Molecular Neurobiology

ISSN0893-7648/05/32(3): 217–222/$30.00

R-Ras As a Key Player for Signaling Pathway of Plexins

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Abstract

Axon guidance represents an important step in the formation of neuronal networks. Axons are guided by various guidance factors, such as semaphorins, slits, ephrins, and netrins. Plexins are cell surface receptors for the repulsive molecules of the semaphorin family. Cytoplasmic regions of plexins are responsible for initiating cellular signal transduction, resulting in axon repulsion. Recent advances have shed light on the signal transduction mechanism of plexins and the mechanisms by which it leads to a repulsive response. Plexin-B1 possesses an intrinsic guanine triphosphate (GTP)ase activating protein activity for R-Ras, a member of Ras family of small GTPases that has been implicated in promoting cell adhesion and neurite outgrowth through integrin activation. Stimulation of Plexin-B1 by Sema4D induces collapse of the growth cone through down-regulation of R-Ras activity. This article summarizes current understanding of the signaling mechanisms of plexins.

Index Entries: Plexin; semaphorin; R-Ras; axon guidance; neuron; integrin.

Introduction

Neurons form a complex neuronal network to function properly. Formation of this network includes several important 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. Of these steps, axon guidance is one of the critical steps for the accurate neuronal network formation. During development of the nervous system, axons are guided to their proper targets by sensing various extracellular cues in the local environment. Neuronal growth cones, which are located at the tip of the growing axon, act as a sensor for guidance cues.

Various families of axon guidance molecules, including netrins, slits, semaphorins, and ephrins, have been identified *(1)*. These guidance molecules bind to their specific receptors expressed in the growth cones of

Received April 6, 2005; Accepted May 16, 2005.
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neurons and steer axons by regulating adhesion, protrusion, or repulsion of the growth cones. Guidance molecules are expressed in various regions of brains, and neurons expressing specific receptors recognize these guidance molecules and correctly project their axons to target cells. Based on the response directions, axon guidance molecules are divided into two functionally different categories known as the attractive and repulsive cues; axons move toward the source of attractive cues and avoid the source of repulsive cues.

Semaphorins are a large family of secreted or membrane-bound proteins, which function as axon repulsive factors *(2)*. Currently, more than 20 semaphorins have been identified, and they are now classified in eight subclasses based on sequence similarity. Classes 1 and 2 are invertebrate semaphorins; classes 3 through 7 are vertebrate semaphorins; and V is viral-encoded semaphorin. The function of semaphorins is mediated by transmembrane receptors known as plexins, and mammalian plexins are classified into four subfamilies: Plexin-A1 to -A4, Plexin-B1 to -B3, Plexin-C1, and Plexin-D1 *(3)*. Of these plexins, Plexin-B1 has been extensively investigated as a receptor for Sema4D.

R-Ras GAP Activity of Plexin-B1

Signaling pathways of Plexin-B1 have been extensively studied *(4)*. Of the plexin families, Plexin-B subfamily has PSD-95/Dlg/ZO-1 (PDZ) domain-binding motif at the C-terminus of the cytoplasmic tail, and Plexin-B1 has been shown to activate Rho through association of PDZ–RhoGEF–LARG, Rho-specific guanine nucleotide exchange factors (GEFs), with the C-terminal PDZ-binding motif of Plexin-B1 inducing contraction *(5–9)*. However, C-terminal PDZ-binding motif is found only in the Plexin-B subfamily, and this motif is not found in invertebrate Plexin-B. Therefore, PDZ–RhoGEF-mediated Rho activation is not likely to be a common signaling pathway for a plexin family. On the other hand, the cytoplasmic tails of plexins have two domains denoted, C1 and C2, which are highly conserved among all plexin families *(10)*. These domains show weak sequence similarity to guanine triphosphate (GTP)ase activating proteins (GAPs) for small GTPases—especially those of the Ras family *(11)*. This homology has suggested that plexins act as GAPs for small GTPases, inactivating G proteins. However, GAP activities of plexins has been unclear, and the significance of this similarity has also been unclear.

Meanwhile, an increasing number of GAPs for small GTPases (including Ras and Rho families) has been identified, and structural features of various GAPs have been extensively studied *(12)*. Two highly conserved motifs containing Arg or Lys residue, primary Arg motif, and secondary Arg–Lys motif are found in catalytic domains of GAPs for small GTPases. Arg (Ras GAP) or Lys (Rho GAP) residue in the secondary motif stabilizes Arg residue in the finger loop of the primary Arg motif, and this bridge of Arg motifs contacts GTP bound to small GTPases.

