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Regulation of neurite morphogenesis by interaction between R7 regulator of G protein signaling complexes and G protein subunit Ga\(_{13}\)

Stephanie L. Scherer, Matthew D. Cain, Stanley M. Kanai, Kevin M. Kaltenbronn, and Kendall J. Blumer

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The R7 regulator of G protein signaling family (R7-RGS) critically regulates nervous system development and function. Mice lacking all R7-RGS subtypes exhibit diverse neurological phenotypes, and humans bearing mutations in the retinal R7-RGS isoform RGS9-1 have vision deficits. Although each R7-RGS subtype forms heterotrimeric complexes with G\(_{\alpha}\)-subunits, several neurological phenotypes of R7-RGS knock-out mice are not readily explained by dysregulated G\(_{\alpha}\) signaling. Accordingly, we used tandem affinity purification and LC-MS/MS to search for novel proteins that interact with R7-RGS heterotrimers in the mouse brain. Among several proteins detected, we focused on Ga\(_{13}\) because it had not been linked to R7-RGS complexes before. Split-luciferase complementation assays indicated that Ga\(_{13}\) in its active or inactive state interacts with R7-RGS heterotrimers containing any R7-RGS isoform. LARG (leukemia-associated Rho guanine nucleotide exchange factor (GEF)), PDZ-RhoGEF, and p115RhoGEF augmented interaction between activated Ga\(_{13}\) and R7-RGS heterotrimers, indicating that these effectors can engage Ga\(_{13}\)-R7-RGS complexes. Because Ga\(_{13}\)-R7-RGS interaction required R7BP, we analyzed phenotypes of neuronal cell lines expressing RGS7 and G\(_{\beta}\) with or without R7BP. We found that neurite retraction evoked by Ga\(_{12/13}\)-dependent lyso phosphatidic acid receptors was augmented in R7BP-expressing cells. R7BP expression blunted neurite formation evoked by serum starvation by signaling mechanisms involving Ga\(_{12/13}\) but not Ga\(_{13}\). These findings provide the first evidence that R7-RGS heterotrimers interact with Ga\(_{13}\) to augment signaling pathways that regulate neurite morphogenesis. This mechanism expands the diversity of functions whereby R7-RGS complexes regulate critical aspects of nervous system development and function.

Regulator of G protein signaling (RGS) proteins regulate the amplitude and kinetics of G protein-coupled receptor signaling by functioning in part as GTPase-activating proteins (GAPs) that accelerate the rate that G protein \(\alpha\)-subunits hydrolyze GTP to GDP (1). Among ~30 mammalian proteins containing an RGS domain, the R7 regulator of G protein signaling family (R7-RGS), consisting of RGS6, RGS7, RGS9, and RGS11, is particularly important. R7-RGS proteins are highly expressed in the nervous system and exhibit GAP activity in vitro only for K. J. B. (2–7). Humans bearing mutations in the retinal RGS9-1 isoform exhibit a vision deficit termed bradypopsis (8), and mice lacking selected or all R7-RGS proteins exhibit various neurological phenotypes manifested by impairment of perinatal viability, weight gain, retina structure and function, neurobehavioral development, motor coordination, cerebellar and hippocampal development, and analgesic response to opioids (9–12), thereby establishing these regulators as crucial players in neurological development and function.

Evidence suggests that R7-RGS proteins have diverse mechanistic functions beyond serving as Ga\(_{\alpha}\)-specific GAPs. First, in contrast to several other classes of RGS proteins that are GAPs for G\(_{\alpha}\)-subunits (13), R7-RGS proteins are structurally complex. Each R7-RGS isoform possesses N-terminal disheveled, Egl-10, and pleckstrin (DEP), DEP helical extension (DHEX), and G protein \(\gamma\)-like (GGL) domains followed by a C-terminal RGS domain that is necessary and sufficient for GAP activity. The GGL domain binds the most diverged member of the G\(_{\beta}\) family, G\(_{\beta}\) (4, 14), to form obligate heterodimeric complexes structurally similar to classical G\(_{\beta}\)y dimers (15). The DEP domain interacts with either of two SNARE-like membrane anchor proteins (16–21), R7-RGS-binding protein (R7BP) and RGS9 anchor protein (R9AP), to form R7-RGS heterotrimers. Whereas R9AP is a transmembrane protein local-

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ized to photoreceptor disk membranes, R7BP is reversibly and dynamically palmitoylated to regulate plasma membrane localization of R7-RGS heterotrimers throughout much of the nervous system (17, 22–24). Second, as shown in Caenorhabditis elegans, the N-terminal DEP/DHEX domain rather than the RGS domain of R7-RGS proteins determines which neurobiological processes mediated by distinct G proteins are regulated (25). Third, RGS7-Gβ5 and RGS9-2-Gβ5 complexes, respectively, can attenuate Gq-coupled type 3 muscarinic or Gi/o-coupled type 3 dopamine receptors by Gα-dependent mechanisms (19, 21), similar to other structurally complex G proteins that serve as multifunctional regulators, integrators, and/or effectors in signaling networks (26, 27). Lastly, RGS6 can scaffold DNA methyltransferase 1 to the acetyltransferase Tip60 to promote DNA methyltransferase 1 degradation in response to Ras activation in mouse embryonic fibroblasts (28, 29).

Despite such evidence, whether the membrane anchor R7BP is required for functions of R7-RGS complexes other than Gαi/o-specific GAP activity has not been investigated. Here we have addressed this question by using a proteomics approach to identify proteins that interact with R7BP-bound R7-RGS complexes in mouse brain. Our results expand understanding of R7BP-bound R7-RGS complexes by indicating that they facilitate Gαi/o signaling to regulate neurite morphogenesis.

