

EPHB RECEPTOR SIGNALING IN DENDRITIC SPINE DEVELOPMENT

Fumitoshi Irie and Yu Yamaguchi

The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, California 92037

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1. ABSTRACT

Dendritic spines are small bulbous protrusions on the surface of dendrites that serve as principle postsynaptic targets for excitatory synapses (1-3). Structural modifications of dendritic spines have been implicated as a cellular basis for learning and memory. Morphological abnormalities of spines are observed in some neurological diseases such as mental retardation and schizophrenia (4). Thus, studies on the morphological regulation of dendritic spines could have strong relevance to our understanding of the molecular mechanisms of the higher brain function and the pathophysiology of neurological disorders. Here we review recent progress on the role of the cell surface heparan sulfate proteoglycan syndecan-2 and ephrin-Eph signaling in dendritic spine development. Information from these new developments suggests a model in which cell surface ephrin-Eph signaling induces clustering of syndecan-2 and recruitment of cytoplasmic molecules, which leads to localized actin polymerization via Rho family GTPases, N-WASP, and the Arp2/3 complex.

2. DEVELOPMENT OF DENDRITIC SPINES

Dendritic spines are small membrane appendages on the surface of dendrites (1-3). In the case of the hippocampal CA1 region, a single pyramidal neuron contains ~30,000 dendritic spines. In the adult brain, spines typically have mushroom-like and stubby shapes. Morphology of dendritic spines exhibits remarkable changes during development and in the adult brain (Figure 1a). Various molecular and physiological factors have

been implicated in morphological changes of spines, including synaptic activity and plasticity (5, 6), actin filament reorganization (7, 8), calcium dynamics (9, 10), and protein phosphorylation (11). Most of the dendritic protrusions in early postnatal brain are long, filopodia-like ones. These filopodia-like protrusions are highly motile. As the brain undergoes postnatal maturation, the number as well as the length of these highly motile filopodia-like protrusions decreases. In the meantime, protrusions with mushroom-like or stubby shapes increase in number and frequency. In the mature brain, the majority of dendritic protrusions exhibit typical spine morphology (see e.g., reference 3 for review).

There are at least two proposed models concerning how mature spines are formed. In one model, highly motile dendritic filopodia make contacts with nearby axons, and these initial contacts evolve into mature synapses as the tip of the filopodia expands to become a spine head (12)(Figure 1b). In the other model, filopodia make contacts with axons, but synapses are formed not on the tip of filopodia but on the surface of the dendritic shaft in the vicinity of the filopodia that made the initial contact with the axon. In this model, a spine is not a transformed filopodium, but formed directly from the surface of dendritic shafts as a new membrane appendage independent of the filopodium that initiated the axonal contact (13). While there is still no consensus view of how dendritic spines develop, it is noteworthy that in both models contact formation between axons and dendrites is a key event for

