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B plexins activate Rho through PDZ-RhoGEF

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Abstract Plexins are receptors for the repulsive axon guidance molecules semaphorins. Previously, we have shown that plexin-B1 binds activated Rac, but that clustering of plexin-B1 causes Rho activation, resulting in stress fiber formation. Using the yeast two-hybrid system, we found that the C-terminus of B plexins interacted directly with Rho-specific exchange factors, via their PDZ domain. Mutation of the carboxy-terminal amino acids of plexin-B1 or coexpression of a dominant negative PDZ-RhoGEF abrogated the ability of plexin-B1 to cause stress fiber formation. Our results demonstrate a role for PDZ-RhoGEF in B plexin-mediated activation of Rho/Rho kinase signaling, implicated in the regulation of axon guidance and cell migration.

Key words: Plexin; Semaphorin; Rho; PDZ; Guanine nucleotide exchange factor; Axon guidance

1. Introduction

Signaling pathways that link extracellular factors to activation of Rho GTPases control cytoskeletal rearrangements and cell growth [1]. Rho regulates the assembly of contractile actin-myosin filaments [2], Rac induces actin polymerization to produce lamellipodia [3], while Cdc42 induces actin assembly leading to filopodia [4,5]. Guanine nucleotide exchange factors (GEFs) promote the exchange of GDP for GTP, thus activating Rho-like proteins [6], whereas GTPase-activating proteins increase the low intrinsic rate of GTP hydrolysis of small GTPases and are negative regulators [1]. In their active, GTP-bound state Rho-like GTPases interact with and activate effectors, amongst which protein or lipid kinases and scaffold proteins [7]. GEFs possess a tandem Dbl homology (DH) and pleckstrin homology (PH) domain that catalyzes the GDP–GTP exchange reaction of specific Rho proteins. Moreover, GEFs may contain a variety of other domains, which allow them to participate in different signaling processes [7].

Plexin-B1 is a semaphorin receptor [8], which signals towards Rho proteins [9]. Since plexins and semaphorins are widely expressed they are likely to have a variety of biological functions. Their best characterized role to date is in axonal growth cone guidance (for review [10,11]). The secreted semaphorin Sema3A inhibits neurite outgrowth and induces the collapse of lamellipodia and filopodia in growth cones [8,11,12]. Sema4D is a membrane-bound ligand for plexin-B1 that appears to be involved in adhesion and proliferation of lymphocytes [8,13,14]. Previously, we and others have found that plexins signal directly to different Rho GTPases [9,15,16]. Plexin-B1 interacts directly with Rac in its active GTP-bound state. However, clustering of plexin-B1 caused formation of stress fibers via Rho, suggesting that plexin-B1 activates Rho and not Rac [9]. Here we present evidence that plexin-B1 activates Rho via PSD-95/Dlg/ZO-1 (PDZ)-RhoGEF, an activator of Rho also implicated in G protein-coupled receptor signaling towards Rho via Gα12 and/or 13 [17–21].

2. Materials and methods

2.1. Vectors

The expression vectors for CD2/plexin-B1 [9], for myc-tagged PDZ-RhoGEF [18], for FLAG-tagged PDZ-RhoGEF [22], for VSV-tagged plexin-B1 [8] and VSV-tagged DCC [8] have been described before. The yeast bait vectors pYTH or pGBT9 containing PDZ-RhoGEFΔDH amino acids 1–1522, except for a deletion of amino acids 735–958); PDZ (amino acids 1–300); DH/PH (amino acids 735–1120); DH (amino acids 735–958); regulator of G protein signaling (RGS) (amino acids 301–592) and C1 (amino acids 1121–1522) or the PDZ domain of LARG (amino acids 1–182) and the yeast prey vectors pACTII containing plexin-B1, plexin-B1Δ3aa, B2, A2 or D1 cytoplasmic domain were generated by PCR. In PDZ-RhoGEF-RGSm/ΔDH amino acids I364L, W365G in the RGS domain were mutated by site-directed mutagenesis.