**Results**

**RGS7, Gβ5, and R7BP form macromolecular complexes larger than simple heterotrimers in neuronal cells**

If R7BP-bound R7-RGS proteins have novel functions, they would be expected to participate in novel protein–protein interactions. Accordingly, we investigated whether RGS7, Gβ5, and R7BP exist in macromolecular complexes larger than simple heterotrimers in living cells. We used Neuro2a (N2a) cells endogenously expressing RGS7 and Gβ5 and stably transfected with N-terminally FLAG-tagged R7BP at physiologically normal levels (5) that were treated with vehicle or disaccharimindyl suberate (DSS), a cell-permeable, amine-reactive cross-linker. Cell lysates were immunoprecipitated with FLAG antibody, resolved via SDS-PAGE, and immunoblotted to detect Gβ5, the obligate core subunit of all R7-RGS complexes. As expected if additional proteins associate with R7-RGS heterotrimers, this approach detected several distinct species with relative molecular weights larger than R7-RGS heterotrimers (Fig. 1A). These experiments also detected non-cross-linked Gβ5 and cross-linked R7-RGS-Gβ5-FLAG-R7BP heterotrimers (Fig. 1A). These results are consistent with an independent study in which complexes containing R7BP were detected in lysates of transfected HEK293T cells and mouse brain lysates cross-linked with paraformaldehyde (30).

**Generation of transgenic mice expressing tandem affinity purification (TAP)-tagged R7BP**

To identify potentially novel proteins that associate with R7BP-containing R7-RGS heterotrimers, we isolated these complexes from brain tissue by affinity purification and identified proteins recovered with these complexes by liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS). This approach was motivated by prior studies showing that the membrane anchor R9AP and transducin α-subunits co-purify with RGS9-1-Gβ5 heterodimers in photoreceptor membranes (20) and that an orphan G protein-coupled receptor (GPR158) associates with RGS7-Gβ5 heterodimers in brain lysates (18, 31). However, our approach was novel because it identified proteins that associate with heterotrimers containing any R7-RGS isofrom, Gβ5, and R7BP.

We developed a TAP strategy in which the FLAG-StrpII-StrepII tag (32) was appended to the N terminus of R7BP (SF-R7BP; Fig. 1B) and generated transgenic mice expressing this protein specifically in neurons. This was accomplished by driving SF-R7BP expression from the mouse prion promoter upon integration of the transgene in single copy at the X-linked Hprt locus (33, 34) (Fig. 1B). As indicated by immunoblotting with anti-R7BP antibodies, SF-R7BP and endogenously expressed R7BP were expressed similarly in various brain regions and were undetectable in heart and kidney (Fig. 1C). Importantly, SF-R7BP was not overexpressed relative to endogenous R7BP, reducing concern of identifying biologically irrelevant protein–protein interactions due to overexpression. SF-R7BP protein expression profiles were similar in three independently derived transgenic lines, all of which were used for TAP experiments. In all subsequent experiments, SF-R7BP-expressing adult male mice were used to avoid potentially mosaic expression of SF-R7BP due to X chromosome inactivation in females.

**Tandem affinity purification and LC-MS/MS analysis of R7BP-containing protein complexes**

R7BP-containing protein complexes were isolated from the combined lysates of three SF-R7BP adult male mouse brains as detailed under “Experimental procedures.” The purification...
procedure was optimized to achieve low nonspecific protein binding as indicated by SYPRO Ruby staining of TAP eluates from control non-transgenic mice relative to SF-R7BP transgenic mice (Fig. 2A). Moreover, the purification conditions preserved R7-RGS heterotrimers as indicated by immunoblotting showing that Gβ5 and RGS7 co-purified with SF-R7BP (Fig. 2B).

Proteins that co-purified with R7-RGS heterotrimers were identified by resolving TAP FLAG eluates on SDS-PAGE, excising and extracting SYPRO Ruby-stained gel bands, and digesting with Glu-C and trypsin (Fig. 2C). The resulting peptides were sequenced by LC-MS/MS, and the corresponding proteins were identified as described under “Experimental procedures.” Detailed peptide and protein identification data are summarized in Table 1 and supplemental Table 1. As expected, SF-R7BP (denoted in Table 1 as Rgs7bp protein), Gβ5, and three of the four R7-RGS isoforms (RGS11 was not detected, presumably due to limited expression in brain) were identified (Table 1 and supplemental Table 1). Gαq, which is a substrate for the GAP function of R7-RGS heterotrimers, also was identified with high confidence.

**Gαq is a novel binding partner of R7-RGS heterotrimers**

Gαq was identified with high confidence in our LC-MS/MS analysis of proteins that co-purified with SF-R7BP-bound R7-RGS complexes (Table 1 and supplemental Table 1). Similarly, Go13 was identified in TAP-LC-MS/MS experiments using N2a cells expressing SF-R7BP (data not shown). Although unique peptides diagnostic of other Ga isoforms or classical Gβγ subunits were not detected, we cannot exclude that these proteins were present in R7-RGS complexes bound to SF-R7BP.

Recovery of Go13 was intriguing for two reasons. First, Go13 is not a substrate for the GAP function of R7-RGS heterotrimers that is specific in vitro for Gαi/o subunits (2–4). Therefore, co-purification of Go13 with R7-RGS complexes suggested that R7-RGS heterotrimers potentially influence the function of this Ga subunit by GAP-independent mechanisms. Second, mice deficient in all R7-RGS heterotrimers due to knock-out of the shared obligate subunit Gβγ have abnormal dendritic morphology as seen in retinal ON-bipolar and Purkinje neurons (10, 11). Because Go13 is a well established regulator of the actin cytoskeleton, which regulates dendritic morphogenesis, a functional relationship between R7-RGS heterotrimers and Go13 might account in part for the dendritic morphology phenotypes of Gβ5−/− mice. Accordingly, the remainder of the present study focused on the interaction between R7-RGS heterotrimers and Go13.

