CMLS, Cell. Mol. Life Sci. 62 (2005) 1363–1371
1420-682X/05/121363-09
DOI 10.1007/s00018-005-5018-2

CMLS Cellular and Molecular Life Sciences

Review

Plexins: axon guidance and signal transduction

M. Negishi*, I. Oinuma and H. Katoh

Laboratory of Molecular Neurobiology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8502 (Japan), Fax: +81 75 753 7688, e-mail: mnegishi@pharm.kyoto-u.ac.jp

Received 13 January 2005; received after revision 3 February 2005; accepted 15 February 2005
Available online 07 April 2005

Abstract. Axon guidance represents a key stage in the formation of neuronal network. Axons are guided by a variety of guidance factors, such as semaphorins, ephrins and netrin. Plexins function as receptors for the repulsive axonal guidance molecules semaphorins. Intracellular domains of plexins are responsible for initiating cellular signal transduction inducing axon repulsion. Recent advances have revealed molecular mechanisms for plexin-mediated cytoskeletal reorganization, leading to repulsive responses, and small GTPases play important roles in this signaling. Plexin-B1 activates Rho through Rho-specific guanine nucleotide exchange factors, leading to neurite retraction. Plexin-B1 possesses an intrinsic GTPase-activating protein activity for R-Ras and induces growth cone collapse through R-Ras inactivation. In this review we survey current understanding of the signaling mechanisms of plexins.

Key words. Plexin; semaphorin; Rho; R-Ras; axon guidance; neuron.

Introduction

Neurons form a complex network to function properly. Formation of this network includes many steps: neuronal migration to proper regions, neurite outgrowth, formation of polarity, guidance of axons and dendrites to proper targets, dendritic maturation and synapse formation with appropriate partners. Among them, axon guidance is one of the critical steps for the proper formation of a neuronal network. During development of the nervous system, axons are guided to their proper targets by sensing a variety of extracellular cues in the local environment. Neuronal growth cones, located at the tip of the growing axon, are highly motile structures that respond to guidance cues by selectively altering the stability of the actin cytoskeleton and microtubules.

Biochemical and genetic studies have revealed a variety of families of axon guidance molecules, including netrins, slits, semaphorins and ephrins [1]. These guidance molecules bind to their specific receptors expressed in the growth cones of neurons and steer axons by regulating cytoskeletal reorganization in the growth cones. Guidance molecules are expressed in various regions of the brain, and neurons expressing specific receptors recognize these molecules and correctly project their axons to target cells according to the guiding map of guidance molecules. Axon guidance molecules are categorized into two groups, attractive and repulsive cues, based on the direction of response: axons move toward the source of attractive cues and avoid the source of repulsive cues. Netrins are attractive guidance molecules, while slits, semaphorins and ephrins belong to repulsive cues. However, netrins and semaphorins are bifunctional and can act as axon attractants. Receptors for these guidance molecules have been also identified, and DCC and UNC-5, robos, plexins and neuropilins, and Ephs are receptors for netrins, slits, semaphorins and ephrins, respectively.

* Corresponding author.

Semaphorins and plexins

Semaphorins are a large family of secreted or membrane-bound proteins [2], which have been shown to regulate axonal pathfinding during the development of the nervous system [3, 4]. To date, more than 20 semaphorins have been identified, and they are now classified into eight subclasses on the basis of sequence similarity and distinctive features [2]. Classes 1 and 2 are invertebrate semaphorins, classes 3 through 7 are vertebrate semaphorins, and class V are viral-encoded semaphorins, found in the genome of non-neurotrophic DNA viruses. Among them, classes 1, 4, 5 and 6 are transmembrane molecules, and class 7 is a membrane-associated form through a glycosylphosphatidylinositol-anchor motif. On the other hand, classes 2, 3 and V are secreted proteins. A distinctive protein module of about 500 amino acids, called the sema domain, characterizes all semaphorins, and this domain is located in amino (N)-terminal regions. Sema domains are also found in the extracellular regions of plexins, semaphorin receptors. In contrast, the carboxyl (C) regions of semaphorins are more variable, and some of them have immunoglobulin-like loops. Classes 3 and 4 semaphorins are homodimerized through cysteine disulfide bonds, and dimerization appears to be critical for their physiological function [5]. In the case of Sema4D, membrane-bound Sema4D is proteolytically cleaved into a soluble form, although it remains unclear whether the transmembrane-type Sema4D requires conversion into a soluble form to exert its functions [6]. The function of semaphorins is mediated by plexins, and mammalian plexins are classified into four subfamilies: Plexin-A1–4, Plexin-B1–3, Plexin-C1 and Plexin-D1 [7]. Neuropilins, neuropilin-1 and neuropilin-2, are another type of receptor and were initially identified as direct binding receptors for class 3 semaphorins [8]. However, neuropilins have very short cytoplasmic tails, and they alone have no ability to transduce the signals of Sema3. Neuropilins form complexes with Plexin-As, and associated Plexin-As are signaling moieties. Classification of semaphorins and their receptors are listed in table 1.

Among class 3 semaphorins, Sema3A has been extensively studied. Sema3A shows repulsive effects on axons of dorsal root ganglion (DRG), sympathetic ganglion, spinal motoneurons, cerebral cortical neurons and hippocampal neurons [2]. Sema3A also inhibits migration of neural crest cells at early developmental stages [7]. Sema3A binds to neuropilin-1/Plexin-A complex and induces repulsive responses. On the other hand, Sema3F binds to neuropilin-2/Plexin-A, and has been shown to induce pruning of hippocampal mossy fibers [9]. In contrast to class 3 semaphorins, transmembrane-type Sema6D directly binds to Plexin-A1 and inhibits endocardial cell migration [10]. Interestingly, Sema6D provides reverse signaling, enhancing the migration of Sema6D-expressing myocardial cells into the trabeculae [11]. Among class 4 semaphorins, Sema4D/CD100 has been extensively investigated [12]. Sema4D is a transmembrane protein and utilizes Plexin-B1 and CD72 as receptors. Plexin-B1 is expressed in chick retinal ganglion neurons and hippocampal neurons, while Sema4D is expressed in oligodendrocytes throughout the central nervous system (CNS) white matter, with a peak during the myelination period [13]. Sema4D induces growth cone collapse in hippocampal neurons and neurite retraction in PC12 cells [14, 15]. Sema5A is also a transmembrane semaphorin, and Plexin-B3 is a specific and functional receptor for Sema5A [16]. Sema5A is expressed in oligodendrocytes and their precursors, and it induces growth cone collapse of retinal ganglion cells, probably through Plexin-B3 [17]. Sema7A was originally identified to bind to Plexin-C1 in vitro. In contrast to other semaphorins, Sema7A has been shown to promote out-

Table 1. Specificity of semaphorin receptor interactions.