2.2. Yeast two-hybrid analysis

A human brain cDNA library fused to the GAL-4 activation domain in the pACTII vector was screened using PDZ-RhoGEFΔDH fused to the GAL-4 DNA binding domain in pYTH9 vector as a bait [23]. Approximately 6×10^6 yeast colonies were screened for their ability to grow on selective medium containing 25 mM 3-aminotriazole. Plasmids were rescued from 12 β-galactosidase positive clones and sequence analysis showed that they encoded six different cDNAs. One plasmid encoded the last 106 amino acids of plexin-B1.

2.3. Co-immunoprecipitation assay

HEK293 cells were transfected by calcium phosphate coprecipitation and lysed after 20 h of culture in lysis buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris, pH 8.0 containing protease inhibitors) for 30 min. Cleared lysates were incubated with anti-VSV antibodies and proteins were precipitated using protein G Sepharose. After three washes proteins were eluted with SDS sample buffer, separated by SDS–PAGE, blotted onto polyvinylidene difluoride membrane and

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Abbreviations: DH, Dbl homology; GEF, guanine nucleotide exchange factor; PDZ, PSD-95/Dlg/ZO-1; PH, pleckstrin homology; RGS, regulator of G protein signaling; S1P, sphingosine-1-phosphate

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revealed with anti-FLAG (M2 monoclonal antibody (mAb), Sigma) or anti-VSV antibodies.

2.4. CD2/plexin-B1 chimera crosslinking assay

Microinjection of serum-starved, quiescent Swiss 3T3 cells, the crosslinking and staining procedure have been described before [9]. Briefly, nuclei of 100 cells were injected with an expression vector containing CD2/plexin-B1 or CD2/plexin-B1-FLAG at a concentration of 0.1 mg/ml. After 5 h incubation, membrane-localized CD2 proteins were crosslinked using the OX34 mouse anti-rat CD2 mAb, followed by donkey anti-mouse IgG for 15 min at 37°C. Cells were fixed with 4% paraformaldehyde. CD2-positive cells were identified using the OX34 mAb, followed by goat anti-mouse FITC (Jackson Immunoresearch), actin was stained using rhodamine-phalloidin and myc-positive cells were identified using rat anti-myc Jac6 mAb (Serotech), followed by goat anti-rat TR (Jackson Immunoresearch). For double staining for CD2 and myc, first the CD2 staining was completed, the sample was blocked using mouse IgG and then stained for myc. Coverslips were mounted in Moviol and scored on a Zeiss Axiophot microscope with 40× and 63× oil immersion lenses. Fluorescent images were recorded on a confocal microscope (Zeiss).

3. Results

3.1. PDZ-RhoGEF binds to the C-terminus of B plexins in yeast two-hybrid analysis

Previously, we and others have shown that PDZ-RhoGEF, also know as kiaa0380, is a Rho-specific GEF, which causes cell rounding upon microinjection into Swiss 3T3 cells [17,18,21]. To identify proteins that interact with PDZ-RhoGEF we performed a yeast two-hybrid screen with a human brain cDNA library and a deletion mutant of PDZ-RhoGEF, missing the DH domain, as bait. Sequence analysis of a plasmid rescued from yeast clones showed complete sequence identity to the C-terminal 106 amino acids (residues 2031–2135) of the cytoplasmic domain of human plexin-B1, a transmembrane receptor for semaphorins [8] (Fig. 1A).

To map the domain by which PDZ-RhoGEF interacts with plexin-B1, the separate domains were tested in yeast two-hybrid analysis. As shown in Fig. 1A, plexin-B1 binds to the PDZ domain of PDZ-RhoGEF. PDZ domains bind specific recognition sequences, such as (S/T)-X-(V/I/L), at the carboxy-termini of certain proteins [24]. Four classes of plexins (A, B, C and D) have been described [8]. Examination of the carboxy-termini showed that all B plexins contain a class I consensus sequence for PDZ binding (TDL). Therefore, we tested whether other plexins interacted with the PDZ domain of PDZ-RhoGEF in yeast two-hybrid analysis. As shown in Fig. 1B, plexin-B1 and -B2 interacted with the RhoGEF PDZ domain, whereas plexin-A2 or plexin-D1 did not. No interaction of the plexins was found with the RGS domain of PDZ-RhoGEF or the empty pACTII vector (not shown). Mutation of the putative PDZ binding sequence in the plexin-B1 C-terminus by deleting the last three amino acids abrogates the interaction between plexin-B1 and the PDZ domain, indicating a direct interaction between the PDZ domain and the PDZ binding motif in the plexin-B1 C-terminus. Plexin-B1 and -B2 interacted as well with the PDZ domain of LARG (Fig. 1C), another Rho-specific GEF very similar to PDZ-RhoGEF [25].