To provide independent evidence whether Go13 can associate with R7-RGS heterotrimers, we adopted split-luciferase complementation assays to assess protein-protein interactions in living cells (35, 36). Because split-luciferase complementation apparently had not been used before with Ga subunits, we first determined whether this technique is appropriate for our purposes. We inserted the N-terminal fragment of click beetle green luciferase (CBGN; Ref. 36) into the αB-αC loop of the helical domain in wild-type and constitutively active (i.e. GTPase-defective Gln → Leu (Q/L) mutants) forms of Gaq and Go13, because insertion of GFP at this site preserves Ga function (37). The C-terminal fragment of click beetle green luciferase (CBGC) was fused to the N termini of the following proteins: RGS2 (CBGC-RGS2), a GAP for Gaq but not Go13; the RH-RGS domain of LARG (CBGC-LARG-RGS), a GAP for Go13 but not Gaq; and Gβ3 (CBGC-Gβ3). These constructs were used to determine whether split-luciferase complementation detects specific and activity-dependent interaction between Gaq or Go13 and their cognate binding partners in intact cells.

This expectation was confirmed by results of luciferase assays performed with HEK293 cells co-transfected with various combinations of split luciferase-tagged proteins (Fig. 3A). Wild-type CBGN-tagged Gaq or Go13 complemented strongly with CBGC-Gβ3 co-transfected with Gγ2. Constitutively active GaqQ/L-CBGN complemented strongly with CBGC-LARG-RGS but not CBGC-RGS2. Likewise, constitutively active Go13Q/L-CBGN complemented strongly with CBGC-RGS2 but not CBGC-LARG-RGS. Wild-type Go13-CBGN and Go13-CBGN complemented insignificantly, respectively, with CBGC-LARG-RGS and CBGC-RGS2. Thus, split-luciferase complementation detected specific protein-protein interactions determined by Ga subunit identity and activation state.

Split-luciferase complementation was then used to study interaction between Go13 and R7-RGS heterotrimers and to determine whether this interaction is affected by the activity state of Go13. In these experiments, HEK293 cells were cotransfected with CBGC-FLAG-Gβ5/Go13-HA-RGS7 with or without FLAG-R7BP, various Ga-CBGN constructs, and Renilla luciferase as a transfection control. When co-expressed with HA-RGS7 and FLAG-R7BP, CBGC-FLAG-Gβ5 strongly complemented with either wild-type or constitutively active Go13-CBGN but not with wild-type or constitutively active Go13-CBGN (Fig. 3B). These results confirmed that R7-RGS heterotrimers and Go13 interact in a specific manner rather than by nonspecific collisional interaction within the plane of the plasma membrane and indicated that this interaction is independent of Go13 activation. These interactions required the
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Figure 3. R7BP is required to detect interaction between Go13 and R7-RGS complexes containing any R7-RGS family member. A, proof of concept that split-luciferase complementation detects protein-protein interactions specific for Go subunit identity and activity state. Data are represented as the fold difference in normalized split-luciferase signal relative to the positive control for each Go-CBGN construct. Data shown are the average of three independent experiments. B, split-luciferase complementation between the indicated Go-CBGN proteins and CBGC-Gβ, RGS7 with or without R7BP. Each box represents a data point from one of three independent experiments. C, split-luciferase complementation between Go13(Q/L)-CBGN and CBGC-FLAG-Gβo, co-expressed with each R7-RGS isoform with or without R7BP. Luciferase signals were normalized to the level of CBGC-3xFLAG-Gβo, expression determined by immunoblotting. Each box represents a data point from one of five independent experiments. D, representative FLAG Western blot (WB) showing CBGC-3xFLAG-Gβo, and 3xFLAG-R7BP expression for each condition in C. The band marked with an asterisk is a degradation product of CBGC-3xFLAG-Gβo, E, Go13(Q/L)-CBGN-EE expression does not change with co-expression of R7-RGS heterotrimer proteins. Shown are representative immunoblots for C. Error bars indicate standard deviation of the mean. Statistical significance was determined using two-tailed, two-sample Student’s t test assuming equal or unequal variance as deemed appropriate by F-test analysis. *p < 0.05; **p < 0.1; ***p < 0.001; n.s., p > 0.05.

presence of R7BP (Fig. 3B), presumably because palmitoylated R7BP targets R7-RGS-Gβo complexes to the plasma membrane where Go13 localizes.

Next we used split-luciferase complementation to determine whether complexes containing any R7-RGS isoform can interact with Go13 or whether particular R7-RGS family members are preferred. For these experiments, HEK293 cells were transfected with Go13(Q/L)-CBGN and CBGC-FLAG-Gβo with various R7-RGS isoforms in the presence or absence of R7BP. Because the proteolytic stability of Gβo varies depending on which R7-RGS isoform is expressed in the presence or absence of R7BP and because luciferase signals depend on the expression level of CBGC-FLAG-Gβo, we normalized split-luciferase signals in these experiments to the expression of CBGC-FLAG-Gβo determined by immunoblotting (Fig. 3D). Normalization for Go13(Q/L)-CBGN expression was unnecessary because this protein was well expressed with or without RGS7 or R7BP as indicated by immunoblotting of glutamate-glutamate (EE)-

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R7-RGS regulation of neurite morphogenesis

Figure 4. R7BP augments neurite retraction evoked by activation of G\(_{12/13}\)-coupled LPA receptors or serum starvation. Rhodamine-phalloidin staining and fluorescence microscopy were used to visualize neurites and cell morphology. Cells were scored by counting the number of neurites elaborated from the cell body that were greater than half the cell body diameter. At least 50 cells were scored per condition per experiment. A, representative fluorescence images of parental N2a cells transfected with either GFP (control) or HA-p115-RGS and treated with vehicle (veh) or 10 \(\mu\)M LPA. Control experiments confirmed antibody staining specificity (data not shown). B, quantification of neurites formed per cell under conditions shown in A. Data shown are the average of three independent experiments. C, number of cells with neurites under conditions shown in A. Each box represents a data point from one of three independent experiments. D, representative fluorescence images of parental N2a or N2a cells stably expressing FLAG-R7BP treated with the indicated concentrations of LPA. E, quantification of neurite formation under conditions shown in D. Each box represents a data point from one of four independent experiments. Scale bars, 50 \(\mu\)m. Error bars indicate standard deviation of the mean. Statistical significance was determined using two-tailed, two-sample Student’s t test assuming equal or unequal variance as deemed appropriate by F-test analysis. *, \(p < 0.05\); **, \(p < 0.01\). Proj., projection.

tagged G\(\alpha_{13}(Q/L)\)-CBGN (Fig. 3E). Data analyzed in this way showed efficient luciferase complementation when G\(\alpha_{13}(Q/L)\)-CBGN was co-expressed with CBGC-FLAG-G\(\beta_5\), R7BP, and any R7-RGS family member, whereas complementation was low in the absence of either an R7-RGS subunit or R7BP (Fig. 3C). Therefore, G\(\alpha_{13}\) interacted in living cells with heterotrimers bearing R7BP, G\(\beta_5\), and any R7-RGS isoform.