Classes	Semaphorins	Forms	Receptors
1	Sema-1a, 1b	transmembrane	Plexin-A
2	Sema-2a	secreted	?
3	Sema3A, B, C, D, E, F	secreted	neuropilin/Plexin-A
	Sema3A	secreted	neuropilin-1/Plexin-A
	Sema3F	secreted	neuropilin-2/Plexin-A
	Sema3C	secreted	neuropilin /Plexin-D1
	Sema3E	secreted	Plexin-D1
4	Sema4A	transmembrare	Tim-2
	Sema4D	transmembrane	Plexin-B1, CD72
5	Sema5A	transmembrane	Plexin-B3
6	Sema6D	transmembrane	Plexin-A1
7	Sema7A	GPI-anchor	Plexin-C1, β1-integrin
V	Sema VA	secreted	Plexin-C1

Semaphorins exist as secreted, transmembrane or GPI-anchored form shown above. Expression of Tim-2 and CD72, receptors for class 4 semaphorins, is restricted in hematopoietic cells.

growth of central and peripheral axons through integrin activation independent of Plexin-C1 [18]. Plexin-D1 has been shown to be expressed in vascular endothelial cells and regulate proper blood vessel pathfinding probably through Sema3C binding to neuropilins/Plexin-D1 [19, 20]. Therefore, semaphorins exert a variety of biological functions through either direct binding to plexins or binding to neuropilin/plexins complexes.

Signaling pathways of Plexin-A

Guidance molecules, including semaphorins, direct growth cone migration by regulating cytoskeletal reorganization [1]. One of the key regulators of cytoskeletal dynamics in neurons is small GTPases of the Rho family [21, 22]. Like other small GTPases, the Rho family GTPases serve as molecular switches by cycling between an inactive GDP-bound state and an active GTP-bound state, and once activated, they can interact with their specific effectors, leading to a variety of biological functions. Activation of the Rho family GTPases requires GDP-GTP exchange catalyzed by various guanine nucleotide exchange factors (GEFs) in response to a variety of extracellular stimuli, and their activation is turned off by GTPase-activating proteins (GAPs), which stimulate the intrinsic GTPase activities of the G proteins. Extracellular stimuli regulate activities of Rho family GTPases through control of either GEFs or GAPs. Presently, at least 20 mamalian Rho family GTPases have been identified: Rho (A, B and C), Rac (1, 2 and 3), Cdc42, RhoD, RhoG, RhoH/TTF, TC10 and Rnd (1, 2 and 3). Among them, the functions of Rho, Rac and Cdc42 have been characterized extensively, and they are known to induce stress fibers, lamellipodia and filopodia in fibroblasts, respectively [23]. In neurons, Rac and Cdc42 stimulate extension of lamellipodia and filopodia at advancing growth cones, respectively [24], whereas Rho triggers growth cone collapse and neurite retraction [25]. In addition to these well-characterized Rho family GTPases, other members, including RhoG [26, 27], Rnd [28, 29] and TC10 [30], have been shown to play important roles in neuronal network formation.

Sema3A-induced growth cone collapse has been shown to require Rac1 activity in DRG neurons and spinal motoneurons [31, 32]. The active form of Rac1 directly binds to Plexin-A1, and its binding site in the cytoplasmic tail of Plexin-A1 is located in the region between C1 and C2, which are highly conserved among all plexin families [33]. Seam3A was shown to activate Rac1, but its activation mechanism is unclear. Requirement of Rac1 activity for the Sema3A-mediated repulsive response is a surprising result because Rac1 is usually associated with membrane protrusions as mentioned above. Concerning the role of Rac1 in Sema3A signaling, it was reported that Rac1 is required for Sema3A-mediated endocytosis of the growth cone plasma membranes and reorganization of F-acin in DRG neurons [34]. Endocytosis of plasma membranes is supposed to be an important step for growth cone collapse. Sema3A-induced Rac1 activation may drive endocytosis of the plasma membranes instead of membrane protrusions, resulting in growth cone collapse.

As shown in figure 1, tyrosine phosphorylation is involved in Sema3A signaling, and Fes/Fps tyrosine kinase has been implicated in Sema3A-induced collapse [35]. Fes/Fps is a non-receptor-type tyrosine kinase and directly binds to the cytoplasmic region of Plexin-A1. In the resting state, neuropilin associates with Plexin-A1 and blocks the binding of Fes/Fps to Plexin-A1. Sema3A binding to neuropilin permits Fes/Fps to associate with and phosphorylate Plexin-A1. This tyrosine phosphorylation stimulates repulsive action in the receptor. Collapsin response mediator protein 2 (CRMP2) was originally identified as an intracellular signaling molecule of Sema3A [36]. CRAM was identified as another member of CRMPs and was shown to associate with CRMP2, forming a CRMP2/CRAM complex [37]. Fes/Fps also tyrosine-phosphorylates CRMP2/CRAM complex as well as Plexin-A1. In addition to Fes/Fps, another kinase signaling is involved in Sema3A-mediated repulsive response. Fyn, a members of the Src family of tyrosine kinases, associates with and phosphorylates Plexin-A2 in response to Sema3A [38]. Furthermore, serine/threonine kinase Cdk5 associates with Plexin-A2 and is activated through Sema3A-mediated Fyn; activated Cdk5 phosphorylates CRMP2. Another Ser/Thr kinase, GSK3β, also phosphorylates CRMP2, and the phosphorylation of CRMP2 by both Cdk5 and GSK3β is essential for Sema3A-induced growth cone collapse [39, 40]. The activation of Fyn, Cdk5 and GSK3β is involved in Sema3A signaling. CRMP2 binds to tubulin heterodimers, promoting microtubule assembly [41]. It is plausible that phosphorylation of CRMP2 regulates interaction of CRMP2 with tubulin. Actually, phosphorylation of CRMP2 by Rho-kinase has been shown to reduce association of CRMP2 with tubulin [42]. Gdk5 and GSK3β may thus participate in Sema3A signaling through regulation of microtubule dynamics by phosphorylation of CRMP2.