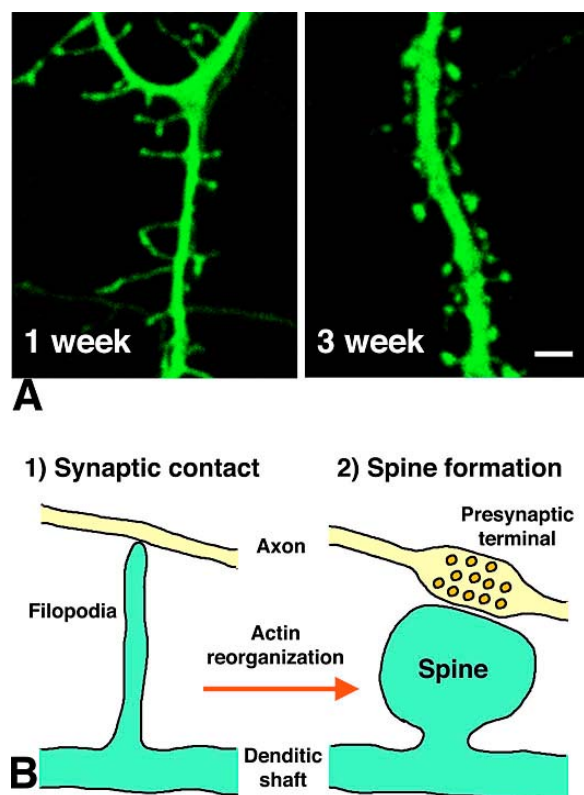


Figure 1. Development of dendritic spines. (a) The morphologies of dendritic protrusions are visualized by green fluorescent protein (GFP) in cultured hippocampal neurons at 1 week (left) and 3 weeks (right). Bar, 2 μ m. (b) A hypothetical model for dendritic spine development. Synaptic contacts between axons and dendritic filopodia are thought to trigger the morphological change of dendritic protrusion to spines. Differentiation of presynaptic boutons might also be triggered by these axo-dendritic contacts.

synaptogenesis and spine formation. It is thus logical to hypothesize that cell surface recognition events that occur between axons and dendrites act as a "trigger" for synaptogenesis and spine formation.

3. SYNDECAN-2 AND DENDRITIC SPINE DEVELOPMENT

3.1. Expression of syndecan-2 in dendritic spines

Heparan sulfate proteoglycans (HSPGs) are a group of proteins carrying heparan sulfate chains. They are expressed mainly on the cell surface and in the extracellular matrix and play critical roles in growth factor/cytokine signaling, the formation of morphogen gradients, cell adhesion, and the assembly of the extracellular matrix. The presence of heparan sulfate in CNS synapses was first suggested as early as the 1960's by electron microscopy combined with cationic dye staining and digestion with heparin/heparan sulfate lyases (14, 15). However, no significant progress was made until the late 1990's, when the presence of syndecan-2 in CNS synapses was demonstrated by two independent studies. By yeast two hybrid screening, Hsueh et al. (16) identified syndecan-2 as a binding partner for CASK, a PDZ domain-containing

protein present in synapses. This report also localized syndecan-2 in the postsynaptic site by immunoelectron microscopy using antibodies that recognize all 4 mammalian syndecan. On the other hand, Ethell and Yamaguchi (17) used an anti-heparan sulfate monoclonal antibody to demonstrate that heparan sulfate is concentrated in dendritic spines of cultured hippocampal neurons. The HSPG concentrated in spines was then identified as syndecan-2 using an anti-syndecan-2 antibody.

Syndecans are one of the two major families of cell surface HSPGs (18-20). The syndecan family has four members, syndecan-1, -2, -3 and -4, whose core proteins consist of a structurally diverse extracellular domain, and highly conserved transmembrane and cytoplasmic domains. Studies with non-neural cells have demonstrated that syndecans tend to be enriched at the sites of cell-cell and cell-matrix adhesion (21) and are colocalized with actin filaments (22). Phosphorylation and protein interactions through the cytoplasmic domain have been implicated in the targeting of syndecans into these specific membrane sites.

Expression of heparan sulfate in dendritic spines is developmentally regulated. In cultured hippocampal neurons, the expression of heparan sulfate is weak and diffuse during the first 2 weeks in vitro, but by 3 weeks, strong heparan sulfate immunoreactivity is detectable as punctate signals distributed on the surface of dendrites (17). The punctate staining becomes more prominent at 4 weeks in vitro. The timing of heparan sulfate expression, which lags behind synaptogenesis, coincides with the widespread formation of dendritic spines. This observation is consistent with the time course of syndecan-2 expression in the postnatal cerebellar cortex (23).

3.2. Spine formation by syndecan-2

The time course of syndecan-2 expression in developing spines suggests that syndecan-2 plays a functional role in spine formation. In fact, in non-neuronal cells syndecans have been implicated in the formation of various membrane microdomains and protrusions (24-26). Consistent with this notion, forced expression of syndecan-2 induces spine formation in immature neurons. Interestingly, introduction of syndecan-2 does not affect the number of synapses (17), indicating that the effect of syndecan-2 is not on synaptogenesis, which precedes spine formation.