**R7-RGS heterotrimers augment neurite retraction evoked by G\(\alpha_{13}\)-coupled lysophosphatidic acid (LPA) receptors**

To determine whether R7-RGS heterotrimers affect biological processes driven by G\(\alpha_{13}\) and its close paralog G\(\alpha_{13}\), we took advantage of studies showing that G\(\alpha_{12/13}\) signaling causes growth cone collapse and neurite retraction in cultured neurons and neuronal cell lines (38–44). Accordingly, we determined whether R7-RGS heterotrimers affect neurite retraction in differentiated N2a cells. N2a cells were chosen as a model because they elaborate neurites when differentiated by serum starvation, and expression of constitutively active G\(\alpha_{13}\) or RhoA in these cells antagonizes neurite formation. To address this question, we differentiated N2a cells by serum starvation and treated them with LPA for 15 min. After fixation and permeabilization, cells were stained with rhodamine-phalloidin to visualize neurites and actin organization. We found that LPA treatment robustly evoked neurite retraction (Fig. 4, A–C). Because LPA receptors can couple to G\(_{12/13}\), G\(_{q/11}\), G\(_s\), and G\(_{12/13}\) (45), we determined whether LPA-induced neurite retraction in differentiated N2a cells is mediated by G\(_{12/13}\) signaling. To address this question, we inhibited G\(_{12/13}\) signaling by overexpressing a truncated HA-tagged version of p115-RhoGEF (HA-p115-RGS) that is targeted to the plasma membrane by a prenylation motif and retains GAP activity for G\(_{12/13}\) but cannot activate RhoA (46). Expression of HA-p115-RGS blocked LPA-induced neurite retraction in differentiated N2a cells (Fig. 4, A–C), indicating that G\(_{12/13}\) signaling is indeed required for this process.

Based on the preceding results, we investigated whether R7-RGS heterotrimers can regulate LPA-induced neurite retraction. Because R7BP facilitates interaction between R7-RGS complexes and G\(\alpha_{13}\), we compared the effects of LPA on N2a cells, which express RGS7 and G\(\beta_5\), but lack R7BP (5), with N2a cells stably expressing FLAG-R7BP at physiological levels (5). Results indicated that LPA-evoked neurite retraction was augmented in cells expressing FLAG-R7BP (Fig. 4, D and E), suggesting that R7BP-bound R7-RGS complexes augment signaling by G\(_{12/13}\)-coupled LPA receptors.
R7-RGS regulation of neurite morphogenesis

In the preceding experiments, we also noted that, prior to LPA stimulation, R7BP expression antagonized neurite formation evoked by serum starvation alone (Fig. 4, D and E). To explore the mechanisms involved, we tested two models. In the first model, R7BP expression enables R7-RGS complexes to augment serum starvation-evoked G_{12/13} signaling, which attenuates neurite formation. If so, inhibiting G_{12/13} activity would be expected to rescue neurite formation in N2a cells stably expressing FLAG-R7BP. To test this prediction, FLAG-R7BP-expressing N2a cells were transiently transfected with HA-p115-RGS or a GFP-expressing control plasmid, differentiated, and analyzed for neurite formation. Results indicated that neurite formation trended higher but was incompletely rescued in cells transiently expressing HA-p115-RGS as compared with controls (Fig. 5, A and B; p = 0.24). Thus, R7BP-bound R7-RGS heterotrimers appeared to regulate neurite morphogenesis under basal conditions in serum-starved cells by mechanisms that depend partly on G_{12/13} signaling.

One potential explanation for the preceding result is provided by a second model in which R7BP-bound R7-RGS complexes regulate neurite morphogenesis in serum-starved cells in part by attenuating G_{12/13} signaling. Indeed, G_{12/13}-coupled cannabinoid type 1 receptor signaling promotes neurite outgrowth in N2a cells (47, 48), which potentially could be negatively regulated by the GAP activity of R7BP-bound R7-RGS complexes (17, 23, 49–51). However, whether G_{12/13} activity in N2a cells promotes neurite formation in response to serum starvation had not been tested. We found that this appeared not to be the case because blocking G_{12/13} activity with pertussis toxin (PTX) did not inhibit neurite formation evoked by serum starvation (Fig. 5, C and D) in contrast to control experiments demonstrating that PTX treatment strongly inhibited the ability of a cannabinoid type 1 agonist (WIN 55,212-2) to blunt forskolin-evoked cAMP accumulation detected with a FRET reporter (Fig. 5, E and F). Therefore, these results argued against models in which R7BP-bound R7-RGS complexes promote neurite retraction by attenuating G_{12/13} signaling.

Lastly, we explored how R7BP augments G_{13}-dependent neurite retraction by studying G_{13}-activated RhoGEFs (LARG, p115RhoGEF, and PDZ-RhoGEF) that mediate RhoA activation and consequent neurite retraction. Our initial approach relied on prior investigations showing that these RhoGEFs can be recruited to the plasma membrane by activated G_{13} (46). Thus, if R7BP-bound R7-RGS complexes promote G_{13} signaling, then expression of R7BP in N2a cells might be expected to augment plasma membrane recruitment of these RhoGEFs. However, by confocal imaging, we were unable to detect differences in plasma membrane recruitment of GFP-tagged LARG, p115RhoGEF, or PDZ-RhoGEF in response to LPA and/or R7BP expression (data not shown).