An increasing number of molecules interacting with intracellular region of Plexin-A have been identified. Among them, MICAL is a unique molecule. MICAL is a flavoprotein oxidoreductase that binds to the C2 domain of Plexin-A in *Drosophila* [43]. MICAL is involved in Sema-1a-Plexin-A-mediated repulsive axon guidance in *Drosophila*, and its oxidoreductase activity is required for the action of MICAL. Recently, it was shown that Sema3A stimulates synthesis of 12(S)-hydroxyeicosatetraenoic acid (HETE); inhibition of this synthesis pre-

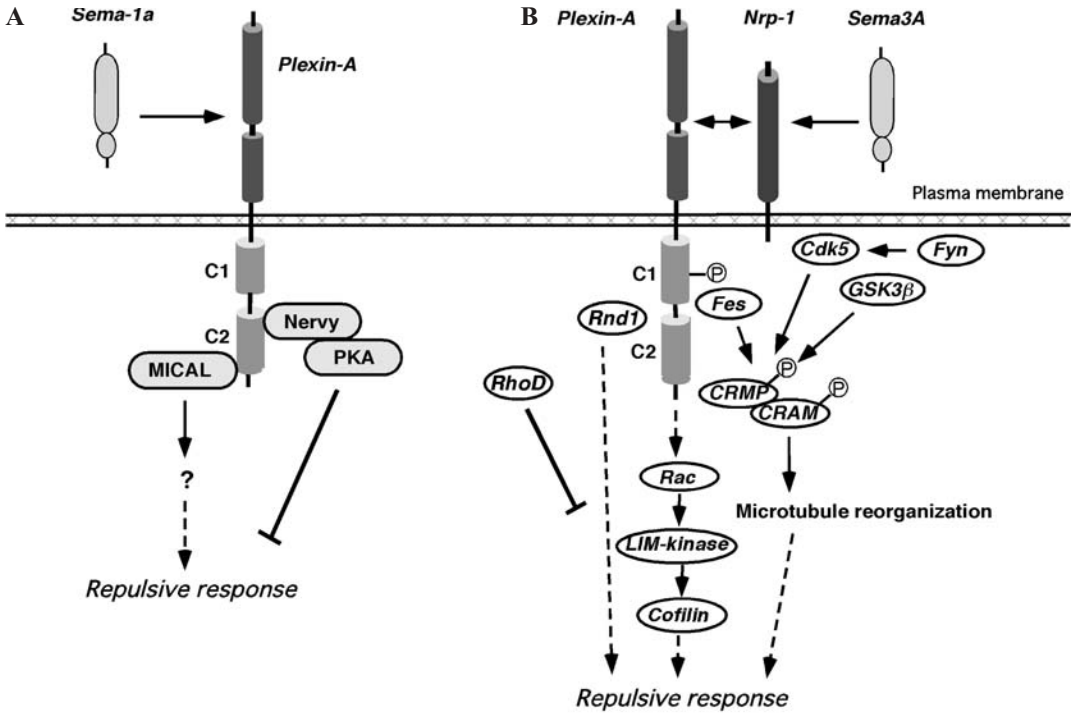

Figure 1. Models for Plexin-A signal transduction. (*A*) In *Drosophila*, Sema-1a directly binds to Plexin-A. A novel protein MICAL directly associates with the C2 domain of Plexin-A and is required for Plexin-A signaling. MICAL is a flavoprotein oxidoreductase, but its substrate is unknown. On the other hand, Nervy and PKA antagonize the repulsion. (*B*) In vertebrates, Sema3A binds to neuropilin-1, which associates with Plexin-A, and Plexin-A transduces repulsive signals in neurons. Several tyrosine and serine/threonine protein kinases, including Fes/Fps, Fyn, Cdk5 and GSK3β, are activated by Sema3A, and eventual phosphorylation of CRMP-2 is involved in Sema3A-induced growth cone collapse.

vents Sema3A-induced growth cone collapse, and 12(S)-HETE mimics Sema3A-induced collapse [44], suggesting the involvement of redox reactions in Sema3A signaling. Flavoprotein oxidoreductases catalyze the oxidation of a variety of substrates and generate reactive oxygen. Although the substrate of MICAL is not identified, redox regulation by MICAL may regulate the Sema3A-induced repulsive response.

Rho family GTPases in Plexin-B signaling

Rho family GTPases are also thought to be required for Plexin-B signaling. Three groups have shown that active Rac1 directly binds to Plexin-B1, the Sema4D receptor, in non-neuronal cells, while neither Rho nor Cdc42 bind to the receptor [45–47]. The binding site of active Rac1 is the Cdc42/Rac interactive binding (CRIB)-like domain located between C1 and C2 in the cytoplasmic tail of Plexin-B1 [46]. However, receptor activation does not show lamellipodia formation, a Rac activation phenotype, but instead induces stress fiber formation, a Rho activation phenotype, in fibroblasts, suggesting that Plexin-B1 action is mediated by Rho activation but not by Rac activation [47]. In *Drosophila*, Plexin-B was shown to suppress Rac activity by sequestering active Rac from its downstream effector p21-activated kinase (PAK), inducing repulsion [48]. Sequestration of active Rac by Plexin-B1 from PAK was also reported in mammalian cells [49]. Inhibition of Rac-mediated PAK activation by Plexin-B may suppress Rac-induced membrane protrusions, supporting the repulsive response. Furthermore, active Rac was shown to stimulate the localization of Plexin-B1 to the cell surface, enhancing Sema4D binding to the receptor in COS-7 cells [49]. Thus, signaling Rac and Plexin-B1 appears to be bidirectional; Rac modulates Plexin-B1 activity, and Plexin-B1 modulates Rac function.

As mentioned above, Plexin-B1 signaling has been suggested to involve Rho activation. Among Plexin families, the Plexin-B subfamily has a PSD-95/Dlg/ZO-1 (PDZ) domain-binding motif at the C terminus. Recent studies indicate that Plexin-B1 directly interacts with two Rho GEF-containing PDZ domains, PDZ-RhoGEF and leukemia-associated Rho GEF (LARG), through the C-terminal PDZ binding motif [14, 15, 50–52]. They have PDZ domains at the N-terminal region and are members of the RGS-RhoGEF family, which has G12-family binding domains and specifically activates Rho through G12-family binding [53]. Activation of Plexin-B1 regulates the activity of PDZ-RhoGEF/LARG, leading to activation of Rho, and this Rho activation is involved in Plexin-B1-induced growth cone collapse and neurite retraction (fig. 2).

