The Activity of the Plexin-A1 Receptor Is Regulated by Rac*

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Plexins constitute a large family of transmembrane proteins that act as receptors for the semaphorin family of ligands. They are best known for their role in growth cone guidance, although they also are widely expressed outside the nervous system. Plexins are thought to control axon guidance by modifying the growth cone cytoskeleton, and Rho GTPases have been strongly implicated in this response. However, the exact contribution of Rho proteins is unclear. Sema3A/Plexin-A1-induced growth cone collapse, for example, requires Rac activity, which is a surprising result given that this GTPase is usually associated with membrane protrusions. We show here that Sema3A-induced collapse of COS-7 cells expressing Plexin-A1 also requires Rac but not Rho activity and that the cytoplasmic tail of Plexin-A1 interacts directly with activated Rac. However, collapse induced by a constitutively activated version of Plexin-A1 does not require Rac. We propose a novel function for Rac, namely that it acts upstream of Plexin-A1 during semaphoring-induced collapse, to regulate the activity of the receptor.

Plexins are a family of transmembrane receptors characterized by the presence of a conserved intracellular domain of ~600 amino acids known as the sex and plexins domain (1). The sex and plexins domain is strikingly conserved across the plexin family (57–97% similarity) and consists of two blocks of high sequence conservation separated by a variable linker. Together with their co-receptors neuropilins, plexins act as functional receptors for members of the semaphorin family of growth cone guidance molecules (2–6). The prototypic semaphorin, Sema3A, has a potent inhibitory effect on sensory axon growth, and it is thought to control axon guidance by modifying the growth cone cytoskeleton (7). In addition, the binding of Sema3A to COS-7 cells expressing Plexin-A1 and neuropilin-1 (NP-1) has been shown to induce a morphological "collapse" response that may correlate with growth cone collapse (5).

To facilitate axon turning, growth cones are thought to translate sensory information into cytoskeletal rearrangements (8–13). Rho GTPases are important modulators of cytoskeletal assembly, and they have been strongly implicated in the regulation of axon growth, guidance, and motility (14). The best-characterized Rho family members, Rho, Rac, and Cdc42, regulate the formation of contractile actin-myosin filaments (15), lamellipodia (16), and filopodia (17) respectively. Rho GTPases function as molecular switches, cycling between an "active" GTP-bound and an "inactive" GDP-bound conformation (18). Active GTPases interact directly with target proteins (also known as effectors) to trigger a downstream response (19), and one of the major criteria used to identify putative effectors is that the protein interacts preferentially with the active (GTP-bound) form of the GTPase.

In dorsal root ganglia and spinal motor neurons, Sema3A-induced collapse has been shown to require Rac activity (20–22). This is a surprising observation, since in other cell types and even in neuronal-like cell lines, Rac mediates membrane protrusion. This result has been observed by several groups, and in addition, plexins co-localize with Rac and F-actin in growth cones following stimulation with Sema3A (23). Sema3A-induced collapse has also been reported to require Cdc42 activity in motor, but not dorsal root ganglia, neurons (20, 21).

Interestingly, Rac-GTP interacts directly with a member of the B-family of plexins, Plexin-B1, through a partial CRIB motif (24–26), and in Drosophila, the binding of dPlex-B to dRac1 results in the inactivation of an important Rac effector, p21-activated kinase 1 (p65PAK-1) (27, 28). It has been proposed that stimulation of Plexin-B1 by its ligand, Sema4D, induces local sequestration of Rac, thereby inhibiting signaling to its effector pathways.

In this study, we show that Sema3A-induced collapse of COS-7 cells co-expressing Plexin-A1 and NP-1 requires both Rac and Cdc42 activity. We find that Rac-GTP associates directly with Plexin-A1 and that Rac is activated following Sema3A stimulation. Rho and Rho kinase (ROCK) are not required for this response, indicating that collapse is not a consequence of excessive contractile forces as has been shown for ephrin-A5-induced growth cone collapse mediated by the Eph family of tyrosine kinase receptors (29). Finally, a constitutively activated version of Plexin-A1 no longer requires Rac activity. Our results support a novel role for Rac, namely that it acts upstream of Plexin-A1 during semaphoring-induced collapse and is required for receptor activation.