Mutation of these conserved Arg–Lys residues within Arg motifs to Ala causes loss of GAP activity of small GTPases. Sequence alignment analyses have revealed that two highly conserved subdomains of plexins, C1 and C2, contain primary and secondary Arg motifs, respectively, suggesting that a pair of two subdomains, C1 and C2, acts as a GAP for small GTPases. On the other hand, two Rho family GTPases, active Rac1 and Rnd1, have been shown to directly bind to Plexin-B1, and this binding domain is located in the linker region between C1 and C2 *(13,14)*. Therefore, the Rnd1/active Rac1 binding site splits two Arg motifs of GAP domain of Plexin-B1. Unlike other Rho family GTPases, Rnd1 is constitutively active and predominantly expressed in cortical and hippocampal pyramidal neurons *(15)*. Rnd1 stably associates with Plexin-B1 independently of ligand Sema4D binding *(14)*.

These facts present the possibility of regulation of GAP activity of Plexin-B1 by Rnd1 binding. It was recently shown that Rnd1-asso-

ciated Plexin-B1 binds to GTP-bound R-Ras, and this binding requires Rnd1 *(16)*. This interaction is specific for R-Ras, and the Rnd1-associated Plexin-B1 binds to neither H-Ras nor Rho family GTPases. 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 the expression of this GAP activity for R-Ras requires Rnd1 association with Plexin-B1. Therefore, a pair of C1 and C2 domains of the C-terminal tail in Plexin-B1 directly encodes a GAP that is specific for R-Ras. Expression of a constitutively active R-Ras known as R-Ras-QL or knockdown of endogenous Rnd1 by Rnd1-specific short interfering RNA suppresses Sema4D-induced collapse of the growth cone in hippocampal neurons, whereas knockdown of endogenous R-Ras by R-Ras-specific short interfering RNA induces collapse of the growth cone in the absence of Sema4D. Therefore, downregulation of R-Ras activity is essential for the Sema4D-induced collapse response, and Plexin-B1 stimulation exhibits R-Ras GAP activity, leading to the repulsive response.

The R-Ras GAP domain is conserved among the Plexin family from *Caenorhabditis elegans* to human; 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 the Sema3A–Plexin-A1-mediated repulsion *(11,17)*. Expression of R-Ras-QL also suppresses the Sema3A-induced collapse of the growth cone in hippocampal neurons, indicating that the downregulation of R-Ras activity is required for the Sema3A–Plexin-A-mediated repulsive response *(15)*. Therefore, downregulation of R-Ras activity by plexins through R-Ras GAP domains is likely to be a major signaling pathway for semaphorin-mediated repulsive responses.

Molecular Mechanism of Expression of R-Ras GAP Activity

The cytoplasmic region of Plexin-B1 directly encodes R-Ras GAP; alone, however, the region cannot display R-Ras GAP activity *(15)*. Both binding of Rnd1 to Plexin-B1 and Sema4D stimulation are indispensable for the expression of this R-Ras GAP activity. In the absence of Rnd1, C1 and C2 domains intramolecularly interact with each other, and this closed conformation cannot associate with GTP-bound active R-Ras *(18)*. When Rnd1 binds to the region between the C1 and C2 domains, Rnd1 disrupts the interaction of C1 and C2 domains and 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 (Fig. 1). However, Rnd1-associated Plexin-B1 can hold the GTP-bound R-Ras but cannot promote GTPase activity.

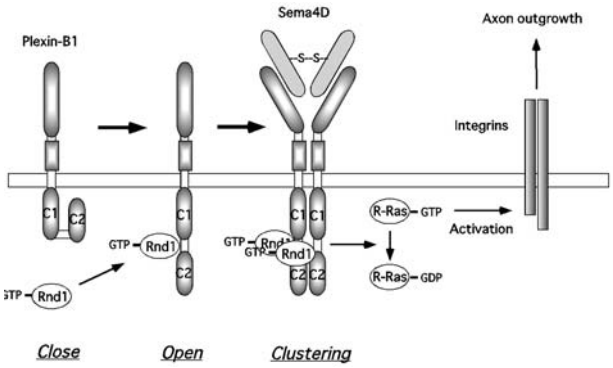

Fig. 1. Model for signal transduction of Sema4D–Plexin-B1–Rnd1 complex through R-Ras guanine triphosphate (GTP)ase-activating protein (GAP) activity. C1 and C2 domains of cytoplasmic tail of Plexin-B1 encode R-Ras GAP. C1 and C2 domains interact with each other, and Rnd1 binding to the region between 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. R-Ras activity stimulates integrin activation, promoting cell adhesion and neurite outgrowth, and downregulation of R-Ras activity reduces integrin-mediated cell adhesion, leading to collapse of the growth cone.