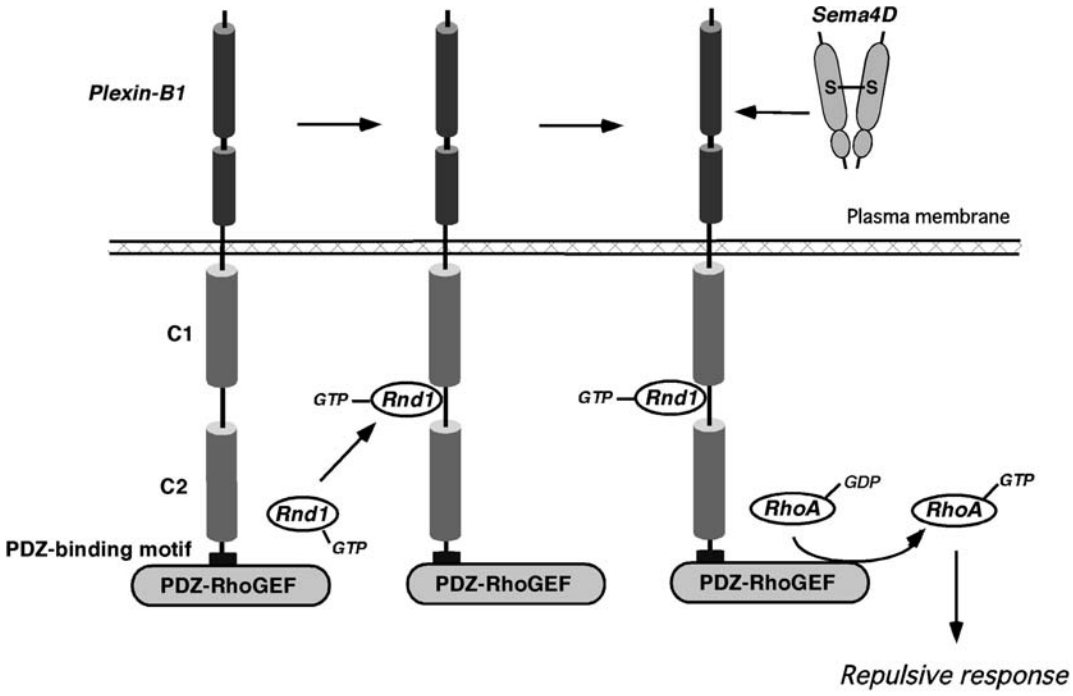

Figure 2. Model for signal transduction of Rho activation by Plexin-B1. The C-terminal tail of Plexin-B1 has a PDZ binding motif, and stably associates with Rho-specific GEFs, PDZ-RhoGEF and LARG through this motif. Rnd1 binding to the cytoplasmic tail of Plexin-B1 may improve the association of PDZ-RhoGEF with Plexin-B1, and Sema4D stimulates RhoA activation through PDZ-RhoGEF, inducing a repulsive response.

Furthermore, it was shown that Sema4D/Plexin-B1-induced growth cone collapse is inhibited by an inhibitor of Rho-kinase, a well-studied Rho effector [14]. Rho-kinase is known to phosphorylate the myosin-binding subunit of myosin phosphatase and myosin light chain, causing contraction of actomyosin [54]. Rho-kinase is located in the growth cones of neurites, which are rich in F-actin and myosin. When growth cones extend forward, actin polymerization is stimulated toward their leading edges, and the pulling action of myosin induces retrograde flow of actin filaments toward the cell interior. The activation of myosin by Rho-kinase and resultant contraction of actomyosin trigger promotion of retrograde flow of actin filaments, causing neurite retraction or growth cone collapse. In addition to Rac1, another Rho family GTPase, Rnd1, has been shown to bind to Plexin-B1 [55]. Rnd1 is a member of a new branch of Rho family GTPases and mainly expressed in brain [56]. Unlike other Rho family GTPases, Rnd1 possesses very low intrinsic GTPase activity and constitutively binds to GTP, indicating that it is constitutively active. Rnd1 is strongly expressed in neurons, including cortical and hippocampal pyramidal neurons, during the early postnatal period. Rnd1 binds directly to the CRIB-like domain of the cytoplasmic tail of Plexin-B1. The Rnd1-Plexin-B1 complex is stable and constitutive regardless of ligand interaction. Rnd1 binding to Plexin-B1 promotes association of PDZ-RhoGEF with Plexin-B1 and potentiates RhoA activation, inducing cell contraction in COS-7 cells. In addition, Rnd1 was shown to promote translocation of Plexin-B1 to the cell surface [57]. Therefore, Rnd1 is a very important regulator in Plexin-B1 signaling.

R-Ras GAP activity of Plexin-B1

Plexin-B1 activates Rho through association of PDZ-RhoGEF with the C-terminal PDZ-binding motif of Plexin-B1 and induces contraction. However, among the entire plexin family, the C-terminal PDZ-binding motif is found only in the Plexin-B subfamily, and it is not found in invertebrate Plexin-B. Thus it is unlikely that PDZ-RhoGEF-mediated Rho activation is a common signaling pathway for the plexin family. Meanwhile, the cytoplasmic tails of plexins show weak sequence similarity to GAPs for small GTPases [58]. This homology has suggested that plexins act as GAPs for small GTPases, inactivating G proteins. However, the GAP activities of plexins have not been demonstrated, and the significance of this similarity has been unclear.

The increasing number of GAPs for small GTPases, including Ras and Rho families, has been identified, and structural features of a variety of GAPs have been extensively studied [59]. Catalytic domains of GAPs for small