Many postsynaptic transmembrane proteins, such as neurotransmitter receptors, ion channels, and receptor tyrosine kinases, interact with PDZ domain-containing proteins (27). PDZ domains typically recognize the C-terminal tails of these transmembrane proteins. Syndecan-2 binds three PDZ domain proteins, namely syntenin (28), CASK (16, 29), and synectin (30), through the C-terminal EFYA motif. Another syndecan-2-binding protein, synbindin, binds to the C-terminal tail but does not contain a typical PDZ domain (31) (Figure 2). A syndecan-2 deletion mutant lacking the C-terminal EFYA motif does not have the spine-inducing activity in immature neurons (17), indicating that the interaction with PDZ domain proteins is essential for syndecan-2 to induce dendritic spines.

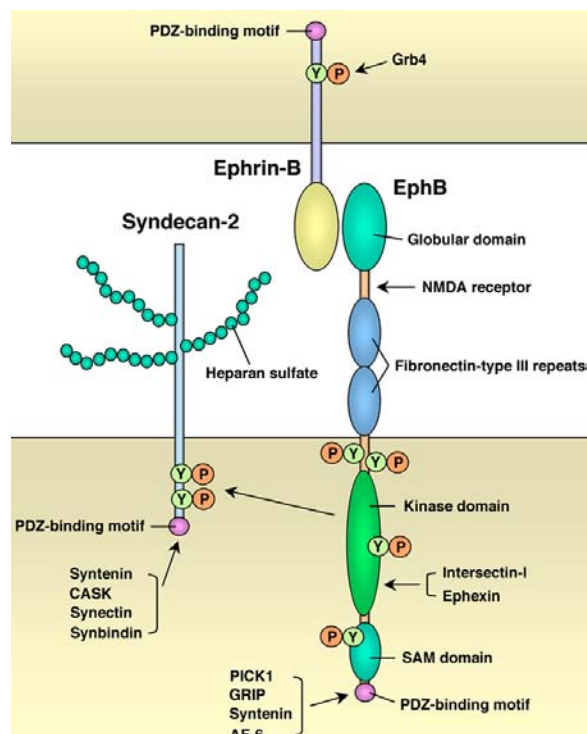


Figure 2. Structures syndecan-2, EphB receptor, and ephrin-B and their putative molecular interactions synapses. See text for details.

It remains to be determined, however, which PDZ domain protein(s) is physiologically involved in this process. There is ample evidence that CASK is concentrated in synapses, but is also present on nonsynaptic membranes and in intracellular compartments (16). Syndecan-2 is not the only postsynaptic protein that has been shown to bind the PDZ domain of CASK. Neurexins, NG2, glycophorin, junctional adhesion molecules, and parkin (32-35) bind CASK through the PDZ domain. Furthermore, CASK interacts with the T-box transcription factor, Tbr-1, in the nucleus. In COS cells, the presence of syndecan-3 can shift the balance of CASK distribution between nucleus and cytoplasm (36). Thus, although CASK is a strong candidate as the PDZ domain protein that mediate the spine-inducing activity of syndecan-2, there are many issues to be investigated to establish its role in dendritic spines. Syntenin binds many transmembrane molecules rather promiscuously. It is noteworthy that its binding partners include molecules known to be concentrated in synapses, including glutamate receptor subunits (GluR1-5, mGluR3, mGluR4a, mGluR6, mGluR7a and mGluR7b) (37, 38) and Eph receptors (39). However, definite immunohistochemical and immunocytochemical evidence for the localization of syntenin in the postsynaptic site is still missing. Little is known about neuronal expression of synectin at this point.

4. EPH RECEPTORS AND SPINE DEVELOPMENT

4.1. Eph receptor tyrosine kinases in the postsynaptic site

Eph receptors are transmembrane tyrosine

kinases involved in various developmental events, including CNS pattern formation, axon guidance, neural crest migration, and vasculogenesis/angiogenesis (40-45). There are two types of Eph receptors, EphA and EphB, which are activated by binding to different classes of ligands, A- and B-class ephrins, respectively. Eph receptors were originally identified as axonal guidance cues in the embryonic brain (43, 46). However, some Eph receptors are expressed in the adult brain (47). Among them, EphA4, EphA7, EphB2, and EphB3 are localized in dendritic spines of hippocampal neurons (48, 49). In addition, Eph receptors bind various PDZ domain proteins present in the postsynaptic site, such as PICK1, GRIP, syntenin, and AF-6 (39, 48) (Figure 2). These observations indicate that Eph receptors are involved not only in axon guidance but also synapse development. Indeed, ephrin-B-induced activation of EphB receptors induces a direct interaction of EphB and NMDA-type glutamate receptors, and perturbation of EphB kinase activity reduces the number of postsynaptic specializations in cultured cortical neurons (50). Furthermore, EphB receptors regulate NMDA receptor-mediated calcium influx and gene expression (51) and synaptic plasticity (52-54).