As an alternative approach that might provide greater sensitivity, we determined whether RhoGEFs affect interaction between constitutively active G_{13} and R7BP-bound R7-RGS complexes in split-luciferase complementation assays. Rather than assessing G_{13} activity, this approach addressed the key question whether complexes containing activated G_{13} and R7-RGS heterotrimeric proteins are capable of physically engaging effector RhoGEFs that trigger RhoA activation and neurite retraction. Accordingly, HEK293 cells were transfected with G_{13}(Q/L)-CBGN, CBGC-FLAG-G_{13} β_{7}, RGS7, and R7BP in the absence or presence of Myc-tagged forms of LARG, p115RhoGEF, or PDZ-RhoGEF. As shown in Fig. 6, split-luciferase complementation resulting from interaction between activated G_{13} and R7BP-bound RGS7-G_{13} β_{7} complexes was increased ~7-fold by LARG, 4-fold by PDZ-RhoGEF, and 2-fold by p115RhoGEF. Accordingly, the results of these and preceding experiments support models in which R7BP-bound R7-RGS complexes interact with G_{13} in neuronal cells to augment signaling mechanisms that promote neurite retraction and blunt neurite formation.

Discussion

Our findings are the first to indicate that R7-RGS heterotrimers form macromolecular complexes with G_{13} and con-
control neurite morphogenesis regulated in part by G_{12/13}-dependent signaling. Accordingly, they expand the functional diversity of R7-RGS heterotrimers beyond their canonical roles as GAPs for G_{i/o}. Because genetic ablation of R7-RGS complexes affects diverse aspects of neurological development and function (9–12), our findings suggest that R7-RGS heterotrimers potentially regulate certain processes in part by augmenting G_{13} signaling rather than solely by attenuating G_{i/o} signaling. Indeed, certain phenotypes of mice lacking R7-RGS heterotrimers are not recapitulated in mice expressing RGS-insensitive mutant forms of G_{13} or G_{i} (52).

Several prior lines of evidence are consistent with the hypothesis that R7-RGS heterotrimers regulate G_{12/13} signaling to control nervous system development. R7-RGS heterotrimers are dramatically up-regulated during postnatal brain development as synapses mature (5, 53). Mice lacking all R7-RGS complexes exhibit abnormal Purkinje cell maturation and dendritic arborization, delayed morphological development of the hippocampus, and increased ectopic granule cells in the hippocampus (10). These postnatal neurological phenotypes potentially could be caused in part by diminished G_{12/13} signaling, which controls similar processes during embryonic brain development. For example, conditional ablation of G_{13}, and its paralog G_{12}, in the nervous system causes overmigration of cortical plate and Purkinje neurons in embryonic mice (41). Accordingly, increased ectopic granule cells in the hippocampus of mice lacking all R7-RGS complexes might arise from overmigration of these cells because G_{13} activity is reduced and fails to provide sufficiently strong migratory “stop” signals, similar to what occurs for cortical plate neurons and cerebellar Purkinje cells in embryonic G_{13}−/− mice. Further evidence linking G_{13} and R7-RGS complexes is provided by the observation that G_{13} mRNA is up-regulated in cerebellum of 2-week-old postnatal mice lacking all R7-RGS complexes (11) potentially to compensate for diminished G_{13} signaling activity caused by the absence of R7-RGS complexes.

Establishing the biological functions of G_{13} signaling regulated by R7-RGS heterotrimers will require understanding the molecular mechanisms involved. Although much remains to be learned, we suggest two mechanistic hypotheses (Fig. 7), which are not mutually exclusive; other models also can be imagined. Both models are consistent with our findings that R7-RGS heterotrimers interact with G_{13} regardless of its activity state and that this interaction is augmented by G_{13}-regulated RhoGEFs. In the first model, R7BP-bound R7-RGS heterotrimers interact with G_{13} to facilitate RhoGEF signaling that promotes neurite retraction (46). Consistent with this mechanism, targeting p115RhoGEF to the plasma membrane by appending a prenylation motif triggers neurite retraction in PC12 cells (46), which express R7BP and R7-RGS complexes (55). In a second model, R7-RGS heterotrimers are proposed to augment neurite retraction by inhibiting deactivation of G_{13} via the GAP activity of RGS domain-containing RhoGEFs. This model is sus-
gested by evidence that certain RGS domains possess allosteric inhibitory sites distal to the surface that binds Ga subunits to stimulate GTP hydrolysis (56, 57). Whether such inhibitory sites exist in the RGS domains of Ga12/13-activated RhoGEFs and are targeted by R7-RGS complexes remains to be investigated. Distinguishing between these two models would require determining whether R7BP-bound R7-RGS complexes regulate the activation or deactivation of Ga13 and RhoGEFs in vitro.

Whether Ga13 signaling is facilitated similarly by complexes containing any R7-RGS isoform is unclear. Although we found that Ga13 can interact with heterotrimeric containing any R7-RGS subtype, our functional studies were limited to N2a cells in which RGS7 appears to be the principal R7-RGS isoform expressed. Indeed, biological processes mediated by distinct G protein subtypes can be regulated by distinct R7-RGS isoforms as indicated by studies of the R7-RGS proteins Egl-10 and Eat-16 in C. elegans (25). Thus, it would be important to determine whether Ga13 signaling also can be augmented by R7-RGS heterotrimers bearing RGS6, -9, or -11.

R7-RGS heterotrimers also may control neurite morphogenesis by regulating signaling mechanisms independent of Ga13. This hypothesis is suggested by our finding that the neurite morphogenesis defect of serum-starved R7BP-expressing N2a cells was rescued incompletely by expression of the Ga13 inhibitor HA-p115-RGS. In contrast, LPA-evoked neurite retraction absolutely required Ga13 signaling as indicated by complete blockade of this process by HA-p115-RGS. Further studies will be required to determine precisely how R7-RGS heterotrimers regulate neurite morphogenesis by Ga13-dependent and -independent mechanisms.