EXPERIMENTAL PROCEDURES

Reagents—Mouse anti-vsv-G (clone P5D4) was from Roche Diagnostics, Inc., mouse anti-Myc (clone 9E10) was from Sigma, mouse anti-Flag (clone M2) and rhodamine-phalloidin were from Sigma, mouse anti-Rac1 (clone 22A8) was from Upstate Biotechnology (Lake Placid, NY), rabbit anti-Myc was from Research Diagnostics (Flanders, NJ), goat anti-human IgG, Fc fragment-specific (goat anti-hFc), and all of the secondary antibodies were from Jackson Immunoresearch Laboratories, Inc. (West Grove, PA), and the Rho kinase...
inhibitor Y-27632 was from Tocris Cookson, Inc. (Ellisville, MO). Recombinant Sema3A-Fc was purified by protein A-Sepharose affinity chromatography from the medium of a stable Chinese hamster ovary K1 cell line (a kind gift from Dr. B. Eickhoff, Kings College, London, United Kingdom) expressing full-length chick Sema3A-Fc (30). For yeast interaction studies, the Saccharomyces cerevisiae Y190 (MATa, gal4Δ-42, gal80Δ-53, his3, trpl-101, ade2-101, ura3-52, leu3-112, ura3::GAL1-to-1, lys2::GAL1-His3::YEp351) yeast strain was used.

DNA Constructs and Cloning Procedures—vsv-Plexin-A1 in pBPK-CMV was provided by Dr. A. Puschel (Westfälische Wilhelms Universität, Münster, Germany) (6). Myc-Plexin-A1SEM and Myc-Plexin-A1ΔECT in pSecTag2 were provided by Dr. S. Strittmatter (Johns Hopkins University, Baltimore, MD) (31). NP-1 in pMT21 was provided by Dr. M. Tomorrow. plasmid pYY79 (4) containing the tetritin intron, a modified form of the tetritin gene (22) was used to stimulate transfected cells for 30 min at 37 °C. Alexa 488-labeled phalloidin (Molecular Probes) and the actin-binding dye calcein-AM (Molecular Probes) were used to monitor actin cytoskeletal changes. Coverslips were mounted in Mowiol mountant (Calbiochem), and images were captured using a Bio-Rad MRC 1000 confocal microscope.

Yeasts Two hybrid Interaction Assays—A cDNA-encoding Plexin-A1 (Acet) fused to the sequence encoding the GAL4 DNA-binding domain of the pYTH9 vector was stably integrated into the genome of the yeast strain Y190. Plexin-A1(cyt):Y190 cells were transformed with cDNA encoding wild-type dRac, dRho, or dCdc42 fused to the sequence encoding the GAL4 activation domain in the pACTII vector. Putative interactions were assessed by growth on 3-AT-selective medium (33).

Immunoprecipitation in Vitro—Plexin-A1 and NP-1 containing glass coverslips 16–25 mm in diameter were fixed in cold 4% paraformaldehyde for 20 min at room temperature. Coverslips were mounted in Mowiol mountant (Calbiochem), and images were captured using a Bio-Rad MRC 1000 confocal microscope.

Immunoprecipitation—Cells were transfected 24 h prior to immunostaining. Cells were incubated in ice-cold buffer A containing 0.1 mM sodium orthovanadate and lysed at 4 °C in radioimmune precipitation buffer (1% w/v Nonidet P-40, 10 mM Tris-HCl, 150 mM NaCl, and 0.1% v/v Tween 20) and bound radiolabeled GTPases were visualized using autoradiography.