4.2. Phosphorylation of syndecan-2 by EphB receptors

Syndecans have been implicated as transmembrane mediators that transduce cell adhesion/contact signals into intracellular downstream pathways. Phosphorylation of the cytoplasmic domains of syndecans, both on serine and tyrosine residues, has been thought to play important roles in this process (55). In fact, syndecan-2 is tyrosine phosphorylated in cultured hippocampal neurons in postnatal mouse brain. Moreover, syndecan-2 coimmunoprecipitates with EphB2 from cultured neurons and synaptosomes from adult mouse brain, suggesting that EphB2 is the key tyrosine kinase that phosphorylates syndecan-2 in the postsynaptic site (56). Indeed, syndecan-2 is phosphorylated by EphB2 at tyrosine-189 (Y189) and tyrosine-201 (Y201) (Figure 2). A syndecan-2 point mutant in which both Y189 and Y201 are mutagenized lacks not only the ability of clustering but also the spine-inducing activity. Moreover, when endogenous EphB receptor kinase activity is blocked by a dominant negative form of EphB2, syndecan-2 cannot induce spine formation. Thus phosphorylation of Y189 and Y201 has functional significance with regard to spine development. On the other hand, serine phosphorylation of syndecan-2 does not seem to play an important role in spine formation. Syndecan-2 is phosphorylated at serine-197 and serine-198 by protein kinase C (57). However, a syndecan-2 point mutant at these serine residues shows normal clustering and the spine-inducing activity (56).

4.3. Eph receptors in dendritic spine development

As discussed above, it is logical to hypothesize that certain cell surface recognition events act as the initial trigger for synaptogenesis and spine formation. Ephrin-Eph receptor signaling, which operates as a contact-based signal transduction system, seems to fit well such a role. In fact, there is evidence supporting this notion. Perturbation of EphB activities by a kinase-inactive EphB2 inhibits endogenous spine development in cultured hippocampal

neurons (56). Furthermore, exogenous application of ephrin-B ligand into hippocampal cultures induces spine formation (58). These findings strongly suggest that ligand-dependent EphB activation is involved in dendritic spine development. However, further studies are needed to establish the physiological role of ephrin-Eph signaling in dendritic spine development and morphological plasticity in the adult brain *in vivo*. Thus far, EphB2 knockout mice have not provided evidence for such a role of EphB2 *in vivo*; these mice exhibit no overt abnormalities in the frequency and morphology of dendritic spines (52, 53). However, since multiple EphB receptors may be co-expressed in the same neurons, several of them may need to be simultaneously inactivated before morphological defects are apparent. Interestingly, EphB2 knockout mice show changes in synaptic plasticity, but these changes are not dependent on the kinase domain of EphB2. This is contrast to the effect of EphB2 on spine formation, which requires kinase activity (56, 58). Thus it appears that EphB receptors modulate synaptic functions at two levels, one of which is dependent on kinase activity and the other is not.

5. SIGNALING PATHWAYS DOWNSTREAM OF EPHB RECEPTORS

5.1. Rho family GTPases and actin polymerization in dendritic spines

The cytoskeleton of dendritic spines consists predominantly of actin filaments (59, 60). Depolymerization reagents block spine development in hippocampal neurons (61), indicating that actin polymerization is essential for spine morphogenesis. Small GTPases of the Rho family, including Rho, Rac, and Cdc42, are known to be involved in actin assembly in both neuronal and non-neuronal cells (62). In transgenic mice expressing constitutive active Rac in Purkinje cells, dendritic spines are much reduced in size but increased in number (63). In rat hippocampal slices, transfection of dominant negative Rac causes a progressive elimination of spines, whereas hyperactivation of RhoA causes drastic simplification of dendritic branch patterns (64). There is also evidence that Cdc42 is involved in spine development. Dominant negative Cdc42 inhibits endogenous spine development in hippocampal neurons in culture (65). More recently, it has been reported that loss-of-function Cdc42 mutation in *Drosophila* vertical system neurons causes a reduction in dendritic spine density (66).