In conclusion, we have identified a novel interaction between R7-RGS heterotrimeric and Ga13, and provided evidence that R7-RGS heterotrimers augment the function of Ga13 as a regulator of neurite morphogenesis. Our findings expand understanding of R7-RGS heterotrimeric as multifunctional complexes critical for diverse aspects of neuronal development and function.

Experimental procedures
Reagents and antibodies
The following commercially produced antibodies were used: mouse anti-GAPDH (GeneTex, catalog number GTX627408, lot 40953), mouse anti-FLAG M2 (Sigma, catalog number F1804, various lots), mouse anti-actin C4 (Millipore, catalog number MAB1501, lot NG1812617), mouse anti-EE (Covance, catalog number MMS-115P, lot E12BF00285), mouse anti-HA.11 (Covance, catalog number MMS-101R), mouse anti-Myc 9E10 (Covance, catalog number MMS-150R, lot 139023001), HRP-conjugated goat anti-mouse IgG (Thermo Scientific, catalog number 31430, various lots), HRP-conjugated goat anti-rabbit IgG (Thermo Scientific, catalog number 31460, various lots), and Alexa Fluor 488-conjugated goat anti-mouse IgG (Invitrogen, catalog number A11001, lot 481679).

Our rabbit anti-R7BP polyclonal antibody has been described (5). Rabbit anti-Ga5 (ATDG) and rabbit anti-RGS7 (R4613) antibodies were generous gifts from Drs. William Simon and Theodore Wensel, respectively (58, 59). Other reagents were as follows: Complete protease inhibitor mixture tablets (Roche Applied Science, catalog number 11697498001), mouse anti-FLAG M2-agarose (Sigma, catalog number A-2220), DSS (Thermo Scientific, catalog number 21555), StrepTactin Superflow (IBA, catalog number 2-1206-002), d-desthiobiotin solution (IBA, catalog number 2-1000-025), 3xFLAG peptide (Sigma, catalog number F4799), SYPRO Ruby stain (Sigma, catalog number S4942), Dual-Luciferase Reporter Assay System (Promega, catalog number E1960), LPA (Avanti Polar Lipids, catalog number 857130P), rhodamine-conjugated phalloidin (Life Technologies, catalog number R415), poly-d-lysine (Sigma, catalog number P0899), pertussis toxin (Calbiochem, catalog number 516561), forskolin (Sigma, catalog number F6886), and WIN 55,212-2 (Sigma, catalog number W102). All remaining reagents were from Sigma unless noted otherwise.

Generation of SF-R7BP transgenic mice
All procedures involving mice were conducted under protocols approved by the Animal Studies Committee of Washington University School of Medicine. To generate mice expressing SF-R7BP in neurons, a vector was generated to target SF-R7BP under control of the mouse prion promoter to the Hprt locus on the X chromosome. The backbone of the targeting vector was a modified version of pHPR1-targeting-Tet-EGFP-protein-entry (generously provided by Dr. Andrey Shaw) in which everything between the HPR1-targeting arms (including the Tet promoter and GFP) was replaced with a multiple cloning site and a PGK-Neo cassette. A transgene consisting of the mouse prion protein (MoPRP) promoter, MoPRP 5’-intron, sequence, SF-R7BP, and MoPRP 3’-untranslated region was inserted in reverse orientation in the multiple cloning site of the parent targeting vector. The MoPRP sequences were originally from the MoPrP-Xhol plasmid (34), and the SF tag sequence was originally from N-SF-TAP-pcDNA3 (a gift from Dr. Christian Johannes Gloeckner; Ref. 60). Standard methods were used to transfet ES cells with the targeting construct by electroporation (Washington University Murine Embryonic Stem Cell Core). Homologous recombination of the transgene causes deletion of exon 1 of Hprt, resulting in loss of HPRT protein expression and resistance to 6-thioguanine. Homologous recombinants were selected using both 4418 and 6-thioguanine. ES cells that survived the selection process were confirmed as homologous recombinants by PCR using a forward primer specific for the PGK-Neo cassette on the transgene and a reverse primer specific for an HPRT genomic sequence outside of the right targeting arm. ES cells that were positive in the PCR test and had a normal karyotype were used for injection into C57BL/6 mouse blastocysts (Washington University School of Medicine, Department of Pathology Microinjection Core Facility) to generate chimeric mice. Chimeric male mice were bred with female C57BL/6 mice (Charles River) to produce offspring that were screened for germ line transmission by PCR. Mice positive for the SF-R7BP transgene were crossbred with R7BP+/− mice (50) to generate R7BP−/− offspring that
also contained the SF-R7BP transgene. These offspring were subsequently crossed to generate R7BP−/− mice that were positive for the SF-R7BP transgene. Because endogenous R7BP is not expected to affect the makeup of the protein interaction profile, SF-R7BP male mice with both R7BP+/− and R7BP−/− backgrounds were used for TAP experiments based on availability.

**Cell culture and transfection**

All cells were maintained in DMEM/F-12 (Gibco, catalog number 11330-032) supplemented with 10% (v/v) FBS (Atlanta Biologicals, catalog number S11150) and penicillin/streptomycin at 37 °C with 5% CO₂ in a humidified incubator. Growth medium for 3xFLAG-R7BP stable cells also contained 200 μg/ml Geneticiin (Gibco, catalog number 10131). Chemical cross-linking experiments used a previously described clonal derivative of N2a cells stably expressing 3xFLAG-R7BP (5). Microscopy experiments used N2a cells transfected with 3xFLAG-R7BP (17) and treated with 400 μg/ml Geneticiin for 16 days to select cells that stably express 3xFLAG-R7BP. Transfections were performed using TransIT-T1 transfection reagent (Mirus, catalog number MIR 2305) according to the manufacturer’s protocol.