3.2. PDZ-RhoGEF is implicated in Rho activation by plexin-B1 clustering in Swiss 3T3 fibroblasts

Plexins are receptors for semaphorins and regulate axon guidance and cell migration [10]. Previously, we have shown

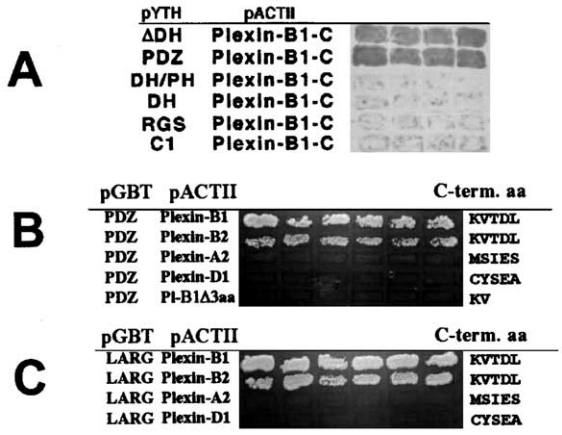

Fig. 1. Interaction between B plexins and PDZ-RhoGEF. A: PDZ-RhoGEF binds to the C-terminus of plexin-B1 via its PDZ domain. Yeast strains containing the indicated domains of PDZ-RhoGEF in the integrated bait vector pYTH were transformed with the pACTII prey vector containing the plexin-B1 C-terminus (amino acids 2031–2153) and plated on minus Leu, minus Trp plates as described [23]. Colonies of equal size were replated in the presence of 3-aminotriazole on minus Leu, minus Trp, minus His, minus Ura plates and allowed to grow for 3 days. B: The PDZ domain of RhoGEF binds to B plexins. Yeast strains were cotransformed with the pGBT9 bait vector containing the PDZ-RhoGEF PDZ domain (amino acids 1–300) and the prey vectors pACTII containing plexin-B1 C-terminus (amino acids 1724–end, plexin-B2 C-terminus (amino acids 1441–end), plexin-A2 (amino acids 1544–end and plexin-D1 (amino acids 1591–end), plexin-B1Δ3aa (amino acids 1724–2132, the last three amino acids have been deleted). C: The PDZ domain of LARG binds to B plexins. Yeast strains were cotransformed with the pGBT9 bait vector containing the LARG PDZ domain (amino acids 1–182) and the prey vectors as described in B.

that clustering of a CD2/plexin-B1 chimera in quiescent Swiss 3T3 cells caused formation of actin bundles and cell contraction, by activation of Rho [9]. Therefore, we used the plexin clustering assay to investigate the effect of mutating the PDZ binding sequence in the plexin-B1 C-terminus.

As shown in Fig. 2A, plexin-B1 clustering caused formation of actin bundles and plexin clusters were found to be concentrated where the actin bundles form apical constrictions. In contrast, clustering of a mutant plexin-B1 chimera in which the last leucine amino acid was replaced with a FLAG tag (CD2/plexin-B1-FLAG) did not cause formation of stress fibers (Fig. 2B). We conclude that Rho activation is mediated via the plexin-B1 C-terminus, by binding and activation of a PDZ domain containing RhoGEF. To substantiate this conclusion, we coexpressed the CD2/plexin-B1 chimera with a myc-tagged full length PDZ-RhoGEF in Swiss 3T3 cells. PDZ-RhoGEF when overexpressed in Swiss 3T3 cells causes cortical actin reorganization and cell rounding [18]. It is localized at the plasmamembrane and a proline-rich motif adjacent to the DH/PH module is essential for the ability of PDZ-RhoGEF to cause this cell rounding [18]. As shown in Fig. 2C, upon crosslinking of CD2-plexin-B1 clear colocalization was observed with myc-PDZ-RhoGEF in the cell cortex at the area of cell–substrate contact, suggesting that PDZ-RhoGEF interacts with plexin-B1 in Swiss 3T3 cells.