Sema4D is known to be a homodimer through cysteine disulfide bonds *(19)*. Binding of Sema4D to Plexin-B1 induces clustering of

the Rnd1-bound monomeric receptor, and this clustering gives the receptor the ability to hydrolyze GTP on R-Ras. Therefore, expression of R-Ras GAP activity of Plexin-B1 consists of two steps: interaction with GTP-bound R-Ras and enhanced GTP hydrolysis in R-Ras. The former is preparation by Rnd1, and the latter is a process induced by the clustering by Sema4D. Various GAPs for small GTPases, including Ras family GTPases, have been identified, and their GAP domains usually show high basal activity in the absence of other factors or interactions *(20)*. Actual expression of GAP activity of these GAP proteins is mediated by interaction with other molecules or receptors via various domains within 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. Similarly to Plexin-B1, the interaction of C1 and C2 domains has also been reported in Plexin-A1 *(21)*. Sema3A induces the aggregation of Plexin-A on dorsal root ganglion growth cones *(22)*. As mentioned earlier, Rnd1 binds to the region between C1 and C2 domains of Plexin-A1 *(16,17)*. Because of the requirement of downregulation of R-Ras activity for Sema3A action, Plexin-A may also exert its function via an R-Ras GAP function regulated by Rnd1 and receptor clustering similar to that of Plexin-B1.

Regulation of Integrins by R-Ras

The Ras family comprises a large group of structurally and functionally related small GTPases *(23)*. In addition to H-Ras, K-Ras, and N-Ras (which are well-known Ras family GTPases), R-Ras, Ral, and Rap are other important Ras family GTPases. Of these, R-Ras is 55% identical to H-Ras in sequence homology but has an N-terminal extension of 26 amino acids. Ras family GTPases serve as molecular switches by cycling between an inactive guanine diphosphate (GDP)-bound state and an active GTP-bound state; once activated, they can interact with their specific effectors, leading to various biological functions. Activation of the Ras family requires GDP–GTP exchange catalyzed by various GEFs, whereas the activation of the GTPases is downregulated by GAPs, which stimulate the intrinsic GTPases activities.

Among the various Ras GEFs, RasGRF1, RasGRP1, RasGRP3, and CalDAG-GEF1 can activate R-Ras, but they also activate many Ras family GTPases (including H-Ras and Rap), and no R-Ras-sepcific GEFs have been identified. Various GAPs also show broader activities toward the many Ras family GTPases, but only p98-R-RasGAP is an identified R-Ras-specific GAP *(24)*.

R-Ras in vitro interacts with many of the same effectors as Ras, including phosphatidylinositol-3-kinase (PI-3-K), Raf, and Ral-GDS. Among them, PI-3-K has emerged as the predominant effector for R-Ras, and R-Ras is a more potent activator of PI-3-K than Ras. R-Ras has been shown to regulate integrin function *(23)*. An active form of R-Ras induces an increase in cell adhesion and migration by activating integrins *(25)*. R-Ras-induced cell migration is sensitive to PI-3-K inhibitors, suggesting that the function of R-Ras is mediated by its downstream effector, PI-3-K *(26)*.

Integrins are cell surface receptors that bind to components of the extracelular matrix and play a central part in regulating cell growth, survival, adhesion, and migration *(27)*. Many neurons use members of the integrin family of cell surface receptors for responses to neurite growth-promoting factors, and integrin activation regulates neurite outgrowth *(28)*. Active R-Ras has been shown to potently promote integrin-dependent neurite outgrowth of retinal neurons on laminin-1, suggesting that R-Ras activity plays an important role in integrin-dependent neurite outgrowth *(29)*. Considering that plexins act as R-Ras GAP, downregulation of R-Ras activity by plexins via R-Ras GAP domains may suppress R-Ras-mediated integrin activation, thereby reducing cell adhesiveness and leading to collapse of the growth cone and inhibition of axon outgrowth.

Semaphorins were originally identified as repulsive axonal guidance molecules; recently,

however, they have been demonstrated to regulate integrin-mediated cell adhesion and cell migration *(30)*. Sema3A exerts an essential permissive role in the execution of vasculature remodeling by inhibiting integrin-mediated adhesion of endothelial cells *(31)*. Activation of Plexin-B1 negatively regulates integrin-based cell adhesion and migration of NIH-3T3 fibroblast cells *(32)*. Plexin-C1 inhibits integrin-mediated adhesion and chemokine-induced migration of dendritic cells *(33)*. Conversely to other semaphorin families, Sema7A promotes axon outgrowth through integrin activation independent of Plexin-C1, which is a receptor for Sema7A *(34)*. Semaphorin–plexin signaling plays an important role in integrin-mediated migration of a variety of cells. Because R-Ras acts as a key player in integrin functions, direct regulation of R-Ras activity by plexins through their R-Ras GAP domains may be a critical signaling mechanism for the cellular function of semaphorins.

Conclusion

This article summarizes recent advances in our understanding of the molecular mechanism for axon guidance mediated by Plexin family through regulation of R-Ras activity. Integrin function and its regulation by R-Ras are deeply implicated in axon outgrowth as well as cell migration. Integrin-mediated semaphorin signaling may be a general mechanism used in the development and function of nervous system. Future research will undoubtedly reveal the entire picture of signaling cascades of semaphorins for their diverse functions.

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