5.2. Regulation of Rho GTPases by Eph receptors

The activities of Rho GTPases are regulated by several factors. Among them, guanine nucleotide exchange factors (GEFs) are the direct activators of GTPases which catalyze the exchange of GDP for GTP (67). Recent work has revealed that GEFs are regulated by Eph receptors. Ephexin, a novel GEF for Rho GTPase, has been cloned as a binding partner of EphA receptors (68) (Figure 2). While ephexin has guanine nucleotide exchange activity toward all three members of the Rho family GTPases, ephrin-mediated activation of EphA receptors selectively enhances RhoA activation, while Rac and Cdc42 activation is inhibited. Perturbation of ephexin in primary neurons inhibits ephrin-A-induced growth cone collapse, which is

mediated by RhoA (68). The specificity toward RhoA, which has not been implicated in spine formation, suggests that EphA receptor-ephexin signaling is not relevant to spine formation. Yet, this finding that EphA receptor modulate a Rho GEF through physical association suggested the possibility that EphB receptors may also regulate GEFs in a similar mechanism.

Interestingly, ephrin-B2 stimulation of hippocampal neurons in culture causes activation of Cdc42 temporally coinciding with EphB2 autoactivation (65), suggesting that some Cdc42 GEFs may be activated by EphB2. Intersectin-1, a Cdc42 GEF that is specifically expressed in neurons (69, 70), is present in dendritic spines and interacts with EphB receptors (65). EphB2 binds the N-terminal region of intersectin-1, which contains two Eps15-homology domains and five SH3 domains (71-75) (Figure 2). This interaction is independent of the kinase activity of EphB2. EphB2, but not EphA4, interacts with intersectin-1. As in the case of several other GEFs, full-length intersectin-1 shows only a negligible level of GEF activity, whereas isolated GEF domain has high GEF activity (70), suggesting the presence of regulatory mechanisms for the expression of GEF activity. Interestingly, EphB unmasks the GEF activity of full-length intersectin-1 (65). This suggests that ephrin-EphB signaling activates Cdc42 through the activation of intersectin-1. Indeed, dominant negative perturbation of intersectin-1 inhibits endogenous spine development in hippocampal neurons (65).

Another GEF that may be regulated by EphB receptors is kalirin. Kalirin is a GEF enriched in dendritic spines and has an activity to regulate spine morphology upon transfection into neurons (76, 77). Activation of EphB receptors by ephrin-B1 induces translocation of kalirin to synapses and activation of Rac1 and its effector PAK (58). Overexpression of catalytically inactive kalirin, dominant negative Rac1, or inhibition of PAK eliminates ephrin-induced spine formation. An interesting difference between the effects of EphB2 on intersectin-1 and kalirin is that unlike intersectin-1, kalirin is tyrosine phosphorylated by EphB. It is not known whether kalirin physically associates with EphB2.

5.3. Role of EphB2-intersectin signaling in N-WASP/Arp2/3-mediated actin polymerization during dendritic spine development

Recent progress has uncovered the mechanism of actin polymerization in various membrane protrusion and microdomains. One of the most important molecules involved in the regulation of actin polymerization is the Wiskott-Aldrich syndrome protein (WASP) (78). WASP and its related molecule, neuronal WASP (N-WASP), promote actin nucleation and branching through interaction with Cdc42 and the Arp2/3 complex (79-82). Upon activation by the WASP family molecules, the Arp2/3 complex initiates the formation of branches from preexisting actin filaments (83, 84).