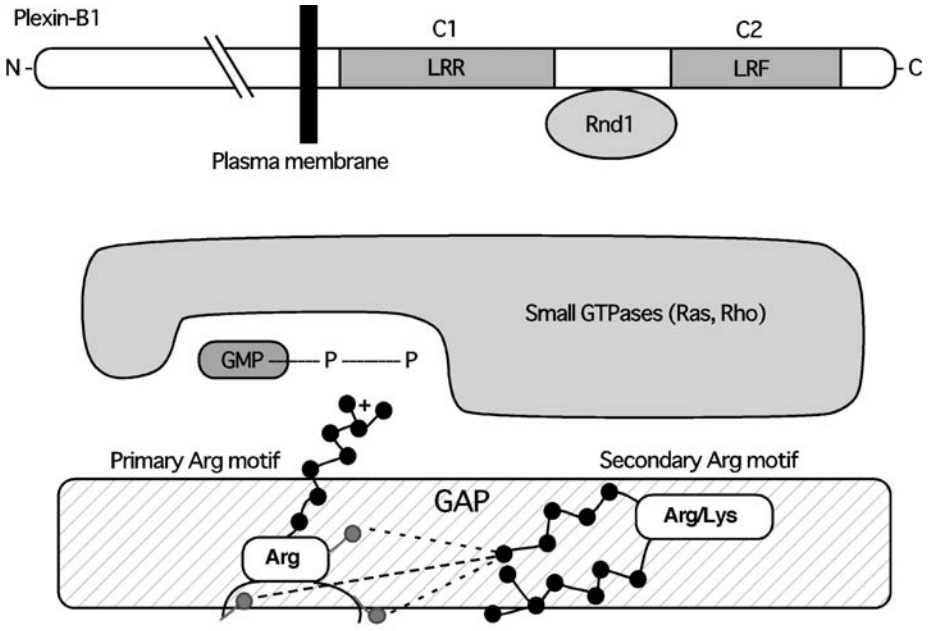

Figure 3. Structure of the complex of catalytic domains of GAPs and the Ras and Rho family of small GTPases. The primary Arg motif is stabilized by the secondary Arg/Lys motif, and the Arg residue of the primary motif interacts with the phosphate group of GTP bound to small GTPases.

GTPases contain two highly conserved motifs consisting of an Arg residue, primary Arg motif and secondary Arg/Lys motif (fig. 3). Arg (Ras GAP) or Lys (Rho GAP) in the secondary motif stabilizes Arg in the finger loop of the primary Arg motif, and this bridge of Arg motifs contacts GTP bound to small GTPases. When these invariant Arg residues are mutated to Ala, this mutation results in loss of GAP activity. Plexins have two subdomains, C1 and C2, showing sequence homology to GAPs for small GTPases within the cytoplasmic tail, and C1 and C2 domains contain primary and secondary Arg motifs, respectively [45]. On the other hand, the Rnd1 binding site in the cytoplasmic tail of Plexin-B1 is located in the linker region between C1 and C2, as mentioned above. Thus, the Rnd1 binding site splits two Arg motifs of the GAP domain of Plexin-B1. Recently, it was shown that Rnd1-associated Plexin-B1 binds to GTP-bound R-Ras, and this binding requires Rnd1 [60]. This interaction is specific for R-Ras, and the Rnd1-associated Plexin-B1 binds neither to H-Ras nor to the Rho family. Plexin-B1 not only binds to GTP-bound R-Ras but also stimulates its intrinsic GTPase activity in vitro and in vivo in response to Sema4D, and this GAP activity for R-Ras requires Rnd1 association with Plexin-B1. Therefore, the C1 and C2 domains of the C-terminal tail in Plexin-B1 encode GAP for R-Ras. Expression of a constitutively active R-Ras, R-Ras-QL, or knockdown of endogenous Rnd1 by Rnd1-specific small interfering RNA (siRNA) suppresses Sema4D-induced growth cone collapse in hippocampal neurons, while knockdown of endogenous R-Ras by R-Ras-specific siRNA causes Sema4D-independent growth cone collapse. Thus, downregulation of R-Ras is essential for Sema4D-induced collapse response, and Plexin-B1 displays R-Ras GAP activity, leading to a repulsive response.

R-Ras GAP domain is conserved throughout the Plexin family, and thus it is speculated that R-Ras GAP activity plays an important role in other Plexin signaling. In addition to Plexin-B1, Rnd1 binds to Plexin-A1 and is required for Sema3A-Plexin-A1-mediated repulsion [45, 61]. Expression of R-Ras-QL also suppresses Sema3A-induced growth cone collapse in hippocampal neurons, indicating that downregulation of R-Ras activity is required for the Sema3A-Plexin-A-mediated repulsive response [60]. R-Ras has been shown to play a key role in cell adhesion and its activation is known to promote cell adhesion, and neurite outgrowth through integrin activation [62–64]. Sema3A and Sema4D have been reported to inhibit integrin-mediated cell adhesion and migration [65–67]. Therefore, downregulation of R-Ras activity by plexins may suppress integrin activation and therby reduce cell adhesiveness, leading to growth cone collapse and neurite retraction. Considering that R-Ras GAP domains are well conserved among different plexin subfamilies, direct regulation of R-Ras activity by plexins is likely to be a major signaling pathway for cellular functions mediated by semaphorins.

Molecular mechanisms for stimulation of Plexin-B1 R-Ras GAP activity have been studied [68]. Both Rnd1 binding to Plexin-B1 and Sema4D stimulation are indis-

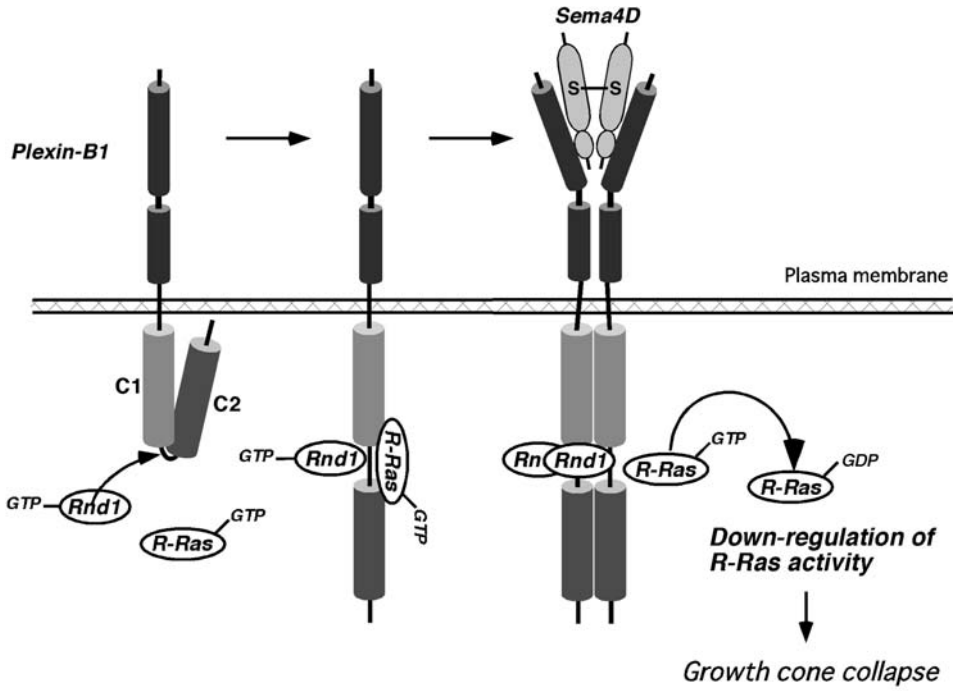

Figure 4. Model for signal transduction of the Sema4D-Plexin-B1/Rnd1 complex through R-Ras GAP activity. The C1 and C2 domains of the cytoplasmic tail of Plexin-B1 encode R-Ras GAP. The C1 and C2 domains interact with each other, and Rnd1 binding to the region between C1 and C2 domains disrupts this interaction, allowing the receptor to associate with GTP-bound R-Ras. Sema4D-induced clustering of the Plexin-B1/Rnd1 complex promotes the hydrolysis of GTP by R-Ras. Downregulation of R-Ras activity reduces cell adhesion, leading to growth cone collapse.