To determine if B plexins interact with PDZ-RhoGEF in vivo, we overexpressed VSV-tagged plexin-B1 and FLAG-tagged full length PDZ-RhoGEF in HEK293 cells and performed a co-immunoprecipitation assay. When VSV-plexin-B1 was precipitated from lysates with the VSV antibody, the

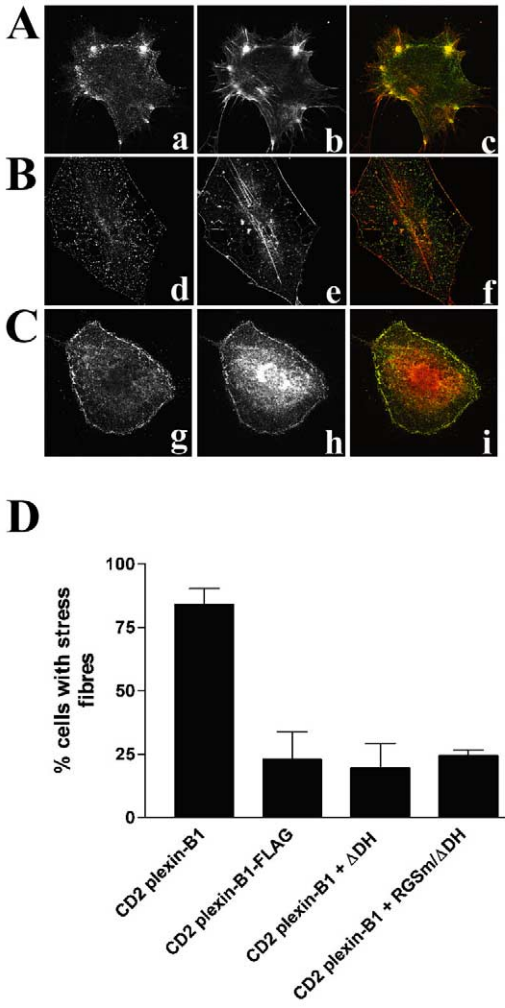

Fig. 2. The effects of crosslinking of a chimeric CD2/plexin-B1 on the actin cytoskeleton. Quiescent Swiss 3T3 cells were injected with an expression vector containing CD2/plexin-B1 (A, a–c) or CD2/plexin-B1-FLAG (B, d–f) or CD2/plexin-B1 was coinjected with myc-tagged PDZ-RhoGEF (C, g–i). After 5 h incubation, membrane-localized CD2 proteins were crosslinked using the OX34 mouse anti-rat CD2 and donkey anti-mouse IgG for 15 min. CD2 expression (a,d,g), F-actin organization (b,e) and myc (h) expression are shown. Images c,f,i are the merged images. D: Mutation of the plexin-B1 C-terminus or coexpression of dominant negative PDZ-RhoGEF constructs abrogates stress fiber formation upon CD2/plexin-B1 crosslinking. Quiescent Swiss 3T3 cells were injected with CD2/plexin-B1, CD2/plexin-B1-FLAG in which the last leucine of the plexin-B1 C-terminus had been replaced by a FLAG tag or CD2/plexin-B1 was co-injected with PDZ-RhoGEF/ΔDH or PDZ-RhoGEF-RGSm/ΔDH plasmids. Five hours later CD2 proteins were crosslinked and F-actin was stained. The % of CD2-positive cells containing actin stress fibers was counted. Results are given as mean+S.D. of three to five experiments.

