are expressed in both neurons and glial cells, while ephrin/Eph signaling is not equally activated in both directions (Lauterbach and Klein, 2006). The signaling machinery in

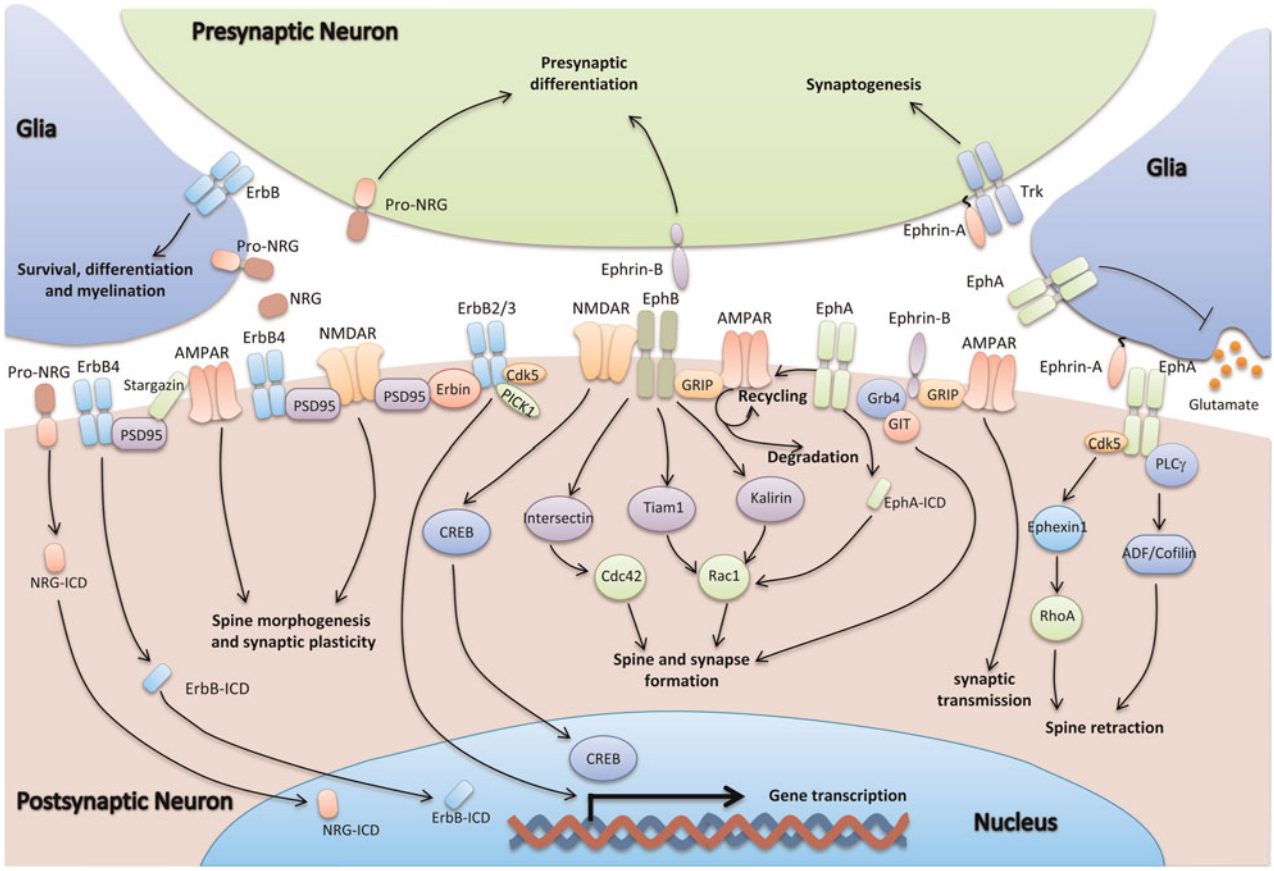

Fig. 1. ErbB and Eph receptors regulate synapse development. A tripartite synapse is composed of presynaptic axon, postsynaptic compartment and the ensheathing glial cells. ErbBs, Ephs and their ligands are widely expressed at synapses and regulate various synaptic functions. At the presynaptic sites, NRG and ephrin-mediated signaling plays a critical role in presynaptic differentiation. Postsynaptically, ErbB/NRG signaling modulates dendritic spine morphogenesis and synaptic transmission through regulating the clustering of cell surface ion channels, such as NMDA receptor (NMDAR) and AMPA receptor (AMPAR). ErbB-ICD and NRG-ICD can translocate into the nucleus to regulate gene transcription, which may contribute to long-term changes of synaptic plasticity. Eph receptors and their ephrin ligands are expressed at postsynaptic membranes, where they regulate AMPAR trafficking and NMDAR-dependent gene expression. In addition, ephrin/Eph signaling regulates dendritic spine formation or retraction through distinct Rho GTPases and actin-binding proteins, including Rac1, Cdc42, RhoA and ADF/cofilin. PDZ proteins (e.g. PSD-95 and GRIP1) and intracellular kinases (e.g. Cdk5) are important for transducing RTK downstream signaling. Furthermore, glial cells actively participate in synapse development by regulating spine morphology and neurotransmitter release through RTK signaling.

neurons, glial cells and extracellular space may help to constitute a specific cellular context that facilitates the trans-endocytosis of EphB2 from neurons to neighboring glial cells. However, the identity of key molecules that control direction of signal transduction of NRG/ErbB or ephrin/Eph is not well understood. Moreover, since the ligands are expressed in both neurons and glial cells, it will be important to understand whether neuron-derived or glia-derived ligands play distinct roles in the regulation of synapse development. Neuron–neuron synapses are ensheathed by glial cells, thus it can be envisaged that neuron-derived ligands regulate the signaling events directly at synapse, while the glia-derived ligands may elicit cellular response at the extrasynaptic regions that fine-tune synaptic functions. Genetic approaches that spatially and temporally manipulate the expression of NRG/ErbB or ephrin/Eph would provide important insights into this issue. Furthermore, since membrane-tethered NRG1 and ephrins can be processed into soluble forms or high molecular weight oligomers, it remains unclear whether these different forms of ligands play differential roles in regulating the downstream signaling events (Hattori *et al.*, 2000; Falls, 2003; Alford *et al.*, 2007). One possible advantage of these processed ligands is to broaden the range of their action so that regulation of receptor function would not be limited to the sites of cell–cell contact. Alternatively, the diffusion of ligands generate local gradients that may function as guidance cues for specific cellular events. Further characterization of the signaling networks of ErbB and Eph receptors would undoubtedly advance our understanding of the molecular mechanisms that underlie synapse development and facilitate our search for therapeutic targets of various diseases.

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