pensable for this activity (fig. 4). In the absence of Rnd1, C1 and C2 domains interact intramolecularly with each other, rendering the receptor inactive for R-Ras GAP activity. Rnd1 binding to the region between C1 and C2 domains disrupts the interaction of C1 and C2 domains, indicating that Rnd1 relieves the closed conformation of the cytoplasmic tail of Plexin-B1. This Rnd1-bound open conformation acquires an ability to associate with GTP-bound R-Ras. The Rnd1-Plexin-B1 complex can hold the GTP-bound R-Ras but not promote GTPase activity. Sema4D is homodimerized through cysteine disulfide bonds [12]. Sema4D ligand binding to Plexin-B1 induces clustering of the Rnd1-bound monomeric receptor, and this clustering triggers the hydrolysis of GTP on R-Ras. Therefore, GAP activation of Plexin-B1 consists of two steps, interaction with GTP-bound R-Ras and promotion of GTP hydrolysis by R-Ras; the former is stimulated by Rnd1, and the latter is a process induced by the clustering by Sema4D. A variety of GAPs for Ras family GTPases have been identified so far, and their GAP domains usually show high basal activity [69]. Regulation of GAP activity is mediated by interaction with other molecules or receptors via various domains of GAPs, such as SH2 and SH3. Considering the lack of basal R-Ras GAP activity of Plexin-B1, regulation of R-Ras activity of Plexin-B1 by Rnd1 and clustering is a novel mechanism. In addition to Plexin-B1, interaction of C1 and C2 domains has also been reported in Plexin-A1 [33]. Sema3A induces the aggregation of Plexin-A on dorsal root ganglion growth cones [65]. Considering Rnd1 binding to Plexin-A1 and the requirement of downregulation of R-Ras activity for Sema3A action, Plexin-A may exert its function via an R-Ras GAP function regulated by Rnd1 and receptor clustering similar to that of Plexin-B1.

Concluding remarks

Here we have summarized recent advances in our understanding of the molecular mechanism for axon guidance mediated by the plexin family. Identifying various molecules involved in plexin signal transduction pathways provided a good understanding of the molecular mechanisms for plexin family-mediated axon guidance and neuronal network formation. Most semaphorins display repulsive effects on axons through the plexin family. However, semaphorin-mediated repulsive responses can be converted to attractive responses by cyclic nucleotides, and cyclic nucleotide-dependent conversions in responses are observed in a variety of guidance cues [70]. Recently, Nervy, an A-kinase anchoring protein, was shown to couple Plexin-A to protein kinase A (PKA),

modulating Sema-1a repulsion in *Drosophila* [71]. In contrast to its repulsive effect on axons, Sema3A acts as a chemoattractant for cortical apical dendrites, and this effect requires guanylate cyclase [72]. Modulation by cyclic nucleotides may provide a mechanism for integrating diverse signaling inputs into the complex wiring of the nervous system. In addition to axon and dendrite guidance, the plexin family controls cell adhesion and migration in a variety of cells, participating in regulation of the immune response, angiogenesis and cancer [73]. Thus plexins are important receptors in a wide range of cellular functions. Future research will undoubtedly reveal the entire picture of signaling cascades of plexins for their diverse functions.