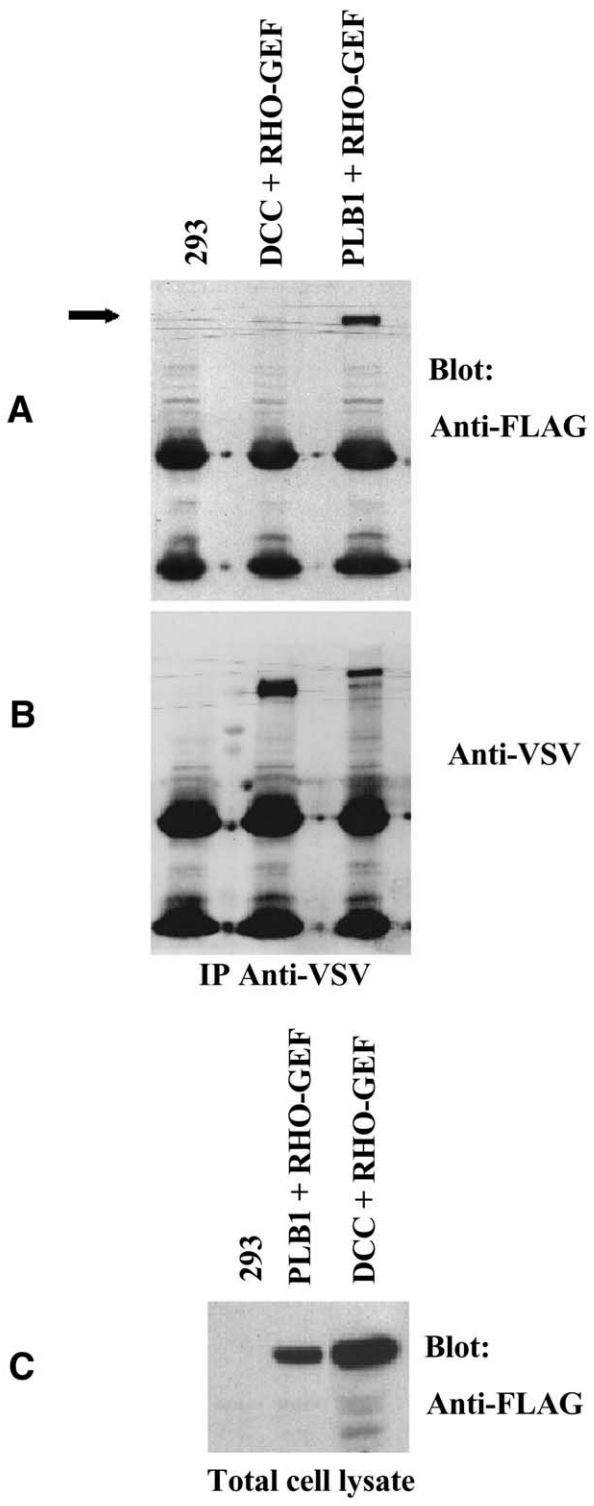

Fig. 3. Interaction of VSV-tagged plexin-B1 with FLAG-tagged PDZ-RhoGEF in vivo. HEK293 cells were cotransfected with VSV-tagged DCC or VSV-tagged plexin-B1 and FLAG-tagged PDZ-RhoGEF. Lysates were incubated with anti-VSV antibody and immunocomplexes were precipitated and subjected to immunoblot analysis with anti-FLAG antibody (A) or anti-VSV antibody (B) In A clear co-immunoprecipitation of FLAG-PDZ-RhoGEF is observed with plexin-B1 (arrow) but not with DCC. In C, it is shown that FLAG-PDZ-RhoGEF is well expressed in the cell lysates.

FLAG-PDZ-RhoGEF was co-immunoprecipitated with the plexin-B1 (Fig. 3). FLAG-PDZ-RhoGEF was not co-immunoprecipitated with VSV-DCC. This suggests that PDZ-RhoGEF interacts with and forms a complex with plexin-B1 in vivo.

PDZ-RhoGEF also plays a role in signaling from G protein-coupled receptors and Gα12/13 proteins to Rho. The RGS domain of PDZ-RhoGEF inhibits stress fiber formation induced by lysophosphatidic acid (LPA) [21]. Previously, we showed that overexpression of the N-terminal fragment of

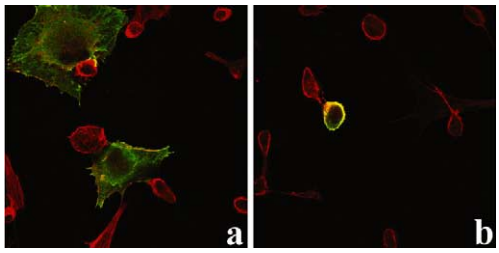

Fig. 4. Dominant negative myc-tagged PDZ-RhoGEF/ΔDH (a) but not the double mutant PDZ-RhoGEF-RGSm/ΔDH (b) blocks cell contraction induced by S1P. Swiss 3T3 cells were microinjected with myc-tagged constructs, left to express for 3 h and then treated with 5 μM S1P for 15 min. Cells were fixed and stained as described. Shown is a confocal image (myc staining in green and actin staining using rhodamine phalloidin in red).