RESULTS

Sema3A-Fc-induced Collapse Requires Rac and Cdc42 but Not Rho or p160ROCK—A COS-7 collapse assay was used to investigate the involvement of Rho GTPases in Sema3A-Fc-mediated collapse. Cells were co-transfected with vsv-Plexin-A1 and NP-1 with or without a dominant negative GTPase mutant (Myc-N17Cdc42, Myc-N17Rac1, or Myc-N19RhoA). After 18 h, cells were stimulated with 2 μg/ml Sema3A-Fc and labeled using a goat anti-hFc antibody and either a mouse anti-Myc antibody or phalloidin. In each experiment, 100 cells positive for both Myc and Sema3A-Fc staining were counted and classified as either collapsed or not collapsed. Dominant negative Cdc42 (Myc-N17Cdc42) completely inhibited Sema3A-Fc-induced collapse as only 30% of expressing cells collapsed in comparison to 70% of control cells (see Fig. 1, a–d and j). Dominant negative Rac (Myc-N17Rac1) also inhibited collapse with 45% of expressing cells collapsed in comparison to 70% of control cells (see Fig. 1, a, b, e, f, and j). This may
be an underestimate of the true inhibition of collapse, because cells expressing dominant negative Rac showed a higher background of spontaneous collapse (40%) compared with control cells (20%). Western blot analysis was used to confirm that the inhibitory effects produced by GTPase mutants were not the result of changes in the expression levels of vsv-Plexin-A1 or NP-1 (Fig. 1i). Furthermore, the inhibitory effects observed were not a consequence of decreased surface expression of vsv-Plexin-A1 and NP-1, because Sema3A-Fc binds as efficiently with or without the dominant negative constructs (data not shown).

Dominant negative Rho (Myc-N19RhoA) did not inhibit Sema3A-Fc-induced collapse (see Fig. 1, a, b, g, h, and i). To confirm that collapse is not a consequence of actin/myosin-induced cell contraction, Y-27632, a small molecule inhibitor of the serine-threonine kinase p160ROCK, was added and this also had no effect on collapse (Fig. 1j).

Sema3A-Fc Induces Rac Activation—To determine whether Rac is activated in response to Sema3A-Fc, a GTPase pull-down assay was used. Cells were transfected with empty vector, NP-1, or vsv-Plexin-A1 and NP-1 and stimulated for 30 min with Sema3A-Fc, and Rac-GTP was precipitated from lysates using GST-PAK/CRIB beads. In COS-7 cells alone or cells expressing NP-1 only, the levels of Rac-GTP were almost undetectable following stimulation, whereas in cells expressing vsv-Plexin-A1 and NP-1, Sema3A-Fc induced a significant increase in the levels of active Rac (see Fig. 2). Additional experiments revealed modest activation 10 min after Sema3A-Fc stimulation, and maximum activation was seen at 30 min (see Fig. 2).

FIG. 1. Sema3A-Fc-induced collapse requires Rac and Cdc42 but not Rho or p160ROCK. Cells were transfected with the plasmids indicated and stimulated 18 h post-transfection with 2 μg/ml Sema3A-Fc for 30 min. In experiments using the p160ROCK inhibitor Y-27632, cells were stimulated 24 h post-transfection. a and b, cells expressing vsv-Plexin-A1 and NP-1, c and d, cells expressing vsv-Plexin-A1, NP-1, and Myc-N17Cdc42, e and f, cells expressing vsv-Plexin-A1, NP-1, and Myc-N17Rac1. g and h, cells expressing vsv-Plexin-A1, NP-1, and Myc-N19RhoA. a, TRITC-conjugated phalloidin. b, d, f, and h, goat anti-hFc. c, e, and g, mouse anti-Myc, clone 9E10. i, Western blot showing the expression levels of vsv-Plexin-A1, NP-1, and Myc-N17Cdc42 or Myc-N17Rac1 in each experiment. Lysates prepared from Sema3A-Fc-stimulated cells were divided into three and probed using mouse anti-vsv, goat anti-NP-1, and mouse anti-Myc antibodies. j, quantification of the effects of dominant negative GTPases and Y-27632 on the Sema3A-Fc-induced collapse. To inhibit Rho kinase, cells expressing vsv-Plexin-A1 and NP-1 were treated with 10 μM Y-27632 30 min prior to stimulation with Sema3A-Fc. 10 μM Y-27632 also was present in the culture medium containing Sema3A-Fc.