1 Dickson B. J. (2002) Molecular mechanisms of axon guidance. Science **298:** 1959–1964
2 Nakamura F., Kalb R. G. and Strittmatter S. M. (2000) Molecular basis of semaphorin-mediated axon guidance. J. Neurobiol. **44:** 219–229
3 Kolodkin A. L., Matthes D. J. and Goodman C. S. (1993) The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. Cell **75:** 1389–1399
4 Luo Y., Raible D. and Raper J. A. (1993) Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. **75:** 217–227
5 Koppel A. M. and Raper J. A. (1998) Collapsin-1 covalently dimerizes, and dimerization is necessary for collapsing activity. J. Biol. Chem. **273:** 15708–14713
6 Elhabazi A., Delaire S., Bensussan A., Boumsell L. and Bismuth G. (2001) Biological activity of soluble CD100. 1. The extracellular region of CD100 is released from the surface of T lymphocytes. J. Immunol. **166:** 4341–4347
7 Tamagnone L., Artigiani S., Chen H., He Z., Ming G.-L., Song H.-J. et al. (1999) Plexins are large family of receptors for transmembrane, secreted and GPI-anchored semaphorins in vertebrates. Cell **99:** 71–80
8 Fujisawa H. (2003) Discovery of semaphorin receptors, neuropilin and plexin, and their functions in neural development. J. Neurobiol. **59:** 24–33
9 Bagri A., Cheng H.-J., Yaron, A., Pleasure S. J. and Tassier-Lavigne M. (2003) Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphorin family. Cell **113:** 285–299
10 Toyofuku T., Zhang H., Kumanogoh A., Takegahara N., Suto F., Kamei J. et al. (2004) Dual roles of Sema6D in cardiac morphogenesis through region-specific association of its receptor, Plexin-A1, with off-track and vascular endothelial growth factor receptor type 2. Gene Dev. **18:** 435–447
11 Toyofuku T., Zhang H., Kumanogoh A., Takegahara N., Yabuki M., Harada K. et al. (2004) Guidance of myocardial patterning in cardiac development by Sema6D reverse signaling. Nat. Cell Biol. **6:** 1204–1211
12 Kumanogoh A. and Kikutani H. (2004) Biological functions and signaling of a tranmembrane semaphorin, CD100/Sema4D. Cell. Mol. Life Sci. **61:** 292–300
13 Moreau-Fauvarque C., Kumanogoh A., Camand E., Jaillard C., Barbin G., Boquet I. et al. (2003) The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. J. Neurosci. **23:** 9229–9239
14 Swierz J. M., Kuner R., Behrens J. and Offermanns S. (2002) Plexin-B1 directly interacts with PDZ-RhoGEF/LARG to regulate RhoA and growth cone morphology. Neuron **35:** 51–63
15 Perrot V., Vazquez-Prado J. and Gutkind J. S. (2002) Plexin B regulates Rho through the guanine nucleotide exchange factors leukemia-associated Rho GEF (LARG) and PDZ-RhoGEF. J. Biol. Chem. **277:** 43115–43120
16 Artigiani S., Conrotto P., Fazzari P., Gilestro G. F., Barberis D., Giordano S. et al. (2004) Plexin-B3 is a functional receptor for semaphorin 5A. EMBO Rep. **5:** 1–5
17 Goldberg J. L., Vargas M. E., Wang J. T., Mandemakers W., Oster S. F., Sretavan D. W. et al. (2004) An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. J. Neurosci. **24:** 4989–4999
18 Pasterkamp R. J., Peschon J. J., Spriggs M. K. and Kolodkin A. L. (2003) Semaphorin 7A promotes axon outgrowth integrins and MAPKs. Nature **424:** 398–405
19 Gitler A. D., Lu M. M. and Epstein J. A. (2004) PlexinD1 and semaphorin signaling are required in endothelial cells for cardiovascular development. Dev. Cell **7:** 107–116
20 Torres-Vazquez J., Gitler A. D., Fraser S. D., Berk J. D., Pham V. N., Fishman M. C. et al. (2004) Semaphorin-Plexin signaling guides patterning of the developing vasculature. Dev. Cell **7:** 117–123
21 Hall A. (1998) Rho GTPases and the actin cytoskeleton. Science **279:** 509–514
22 Negishi M. and Katoh H. (2002) Rho family GTPases as key regulators for neuronal network formation. J. Biochem. **132:** 157–166
23 Nobes C. D. and Hall A. (1995) Rho, rac and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia and filopodia. Cell **81:** 53–62
24 Kozuma R., Sarner S., Ahmed S. and Lim L. (1997) Rho family GTPases and neuronal growth cone remodeling: relationship between increased complexity induced Cdc42Hs, Rac1, and acetylcholine and collapse induced by RhoA and lysophosphatidic acid. Mol. Cell. Biol. **17:** 1201–1211
25 Katoh H., Aoki J., Ichikawa A. and Negishi M. (1998) p160 RhoA-binding kinase ROKα induces neurite retraction. J. Biol. Chem. **273:** 2489–2492
26 Katoh H., Yasui H., Yamaguchi Y., Aoki J., Fujita H., Mori K. et al. (2000) Small GTPase RhoG is a key regulator for neurite outgrowth in PC12 cells. Mol. Cell. Biol. **20:** 7378–7387
27 Katoh H. and Negishi M. (2003) RhoG activates Rac1 by direct interaction with the Dock180-binding protein Elmo. Nature **424:** 461–464
28 Ishikawa Y., Katoh H. and Negishi M. (2003) A role of Rnd1 GTPase in dendritic spine formation in hippocampal neurons. J. Neurosci. **23:** 11065–11072
29 Fujita H., Katoh H., Ishikawa Y., Mori K. and Negishi M. (2002) Rapostlin is a novel effector of Rnd2 GTPase inducing neurite branching. J. Biol. Chem. **277:** 45428–45434
30 Tanabe K., Tachibana T., Yamashita T., Che Y. H., Yoneda Y., Ochi T. et al. (2000) The small GTP-binding protein TC10 promotes nerve elongation in neuronal cells, and its expression is induced during nerve regeneration in rats. J. Neurosci. **20:** 4138–4144
31 Jin Z. and Strittmatter S. M. (1997) Rac1 mediates collapsing-1-induced growth cone collapse. J. Neurosci. **17:** 6256–6263
32 Kuhn T. B., Brown M. D., Wilcox C. L., Raper J. A. and Bamburg J. R. (1999) Myelin and collapin-1 induce motor neuron growth cone collapse through different pathways: inhibition of collapse by opposing mutants of rac1. J. Neurosci. **19:** 1965–1975
33 Turner L. J., Nicholls S. and Hall A. (2004) The activity of the Plexin-A1 receptor is regulated by Rac. J. Biol. Chem. **279:** 33199–33205
34 Jurney W. M., Gallo G., Letourneau P. C. and McLoon S. C. (2002) Rac1-mediated endocytosis during ephrin-A2- and semaphorin 3A-induced growth cone collapse. J. Neuosci. **22:** 6019–6028

35 Mitsui N., Inatomi R., Takahashi S., Goshima Y., Yamamura H. and Yanagi S. (2002) Involvement of Fes/Fps tyrosine kinase in semaphorin 3A signaling. EMBO J. **21:** 3274–3285

36 Goshima Y., Nakamura F., Strittmatter P. and Strittmatter S. M. (1995) Collapsin-induced growth cone collapse mediated by an intracellular protein related to UNC-33. Nature **276:** 509–514

37 Inatomi R., Tsujimura T., Hitomi T., Mitsui N., Hermann P., Kuroda S. et al. (2000) Identification of CRAM, a novel unc-33 gene family protein that associates with CRMP3 and protein-tyrosine kinase(s) in the developing rat brain. J. Biol. Chem. **275:** 27291–27302

38 Sasaki Y., Cheng C., Uchida Y., Nakajima O., Ohshima T., Yagi T. et al. (2002) Fyn and Cdk5 mediate semaphorin-3A signaling, which is involved in regulation of dendrite orientation in cerebral cortex. Neuron **35:** 907–920

39 Brown M., Jacobs T., Eickholt B., Ferrari G., Teo M., Monfries C. et al. (2004) α2-Chimaerin, cyclin-dependent kinase 5/p35 and its target collapsing response mediator protein-2 are essential components in semaphorin 3A-induced growth cone collapse. J. Neuorsci. **24:** 8994–9004

40 Eickholt B., Walsh S. and Doherty P. (2002) An inactive pool of GSK-3 at the leading edge of growth cones is implicated in semaphorin 3A signaling. J. Cell Biol. **157:** 211–217

41 Fukata Y., Itoh T. J., Kimura T., Menager C., Nishimura T., Shiromizu T. et al. (2002) CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. Nat. Cell Biol. **4:** 583–591

42 Arimura N., Inagaki N., Chihara K., Menager C., Nakamura N., Amano M. et al. (2000) Phosphorylation of collapsin response mediator protein-2 by Rho-kinase. J. Biol. Chem. **275:** 23973–23980