PDZ-RhoGEF (amino acids 1–592), containing the RGS domain, was able to inhibit LPA-induced neurite retraction in Neuro2a cells [18]. Therefore, we studied the effect of a PDZ-RhoGEF, in which the Dbl homology domain was removed, in Swiss 3T3 cells.

As shown in Fig. 4a, dominant negative PDZ-RhoGEF blocks cell contraction induced by sphingosine-1-phosphate (S1P), a potent activator of Rho, in Swiss 3T3 cells. Interestingly, a RGSm/ΔDH double mutant PDZ-RhoGEF, containing mutations in the RGS domain preventing interaction with Gα12/13 proteins, did not block the induction of cell rounding by S1P (Fig. 4b). This shows that S1P activates Rho via G protein-coupled receptors involving G12/13 proteins in Swiss 3T3 cells, as shown before for other cell types [26,27]. Co-injection of the dominant negative PDZ-RhoGEF or the RGSm/ΔDH double mutant inhibits the formation of stress fibers by clustering of CD2-plexin-B1, suggesting that an endogenous PDZ-RhoGEF in Swiss 3T3 cells mediates the stress fiber formation (Fig. 2D).

4. Discussion

We report here that the C-terminus of human plexin-B1 or -B2 interacts with the PDZ domain of PDZ-RhoGEF and LARG. The human plexin-B subfamily includes one additional member, plexin-B3, and since the C-terminal amino acids are completely conserved it is likely that plexin-B3 will interact with the PDZ domains as well. Northern blot analysis reveals that plexin-B1 is widely expressed in fetal and adult tissues such as brain, lung, liver and kidney, and in neural, hematopoietic and endothelial cells [28]. PDZ-RhoGEF is highly expressed in brain, testis, heart, ovary and placenta and to a lower level in kidney, pancreas, spleen, prostate, colon, skeletal muscle, lung and liver [17]. LARG is expressed in spleen, prostate, testis, ovary, small intestine and colon, peripheral blood lymphocytes and to a low level in the thymus [25]. The role of B plexins in vivo is unclear at the moment, but as plexin-B1 and the PDZ-containing RhoGEFs are coexpressed in several tissues, it is likely that the interactions play an important role in plexin-B-mediated signaling. During submission of this article a paper by Swiercz et al. was published, in which the authors show that plexin-B1 interacts with PDZ-RhoGEF and LARG in brain and that this interaction is of functional importance in mediating growth cone collapse of hippocampal neurons stimulated by Sema4D, the plexin-B1 ligand [22].

In Fig. 5, we present a model for activation of Rho by PDZ-RhoGEF. Ligand binding to G protein-coupled receptors activates PDZ-RhoGEF by binding of Gα13 to the RGS domain [6,17,18,21,26,27]. We now present evidence for a second activating input signal on PDZ-RhoGEF, namely binding of plexins to the PDZ domain of PDZ-RhoGEF. Sema4D, a ligand for plexin-B1, forms homodimers and dimerization of Sema3A is required to induce growth cone collapse [11,13]. It seems likely, therefore, that semaphorins induce receptor activation through clustering. Clustering of plexin-B1 presumably activates PDZ-RhoGEF or LARG leading to Rho activation and the formation of stress fibers (see Fig. 5). The exact mechanism of activation is unclear, but it might be similar to that of the insulin-like growth factor-1 (IGF-1) receptor, which directly interacts with LARG through the PDZ domain [29]. Similar as the interaction of LARG with the IGF-1 receptor, the interaction of PDZ-RhoGEF with plexin-B1 is observed in the absence of ligand, suggesting that the interaction is constitutive [29]. Physiological activation of the RhoGEF by ligand binding to the receptor is likely to induce full activation of the RhoGEF and G proteins [17,19,29] or phosphorylation of the GEF by kinases like FAK [30] could be involved.