FIG. 2. Rac activation by Sema3A-Fc. Cells were transfected with vector only, NP-1, or vsv-Plexin-A1 and NP-1. After 10 h, cells were serum-starved. Stimulation with 2 μg/ml Sema3A-Fc was carried out 24 h post-transfection for 0, 10, 20, 30, or 60 min. Lysates were prepared, and aliquots were saved to assess total GTPase levels. GST-PAK/CRIB beads were used to pull down Rac-GTP. Equal amounts of beads and total lysates were assessed by Western blot analysis. Blots were probed using mouse anti-Rac1 and mouse anti-vsv antibodies.
**Rac Acts Upstream of Plexin-A1 to Regulate the Activity of the Receptor**—Takahashi and Strittmatter (31) demonstrate that mutants of Plexin-A1 lacking either the Sema domain (Plexin-A1/H9004 SEM) or the whole extracellular region (Plexin-A1/H9004 ECT) are constitutively active when introduced into COS cells (31). To test whether this response is still Rac-dependent, COS cells were transfected with Myc-Plexin-A1/H9004 ECT or Myc-Plexin-A1/H9004 SEM, either alone or together with green fluorescent protein or dominant negative Rac (FLAG-N17Rac1). When expressed alone, Myc-Plexin-A1/H9004 ECT and Myc-Plexin-A1/H9004 SEM induced collapse in 70 and 65% of cells, respectively (see Fig. 3, a and b). However, dominant negative Rac was unable to inhibit collapse induced by constitutively active Plexin-A1 mutants (see Fig. 3, c and d). We conclude that Rac does not participate directly in the pathway leading from Plexin-A1 to the collapse response but it is required for Sema3A-induced collapse.

Since Plexin-B1 has been shown to directly interact with Rac, we examined whether Rac might also interact directly with Plexin-A1. First, DNA encoding the 620 amino acid cytoplasmic tail of Plexin-A1 was subcloned into the yeast two-hybrid bait vector, pYTH9 (see Fig. 4a). The construct produced, hemagglutinin-Plexin-A1(cyt), was subsequently integrated into the genome of the yeast strain Y190, and Western blot analysis confirmed that the protein was expressed. Y190::Plexin-A1(cyt) yeast were then transformed with wild-type Rac, Rho, or Cdc42, and as shown in Fig. 4b, an interaction with Rac was clearly observed. To confirm this observation, a fragment of the cytoplasmic tail of Plexin-A1 was subcloned into the *Escherichia coli* expression vector, pGEX-4T2 (see Fig. 4a). The fragment chosen corresponded to the 149 amino acid stretch of Plexin-B1 previously defined as the minimal region sufficient for interaction with Rac (26). The GST fusion protein (GST-Plexin-A1/frag) was used in a slot blot *in vitro* GTPase binding assay using [γ-32P]GTP-bound Rac. GST-PAK/CRIB was used as a positive control. As shown in Fig. 4, c and d, Rac-GTP interacts with the cytoplasmic tail of Plexin-A1.

**vsv-Plexin-A1-GGA Is unable to Mediate Collapse but Still Activates Rac following Sema3A-Fc Stimulation**—Plexin-A1 does not contain an obvious CRIB motif (24, 37), but a triple substitution mutant (vsv-Plexin-A1-GGA) was constructed analogous to a Plexin-B1 mutant (Plexin-B1-GGA) previously shown to no longer interact with Rac (see Fig. 5a) (24). Cells expressing NP-1 together with either vsv-Plexin-A1 or vsv-Plexin-A1-GGA were stimulated with Sema3A-Fc. In cells expressing NP-1 and vsv-Plexin-A1-GGA, only 7% of cells with bound Sema3A-Fc collapsed compared with 85% of cells expressing NP-1 and vsv-Plexin-A1 (see Fig. 5, b–d). This effect was not a consequence of vsv-Plexin-A1-GGA failing to reach the cell surface as unpermeabilized cells displayed a strong staining of vsv-Plexin-A1-GGA and efficient interaction with Sema3A-Fc (data not shown).