**Plasmids**

pcDNA3.1+ was the backbone for all constructs unless stated otherwise. CBGN-Gα fusion sequences were made using overlap extension PCR to insert a glycine/serine linker (GGGSSGGG) followed by CBGN and another glycine/serine linker (GGGSSGGG) into Gα13 and Gαq, between residues 135 and 136 and between residues 124 and 125, respectively. Split-luciferase fragments were amplified from plasmids containing fragments of click beetle green 99 luciferase (CBGN, amino acids 2–412; CBGC, amino acids 396–542; generous gifts from Dr. David Piwnica-Worms; Ref. 36). Human Gα13 and Gαq were amplified from plasmids purchased from Missouri University of Science and Technology cDNA Resource Center. Constitutively active point mutants (Q226L) of Gα13-CBGN and Gαq-CBGN were generated using site-directed mutagenesis. Gα13-CBGN-EE was generated using overlap extension PCR to mutate Gα13 amino acids 188–193 from DYIPSQ to EYMPTE. CBGC-LARG-RGS was created using overlap extension PCR to insert a glycine/serine linker (GGGSSGGG) followed by CBGN and another glycine/serine linker (GGGSSGGG) into Gα5 (Gα5s) was inserted into the multiple cloning site of pcDNA3.1+. The coding sequence of the short isoform of human Gβ5 (Gβ5s) was inserted in-frame into this plasmid. A plasmid that expresses plasma membrane-localized HA-p115-RGS was constructed by using overlap extension PCR to fuse sequences encoding a glycine/serine linker followed by the last 20 amino acids of human H-Ras to the C terminus of HA-p115-RGS (amino acids 1–252 of human p115RhoGEF). The plasmid expressing 3xFLAG-R7BP has been described (17). All newly described constructs were verified by DNA sequencing. Published plasmids expressing *Renilla* luciferase and HA-tagged R7-RGS proteins were generous gifts from Drs. David Piwnica-Worms and T. Kendall Harden, respectively. Plasmids expressing Myc-LARG, His-Myc-PDZ-RhoGEF, and Myc-p115RhoGEF were generous gifts from Dr. Tohru Kozasa. The plasmid expressing Epac-SH1187-cAMP FRET sensor was generously provided by Dr. Kees Jalink (61).

**In-cell chemical cross-linking**

Wild-type N2 cells or N2a cells stably expressing 3xFLAG-R7BP were trypsinized, pelleted, and washed twice with PBS. Cross-linking reagents were prepared just before use by making a 200 mM stock of DSS in DMSO and then diluting into 100 mM HEPES (pH 8) for a final working solution of 1 mM DSS in 5% (v/v) DMSO. Cells were suspended in 1 mM DSS solution at 2.2 × 10⁶ cells/ml and incubated for 30 min at room temperature. Cross-linking reactions were quenched by adding 20 mM Tris (pH 7.4) and incubating for 15 min at room temperature. Cells were then pelleted and lysed for immunoprecipitation.

**Immunoblotting and immunoprecipitation**

The following immunoprecipitation protocol was used for the data shown in Fig. 1A. Cells were lysed in radioimmuno precipitation assay buffer (150 mM NaCl, 10 mM sodium phosphate, 1% (w/v) sodium deoxycholate, 1% (v/v) IGEPAL CA-630, 0.5% (v/v) SDS, 1 mM DTT, Complete protease inhibitor mixture) by agitation for 30 min at 4 °C and then cleared by ultracentrifugation at 100,000 × g for 10 min at 4 °C. Cross-linked species containing 3xFLAG-R7BP were immunoprecipitated by incubating with mouse anti-FLAG M2-agarose for 2 h at 4 °C followed by three washes with radioimmuno precipitation assay buffer and eluted by boiling with 2× SDS-PAGE sample buffer (50 mM Tris (pH 6.8), 2% (w/v) SDS, 10% (v/v) glycerol, 1% (v/v) 2-mercaptoethanol, 12.5 mM EDTA, 0.02% (w/v) bromphenol blue) for 10 min.

Tissue lysates for experiments shown in Fig. 1C were generated by the following protocol. Mice were euthanized by asphyxiation with CO₂ followed by cervical dislocation. The indicated tissues were isolated, and brains were further dissected in Hanks’ balanced salt solution (Gibco, catalog number 14175) followed by homogenization in TAP lysis buffer (30 mM Tris (pH 7.4), 150 mM NaCl, 0.5% (v/v) IGEPAL CA-630, 0.5% (v/v) deoxycholate, Complete protease inhibitor mixture) using a Dounce homogenizer and then transferred to tubes and rotated end-over-end for 30 min at 4 °C. Lysates were cleared by ultracentrifugation at 100,000 × g for 15 min at 4 °C. A Bradford assay was performed to determine total protein content in the cleared lysates to equalize protein loading for SDS-PAGE.

All Western blot analyses were performed according to the following protocol. Samples were separated by SDS-PAGE, transferred to PVDF (Millipore, catalog number IPVH00010), blocked for at least 1 h using 5% (w/v) milk in TBST (25 mM Tris (pH 7.2), 150 mM NaCl, 2.7 mM KCl, 0.1% (v/v) Tween 20), incubated with the indicated primary antibody in blocking
buffer for at least 2 h, washed three times with TBST, incubated for 1 h with appropriate HRP-conjugated secondary antibodies diluted in blocking buffer, and washed three times with TBST. Signals were detected using ECL solution (GE Healthcare, catalog number RPN2106, or Bio-Rad, catalog number 170-5060) and either film or a ChemiDoc Imaging System (Bio-Rad). In some cases after image acquisition, membranes were rinsed, reblocked, and probed for another protein. All quantified Western blot signals were within the linear range of the detection system as determined by an independent standard curve.