In *Drosophila*, DRhoGEF2, the fly orthologue of PDZ-RhoGEF, LARG and p115RhoGEF, mediates cell shape changes during gastrulation [31,32]. Flies possess two plexins, plexin-A and -B. Genetic evidence suggests that plexin-B mediates axon repulsion in vivo by downregulating Rac and by activating Rho [33]. It will be of interest to determine whether DRhoGEF2 functions downstream of plexins in regulation of axon guidance in *Drosophila*.

Several extracellular signaling molecules like ephrin-A5, thrombin or LPA cause collapse of the growth cone by activating Rho [34–36]. Interestingly, Sema3A requires active Rac to induce growth cone collapse and Rho kinase inhibitors do not block its ability to cause collapse [12,37]. This suggests that Rho activation is not part of the mechanism causing growth cone collapse by Sema3A. In future experiments we

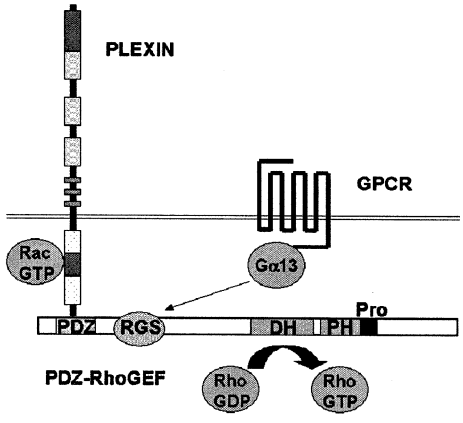

Fig. 5. Proposed mechanism whereby plexins and G protein-coupled receptors stimulate Rho-dependent pathways. Ligand binding of semaphorin to plexins causes activation of PDZ-RhoGEF via its PDZ domain, thereby stimulating the exchange of GDP for GTP on Rho, through its DH and PH domains. A parallel input on PDZ-RhoGEF is established by ligand binding to G protein-coupled receptors activating Gα13, which interacts with PDZ-RhoGEF through its RGS domain, thus catalyzing the exchange of GDP for GTP on Rho.

aim to investigate whether B plexins and PDZ-RhoGEF are involved in axon guidance and cell migration.