43 Terman J. R., Mao T., Pasterkamp R. J., Yu H.-H. and Kolodkin A. (2002) MICALs, a family of conserved flavoprotein oxidoreductases, function in Plexin-mediated axonal repulsion. Cell **109:** 887–900

44 Mikule K., Gatlin J. C., Houssaye B. A. and Pfenninger K. H. (2002) Growth cone collapse induced by semaphorin 3A requires 12/15-lipoxygenase. J. Neurosci. **22:** 4932–4941

45 Rohm B., Rahim B., Kleiber B., Hovatta I. and Püschel A. W. (2000) The semaphorin 3A receptor may directly regulate the activity of small GTPases. FEBS Lett. **486:** 68–72

46 Vikis H., Li W., He Z. and Guan K.-L. (2000) The semaphoring receptor plexin-B1 specifically interacts with active Rac in a ligand-dependent manner. Proc. Natl. Acad. Sci. USA **97:** 12457–12462

47 Driessens M. H. E., Hu H., Nobes C. D., Self A., Jordens I., Goodman C. S. et al. (2001) Plexin-B semaphorin receptors interact directly with active Rac and regulate the actin cytoskeleton by activating Rho. Curr. Biol. **11:** 339–344

48 Hu H., Marton T. F. and Goodman C. S. (2001) Plexin B mediates axon guidance in *Drosophila* by simultaneously inhibiting active Rac and enhancing RhoA signaling. Neuron **32:** 39–51

49 Vikis H. G., Li W. and Guan K.-L. (2002) The Plexin-B1/Rac interaction inhibits PAK activation and enhances Sema4D ligand binding. Gene Dev. **16:** 836–845

50 Aurandt J., Vikis H. G., Gutkind J. S., Ahn N. and Guan K.-L. (2002) The semaphoring receptor plexin-B1 signals through a direct interaction with the Rho-specific nucleotide exchange factor, LARG. Proc. Natl. Acad. Sci. USA **99:** 12085–12090

51 Driessesn M. H. E., Olivo C., Nagata K., Inagaki M. and Collard J. G. (2002) B plexins activate Rho through PDZ-RhoGEF. FEBS Lett. **529:** 168–172

52 Hirotani M., Ohoka Y., Yamamoto T., Nirasawa H., Furuyama T., Kogo M. et al. (2002) Interaction of plexin-B1 with PDZ domain-containing Rho guanine nucleotide exchange factors. Biochem. Biophys. Res. Commun. **297:** 32–37

53 Fukuhara S., Chikumi H. and Gutkind J. S. (2001) RGS-containing RhoGEFs: the missing link between transforming G proteins and Rho. Oncogene **20:** 1661–1668

54 Fukata Y., Amano M. and Kaibuchi K. (2001) Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. Trends Pharm. Sci. **22:** 32–39

55 Oinuma I., Katoh H., Harada A. and Negishi M. (2003) Direct interaction of Rnd1 with Plexin-B1 regulates PDZ-RhoGEF-mediated Rho activation by Plexin-B1 and induces cell contraction in COS-7 cells. J. Biol. Chem. **278:** 25671–25777

56 Nobes C. D., Lauritzen I., Mattei M. G., Paris S., Hall A. and Chardin P. (1998) A new member of the Rho family, Rnd1, promotes disassembly of actin filament structures and loss of cell adhesion. J. Cell Biol. **141:** 187–197

57 Swierz J. M., Kuner R. and Offermanns S. (2004) Plexin-B1/RhoGEF-mediated RhoA activation involves the receptor tyrosine kinase ErbB-2. J. Cell Biol. **165:** 869–880

58 Castellani V. and Rougon G. (2002) Control of semaphorin signaling. Curr. Opin. Neurobiol. **12:** 532–541

59 Scheffzek K., Ahmadian M. R. and Wittinghofer A. (1998) Trends Biochem. Sci. **23:** 257–262

60 Oinuma I., Ishikawa Y., Katoh H. and Negishi M. (2004) The semaphorin 4D receptor Plexin-B1 is a GTPase activating protein for R-Ras. Science **305:** 862–865

61 Zanata S. M., Hovatta I., Rohm B. and Püschel A. W. (2002) Antagonistic effects of Rnd1 and RhoD GTPases regulate receptor activity in semaphorin 3A-induced cytoskeletal collapse. J. Neurosci. **22:** 471–477

62 Zhang Z., Vuori K., Wang H. and Ruoslahti E. (1996) Integrin activation by R-Ras. Cell **85:** 61–69

63 Ivins J. K., Yurchenco P. D. and Lander A. D. (2000) Regulation of neurite outgrowth by integrin activation. J. Neurosci. **20:** 6551–6560

64 Kinbara K., Goldfinger L. E., Hansen M., Chou F. L. and Ginsberg M. H. (2003) Ras GTPases: integrins friends or foes? Nat. Cell Biol. **4:** 767–776

65 Takahashi T., Fournier A., Nakamura F., Wang L.-H., Murakami Y., Kalb R. G. et al., (1999) Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors. Cell **99:** 59– 69

66 Serini G., Valdembri D., Zanivan S., Morterra G., Burkhardt C., Caccavari F. et al. (2003) Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. Nature **424:** 391–397

67 Barberis D., Artigiani S., Casazza A., Corso S., Giordano S., Love C. A. et al. (2004) Plexin signaling hampers integrin-based adhesion, leading to Rho-kinase independent cell rounding, and inhibiting lamellipodia extension and cell motility. FASEB J. **18:** 592–594

68 Oinuma I., Katoh H. and Negishi M. (2004) Molecular dissection of the semaphorin 4D receptor Plexin-B1-stimulated R-Ras GTPase activating protein acitivity and neurite remodeling in hippocampal neurons. J. Neurosci. **24:** 11473–11480

69 Bernards A. and Settleman J. (2004) GAP control: regulating the regulators of small GTPases. Trends Cell Biol. **14:** 377–385

70 Song H.-J. and Poo M.-M. (1999) Signal transduction underlying growth cone guidance by diffusible factors. Curr. Opin. Neurobiol. **9:** 355–363

71 Terman J. R. and Kolodkin A. L. (2004) Nervy links protein kinase A to Plexin-mediated semaphorin repulsion. Science **303:** 1204–1207

72 Polleux F., Morrow T. and Ghosh A. (2000) Semaphorin 3A is a chamoattractant for cortical apical dendrites. Nature **404:** 567–573

73 Tamagnone L. and Comoglio P. M. (2004) To move or not to move? EMBO Rep. **5:** 356–361