To determine whether or not vsv-Plexin-A1-GGA was still capable of mediating Sema3A-Fc-induced Rac activation, pull-down assays were performed. Cells were transfected with NP-1, either alone or in combination with vsv-Plexin-A1 or vsv-Plexin-A1-GGA. These experiments demonstrated that Sema3A-Fc was able to induce Rac activation in cells expressing NP-1 and vsv-Plexin-A1-GGA (see Fig. 5c).

**Potential Autoinhibitory Interactions within the Cytoplasmic Tail of Plexin-A1**—The discovery that Plexin-B1 and Plexin-A1 interact with Rac-GTP raises the possibility that these receptors may be targets of Rac. A characteristic feature of many Rho GTPase effector proteins, *e.g.* WASP or p65PAK, is the...
presence of an autoinhibitory interaction (38–42) between sequences located in the N- and C-terminal parts of the protein, which is relieved upon GTPase binding.

The possibility that a similar interaction might occur within the cytoplasmic tail of Plexin-A1 was explored using immunoprecipitation experiments. Fragments of Plexin-A1 and Plexin-B1 were subcloned into the mammalian expression vectors, pRK5FLAG or pRK5Myc (see Fig. 6a), and cells were transfected with various combinations. Immunoprecipitations were performed using mouse anti-Myc or mouse anti-FLAG antibodies, and precipitated proteins were assessed by Western blot analysis. Blots were probed with mouse anti-Myc and mouse anti-FLAG antibodies. A strong interaction could be seen between the N-terminal (PH1a) and C-terminal (PH2a) cytoplasmic sequences of Plexin-A1 (see Fig. 6a, b, and c). Similar interactions could be seen using the corresponding fragments of Plexin-B1 (Fig. 6, b, and c). This finding demonstrates the potential for an intramolecular interaction within the cytoplasmic tail of Plexin family members or an intermolecular interaction between homodimerized receptors.

The GGA triple point mutation prevents Plexin-A1 from mediating Sema3A-Fc-induced collapse and inhibits Rac binding to Plexin-B1 (24). To determine whether this affects the proposed intra/intermolecular interactions, the PH2a segment of Plexin-A1 was co-expressed with a PH1a-GGA segment. As seen in Fig. 6d, PH1a-GGA still interacted with Myc-Plexin-A1 PH2a, although the level of this interaction was reduced. The Rac-binding region of Plexin-B1 has been mapped to the very end of the fragment corresponding to PH1a shown in Fig. 6a. The fragments lacking this region (PH1aΔRBD) were made from Plexin-A1 and Plexin-B1 (28 and 24 amino acid deletions, respectively) and co-expressed with fragment PH2a. As seen in Fig. 6e, the PH2a fragment of Plexin-A1 was unable to associate with PH1aΔRBD. A similar result was found with the corresponding Plexin-B1 fragments (Fig. 6f). In addition, to examine whether the cytoplasmic regions of Plexin-A1 can interact through an intermolecular interaction, the cytoplasmic tail was subcloned into pRK5FLAG and pRK5Myc (see Fig. 6g). As seen in Fig. 6g, after co-transfection, FLAG-Plexin-A1(cyt) can be efficiently co-precipitated with Myc-Plexin-A1(cyt).