A potential functional linkage between the EphB-intersectin-1 signaling and the N-WASP-Arp2/3-mediated

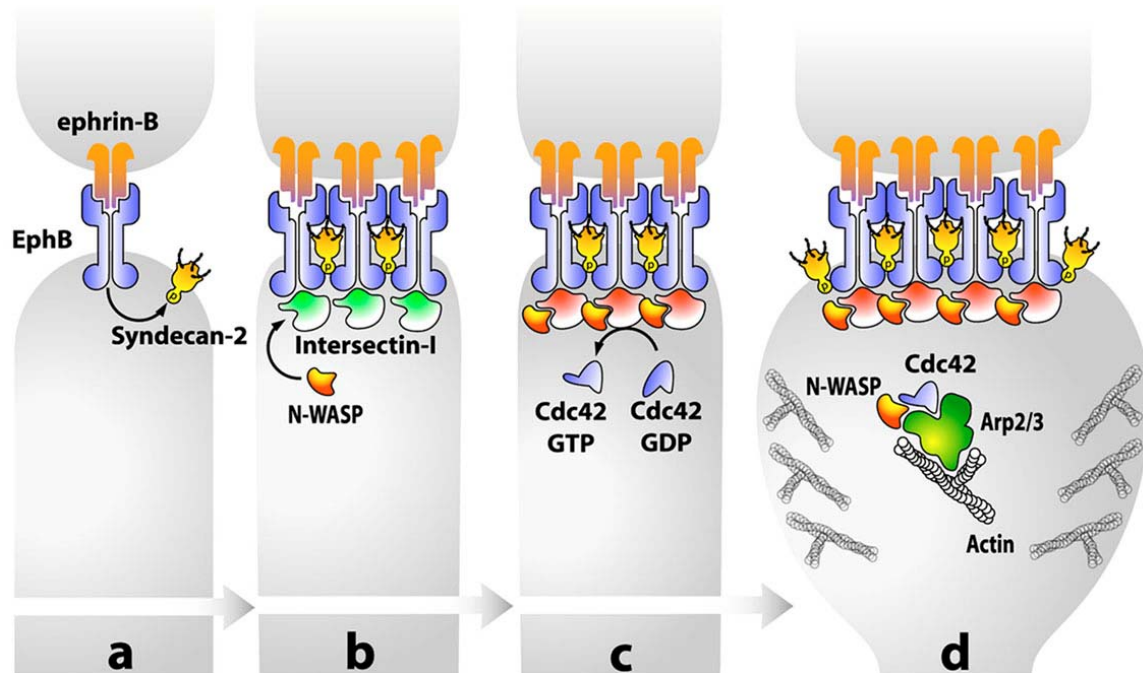


Figure 3. Schematic model for EphB-mediated spine formation. See text for explanation.

actin polymerization during dendritic spine development has recently been uncovered. Hussain et al. (70) have previously demonstrated that N-WASP binds intersectin-1 and upregulates its GEF activity. It has recently been found that the association of both EphB2 and N-WASP confers even higher upregulation of the GEF activity of intersectin-1 than does the association of only N-WASP (65). Thus EphB2 and N-WASP exert a synergistic effect on the GEF activity of intersectin-1. Such an effect should promote the local activation of Cdc42 at the sites of ephrin-Eph signaling, leading to branching polymerization of actin filaments mediated by N-WASP and the Arp2/3 complex at these sites.

A panel of dominant negative mutants has confirmed functional significance of this signaling pathway in dendritic spine development. Overexpression of intersectin-1 mutants (the isolated SH3 domains and the mutant lacking GEF catalytic domain) inhibits spine formation in hippocampal neurons (65). Furthermore, overexpression of the cofilin homology and acidic (CA) domain of N-WASP inhibits spine formation. This domain of N-WASP is the binding site for Arp2/3 (85), and acts as a dominant negative inhibitor that blocks the N-WASP-Arp2/3 interaction as well as Cdc42-dependent actin polymerization (70, 79, 86). This is direct evidence for the role of N-WASP-Arp2/3-mediated actin polymerization in spine formation. Taken together, these results suggest that in developing dendritic spines, cell surface ephrin-B/EphB signaling and the N-WASP-Arp2/3-mediated actin polymerization are functionally integrated by the physical association of EphB2 and intersectin-1. The EphB2-intersectin-1 association and the resulting activation of intersectin-1 could provide an effective mechanism for achieving localized actin filament assembly in the presumptive spines (65).