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References

[1] Hall, A. (1998) Science 279, 509–514.
[2] Ridley, A.J. and Hall, A. (1992) Cell 70, 389–399.
[3] Ridley, A.J., Paterson, H.F., Johnston, C.L., Diekmann, D. and Hall, A. (1992) Cell 70, 401–410.
[4] Kozma, R., Ahmed, S., Best, A. and Lim, L. (1995) Mol. Cell. Biol. 15, 1942–1952.
[5] Nobes, C.D. and Hall, A. (1995) Cell 81, 53–62.
[6] Zheng, Y. (2001) Trends Biochem. Sci 26, 724–732.
[7] Van Aelst, L. and D'Souza-Schorey, C. (1997) Genes Dev. 11, 2295–2322.
[8] Tamagnone, L., Artigiani, S., Chen, H., He, Z., Ming, G., Song, H., Chedotal, A., Winberg, M., Goodman, C.S., Poo, M., Tessier-Lavigne, M. and Comoglio, P.M. (1999) Cell 99, 71–80.
[9] Driessens, M.H., Hu, H., Nobes, C.D., Self, A., Jordens, I., Goodman, C.S. and Hall, A. (2001) Curr. Biol. 11, 339–344.
[10] Tamagnone, L. and Comoglio, P.M. (2000) Trends Cell Biol. 10, 377–383.
[11] He, Z., Wang, K.C., Kopriva, V., Ming, G. and Song, H.L. (2002) Science STKE 119, 1.
[12] Jin, Z. and Strittmatter, S.M. (1997) J. Neurosci. 17, 6256–6263.
[13] Hall, K.T., Boumsell, L., Schultze, J.L., Boussiotis, V.A., Dorfman, D.M., Cardoso, A., Bensussan, A., Nadler, L.M. and Freeman, G.J. (1996) Proc. Natl. Acad. Sci. USA 93, 11780–11785.
[14] Bismuth, G. and Boumsell, L. (2002) Science STKE 128, 4.
[15] Vikis, H.G., Li, W., He, Z. and Guan, K.L. (2000) Proc. Natl. Acad. Sci. USA 97, 12457–12462.
[16] Rohm, B., Rahim, B., Kleiber, B., Hovatta, I. and Püschel, A.W. (2000) FEBS Lett. 486, 68–72.
[17] Fukahara, S., Murga, C., Zohar, M., Igishi, T. and Gutkind, J.S. (1999) J. Biol. Chem. 274, 5868–5879.
[18] Togashi, H., Nagata, K., Takagishi, M., Saitoh, N. and Inagaki, M. (2000) J. Biol. Chem. 275, 29570–29578.
[19] Jackson, M., Song, W., Liu, M., Jin, L., Dykes-Hoberg, M., Lin, C.G., Bowers, W.J., Federoff, H.J., Sternweis, P.C. and Rothstein, J.D. (2001) Nature 410, 89–93.
[20] Wells, C.D., Liu, M., Jackson, M., Gutowski, S., Sternweis, P.M., Rothstein, J.D., Kozasa, T. and Sternweis, P.C. (2002) J. Biol. Chem. 277, 1174–1181.
[21] Rümenapp, U., Blomquist, A., Schworer, G., Schablowski, H., Psoma, A. and Jacobs, K.H. (1999) FEBS Lett. 459, 313–318.
[22] Swiercz, J.M., Kuner, R., Behrens, J. and Offermanns, S. (2002) Neuron 35, 51–63.
[23] Aspenström, P. and Olson, M.F. (1995) Methods Enzymol. 256, 228–241.
[24] Fanning, A.S. and Anderson, J.M. (1999) Curr. Opin. Cell Biol. 11, 432–439.
[25] Kourlas, P.J., Strout, M.P., Becknell, B., Veronese, M.L., Croce, C.M., Theil, K.S., Krahe, R., Ruutu, T., Knuutila, S., Bloomfield, C.D. and Caligiuri, M.A. (2000) Proc. Natl. Acad. Sci. USA 97, 2145–2150.
[26] Windh, R.T., Lee, M., Hla, T., An, S., Barr, A.J. and Manning, D.R. (1999) J. Biol. Chem. 274, 27351–27358.
[27] Hla, T. (2001) Prostaglandins Other Lipid Mediat. 64, 135–142.
[28] Maestrini, E., Tamagnone, L., Longati, P., Cremona, O., Gulisano, M., Bione, Tamanini, F., Neel, B.G., Toniolo, D. and Comoglio, P.M. (1996) Proc. Natl. Acad. Sci. USA 93, 674–678.
[29] Taya, S., Inagaki, N., Sengiku, H., Makino, H., Iwamatsu, A., Urakawa, I., Nagao, K., Kataoka, S. and Kaibuchi, K. (2001) J. Cell Biol. 155, 809–819.
[30] Chikumi, H., Fukuhara, S. and Gutkind, J.S. (2002) J. Biol. Chem. 277, 12463–12473.
[31] Barrett, K., Leptin, M. and Settleman, J. (1997) Cell 91, 905–915.
[32] Häcker, U. and Perrimon, N. (1998) Genes Dev. 12, 274–284.
[33] Hu, H., Marton, T.F. and Goodman, C.S. (2001) Neuron 32, 39–51.
[34] Shamah, S.M., Lin, M.Z., Goldberg, J.L., Estrach, S., Sahin, M., Hu, L., Bazalakova, M., Neve, R.L., Corfas, G., Debant, A. and Greenberg, M.E. (2001) Cell 105, 233–244.
[35] Wahl, S., Barth, H., Ciossek, T., Aktories, K. and Mueller, B.K. (2000) J. Cell Biol. 149, 263–270.
[36] Hirose, M., Ishizaki, T., Watanabe, N., Uehata, M., Kranenburg, O., Moolenaar, W.H., Matsumura, F., Maekawa, M., Bito, H. and Narumiya, S. (1998) J. Cell Biol. 141, 1625–1636.
[37] Arimura, N., Inagaki, N., Chihara, K., Nakamura, N., Amano, M., Iwamatsu, A., Goshima, Y. and Kaibuchi, K. (2000) J. Biol. Chem. 275, 23973–23980.