Finally, to determine whether or not active GTPases influence the proposed intra/intermolecular interactions, the PH1a and PH2a segments of Plexin-A1 were co-expressed with either constitutively activated Cdc42 (FLAG-L61Cdc42) or Rac (FLAG-L61Rac1). The presence of FLAG-L61Cdc42 had no effect on the interaction between Plexin-A1 PH1a and PH2a, whereas the addition of FLAG-L61Rac1 significantly reduced the amount of FLAG-Plexin-A1 PH1a precipitated (see Fig. 7).

**DISCUSSION**

It has been reported previously that Rac plays a role in signaling downstream of Sema3A in the growth cone collapse assay of primary neurons (20–22). Here we show that Rac activity is required during Sema3A-induced collapse of COS-7 cells expressing the Plexin-A1 and NP-1 co-receptors and furthermore that Rac is activated following Sema3A stimulation. Intriguingly, however, collapse induced by constitutively active Plexin-A1 does not require Rac activity. Therefore, it appears that Rac is not required for receptor-induced collapse per se but is required for a ligand-induced collapse. One possible way to account for this is that Plexin-A1 is a Rac target and that Rac is required for receptor activation. This explanation fits well...
with our observations that (i) the cytoplasmic tail of Plexin-A1 interacts directly with Rac-GTP, (ii) the cytoplasmic region of Plexin-A1 can form intramolecular or intermolecular interactions through sequences located in the N- and C-terminal parts of the tail that overlap with the region required for Rac binding, and (iii) constitutively activated Rac can inhibit this intra/intermolecular interaction. Many of the characterized Rho GTPase targets such as p65PAK, WASp, and mDia are known to form autoinhibitory intramolecular interactions, which can be relieved upon binding of an active GTPase. Therefore, we propose that the binding of Rac-GTP relieves an autoinhibitory conformation of the cytoplasmic tail of Plexin-A1.

The requirement for Rac but not Rho or p160ROCK downstream of Sema3A suggests that the collapse we observe in COS cells is not a consequence of excessive actin/myosin contractile forces driven by Rho. Others have reached the same conclusion using neurons (43). To date, many of the ideas surrounding neuronal morphogenesis such as growth cone collapse and neurite extension have assumed that Rho acts antagonistically to Rac/Cdc42, the former producing contraction/retraction responses and the latter producing protrusive/extension processes (14, 45, 46). Thus lysophosphatidic acid-induced neurite retraction is mediated by Rho (47) as is ephrin-A5-induced growth cone collapse (29). The observation that Sema3A-induced collapse requires active Rac suggests that this simple model may be an oversimplification. Indeed, Fan et al. (48) have provided in vivo evidence that Rac is required in slit-mediated midline repulsion in Drosophila, whereas Journey et al. (49) and Marston et al. (50) demonstrate the involvement of Rac in ephrin-A2- and ephrin-B2-induced repulsive responses, respectively. It appears that growth cone guidance may be a more complicated process than a simple Rho/Rac antagonism at the level of the actin cytoskeleton.

The activation of Rac by Sema3A that we describe here is in contrast to reports by others that stimulation of another plexin family member, Plexin-B1, by its ligand Sema4D does not result in Rac activation (51). In addition, others (24, 25, 32)
have failed to detect an association of Rac with Plexin-A1. The reasons for these discrepancies are not known. Through the use of the Plexin-A1-GGA mutant, we do know that Rac activation is not a consequence of the collapse-induced response. As for the direct interaction between Plexin-A1 and Rac, this is clearly much more difficult to detect than the interaction of Rac with some of its better-characterized targets such as FAK. However, in our hands it is comparable to the interaction seen between Rac and Plexin-B1. Sequence analysis demonstrates that unlike Plexin-B1, Plexin-A1 does not contain a recognizable CRIB motif. However, three critical amino acids (LVP) within the CRIB region of Plexin-B1 are conserved at a similar location in Plexin-A1. These residues have been previously shown to be required for the association between Plexin-B1 and Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24). Mutation of these residues in Plexin-A1 (Plexin-A1-GGA) produces a receptor that is unable to mediate Sema3A-induced Rac (24).