6. A MODEL FOR EPHB SIGNALING IN DENDRITIC SPINE DEVELOPMENT

Figure 3 depicts a schematic model for EphB-mediated spine formation based on the findings described above (56, 65). Upon the initial cell-cell contact between axons and dendrites, EphB receptors (presumably in dendritic filopodia) are activated by B-ephrins (presumably in axons). This leads to phosphorylation of target molecules, including syndecan-2, in the presumptive postsynaptic site (a). Phosphorylated syndecan-2 acquires the ability to cluster, which in turn facilitates co-clustering of EphB2 (56). Clusters of EphB2 recruit molecules that bind to the cytoplasmic domain of EphB2. Intersectin-1 is one of such molecules recruited to EphB2/syndecan-2 co-clusters (b). In cooperation with N-WASP and EphB2, the GEF activity of intersectin-1 is turned on, which then stimulates local activation of Cdc42 in the vicinity of ephrin-EphB signaling (c). Activation of Cdc42 then stimulates actin polymerization via N-WASP and Arp2/3 complex to initiate spine formation (d).

Admittedly, this is a highly simplified model. Presumably, spine formation involves other molecules known to regulate actin polymerization, such as Ena/VASP proteins (87) and FERM proteins (88). Also the figure does not incorporate the recent finding on EphB2-kalirin-Rac signaling in spine formation (58). In non-neural cells, Rac mediates lamellipodia formation, whereas Cdc42 induces filopodia formation (89). However, the situation appears more complicated in spine formation. Perturbation of either Cdc42 (65, 66) or Rac (58, 64) results in the loss of spines. With a stalk and a head, formation of mature spines may require the participation of multiple Rho family pathways. It is likely that future studies reveal crosstalks among these pathways. It is also possible that these

pathways are differentially regulated between developmental spine formation and morphological spine plasticity in the adult brain.

7. PERSPECTIVES

In addition to the effects of EphB receptors, it has recently been demonstrated that interaction between ephrin-A3 on astrocytes and EphA4 on postsynaptic sites controls the retraction of dendritic spines in the adult hippocampus (49). Thus ephrin-A/EphA receptor signaling is also likely to play a physiological role in the regulation of dendritic spine morphology. Although the molecular mechanism of this phenomenon remains to be determined, it is possible that retraction of spine neck is also mediated through changes in actin polymerization.

Although not directly relevant to dendritic spine formation, it is interesting to explore the effect of ephrin-Eph reverse signaling on the maturation of the presynaptic terminal. Ephrin-Eph interactions induce not only Eph signaling but also tyrosine phosphorylation of ephrin-B in its short cytoplasmic domain. Phosphorylated ephrin-B induces actin reorganization through interaction with the SH2/SH3 adaptor protein Grb4 (90) (Figure 2). Although little is known about the expression of ephrin-B ligands in the presynaptic terminal, ephrin-Eph interactions at synaptic contacts may also have effects on the maturation of presynaptic terminals.

Finally, an issue with broad implications is the potential involvement of ephrin-Eph signaling in higher brain function and human mental disorders. Morphology of dendritic spine is closely associated with mental disorders (4). Abnormal morphologies of spines have been reported in mental retardation, autism, and other cognitive disorders (91-95). Mutations of molecules involved in Rho GTPases pathways, such as oligophrenin-1 (96), α Pix (97), and Pak3 (98) have been identified as the putative causative genes for nonsyndromic X-linked mental retardation. These observations suggest that regulation of the actin cytoskeleton via Rho GTPase signaling is involved in higher brain function and the development of mental disorders (99). It is thus tempting to speculate that ephrin-Eph receptor signaling may also be involved in these processes. Future studies toward this direction may reveal the molecular linkage between contact-based cell signaling mediated by ephrin-Eph signaling and human cognitive function.

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Send correspondence to: Yu Yamaguchi, M.D., Ph.D., Professor, Developmental Neurobiology Program, The Burnham Institute, 10901 N. Torrey Pines Road, La Jolla, CA 92037, Tel: 858-646-3124, Fax: 858-646-3197, E-mail: yyamaguchi@burnham.org, URL: www.burnham.org