**Tandem affinity purification**

TAP was performed as described previously with following modifications (32). Three adult male mice expressing SF-R7BP (one R7BP−/− and two R7BP+/−) were euthanized by asphyxiation with CO2 followed by cervical dislocation. All subsequent steps were performed on ice or at 4 °C. Whole brains were dissected, homogenized with a Dounce homogenizer in lysis buffer (30 mM Tris (pH 7.4), 150 mM NaCl, 0.5% (v/v) IGEPAL CA-630, 0.5% (v/v) deoxycholate, Complete protease inhibitor mixture), rotated end-over-end for 30 min, cleared by ultracentrifugation at 100,000 × g for 15 min, and cleared further by passing through a 0.22-μm polyethersulfone filter. Cleared lysates were incubated with StrepTactin resin overnight with end-over-end rotation and washed five times in batch with a 10× column volume of wash buffer (30 mM Tris (pH 7.4), 150 mM NaCl, 0.1% (v/v) IGEPAL CA-630, Complete protease inhibitor mixture). Protein complexes were eluted two consecutive times by incubating with 3× column volumes of elution buffer (1× desthiobiotin buffer E (IBA), 0.1% (v/v) IGEPAL CA-630, Complete protease inhibitor mixture) for 30 min in batch. StrepTactin elution fractions were combined and incubated with anti-FLAG M2-agarose in batch for 1 h and washed five times in batch with 10× column volumes of wash buffer. Protein complexes were eluted from FLAG-agarose by incubating with 4× column volumes of FLAG elution buffer (200 μg/ml 3xFLAG peptide in wash buffer) for 30 min in batch. The final eluate was concentrated in preparation for gel electrophoresis using an Amicon Ultra-0.5 10,000 centrifugal filter device (Millipore, catalog number UFC501008).

**LC-MS/MS**

Mass spectrometry analysis was performed by the Proteomics and Mass Spectrometry Facility at the Donald Danforth Plant Science Center (St. Louis, MO). The final TAP eluate described above was resolved by SDS-PAGE, and protein bands were visualized with SYPRO Ruby (Fig. 2C). Prominent bands were excised, and proteins were digested using trypsin and Glu-C and analyzed by LC-MS/MS using a 1-h gradient and an LTQ-Orbitrap mass spectrometer. Peak lists were generated using Mascot Distiller version 2.4. Mass spectrometric data were searched using Mascot version 2.4.1 search engine and NCBInr Mammalia database (April 2013 version; 1,392,029 entries). The following parameters were used: trypsin and Glu-C allowed proteases, two missed cleavages allowed, no fixed modifications allowed, +16 on Met (oxidation) allowed as a variable modification, 15-ppm mass tolerance for precursor ions, and 0.80-Da mass tolerance for fragment ions. The database cRAP_20110301 (118 entries) was used to exclude known contaminants. Scaffold (version 4.0.5) was used to validate peptide and protein identities. Peptide identifications were accepted if they could be established at greater than 80% probability by the Scaffold local false discovery rate algorithm. All reported proteins have at least 99% protein identification probabilities as determined using the Protein Prophet algorithm and at least two exclusive unique peptides assigned. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Proteins sharing significant peptide evidence were grouped into clusters. Glu-C, trypsin, and keratin proteins were removed manually from the results. Reported are the proteins identified from a single TAP experiment starting with three SF-R7BP mouse brains.

**Split-luciferase complementation**

HEK293 cells were plated in 24-well dishes and transfected the next day with the indicated split-luciferase plasmids, *Renilla* luciferase for signal normalization, GFP to assess transfection efficiency, and pcDNA to normalize total DNA amounts across samples. At least three replicate wells of cells were transfected per condition in each independent experiment. Luciferase assays were performed 24–26 h after transfection using the Dual-Luciferase Reporter Assay System according to the manufacturer’s protocol. A GloMax96 luminometer equipped with two injection pumps was used to measure luciferase activity on a per-well basis by first injecting the substrate solution for CBG and then injecting a quencher for click beetle green as well as the substrate for *Renilla* luciferase. Data collection started 0.4 s after substrate addition with a 10-s integration time.

**Fluorescence microscopy**

For microscopy experiments using HA-p115-RGS, cells were transfected and then replated at the next day at low density on poly-D-lysine (PDL)-coated glass coverslips. For experiments not requiring transfection, cells were simply plated at low density on PDL-coated glass coverslips. Three hours after plating, the medium was exchanged for differentiation medium (DMEM/F-12 plus penicillin/streptomycin). For experiments using PTX, cells were plated at low density on PDL-coated glass coverslips and treated with 100 ng/ml PTX overnight. The next morning, the medium was exchanged for differentiation medium containing 100 ng/ml PTX, and this was repeated again 24 h later. In all cases, cells were allowed to differentiate for 48 h. If indicated, cells were treated with various concentrations of LPA (in 5 mg/ml fatty acid-free BSA in DPBS) for 15 min prior to fixing. Cells were fixed by incubation with 4% paraformaldehyde in DPBS (pH 7.2) for 10 min at room temperature followed by washes with DPBS. Fixed cells were permeabilized with 0.5% (v/v) Triton X-100 in DPBS for 10 min at room temperature, washed with DPBS, and blocked with blocking buffer (1% (w/v) BSA, 0.1% (v/v) Tween 20 in DPBS) for at least 30 min at room temperature. If immunostaining was required, coverslips were incubated with primary antibody (diluted in blocking buffer) in a humidified chamber overnight at 4 °C, washed three
times with wash buffer (0.1% v/v) Tween 20 in DPBS), and incubated with appropriate Alexa Fluor-conjugated secondary antibody and rhodamine-conjugated phalloidin in a humidified chamber for 1 h at room temperature. For experiments that did not require immunostaining, rhodamine-phalloidin was incubated with the coverslips for 30 min in a humidified chamber at room temperature immediately following the permeabilization step. All coverslips were washed three times with wash buffer, rinsed briefly in water, and mounted on glass slides using Vectashield mounting medium. Microscopy images were acquired with an Olympus IX81 inverted fluorescence microscope equipped with a UPlanSApo 60× oil objective (numerical aperture, 1.35) and an Exi BLUE digital camera (Q Imaging). Images were acquired using MetaMorph for Olympus Advanced and processed using Fiji software. Brightness and contrast were adjusted on an entire-image basis. Images of rhodamine-phalloidin fluorescence were used to score neurite projections, defined as any process extending from the cell body that was longer than half the cell body length. Cell body lengths were measured using the line tool, and neurite lengths were measured using the segmented line tool in Fiji.

**cAMP FRET sensor assay**

N2a cells were transfected with the Epac-3H187/CAMP FRET sensor. After 24 h, cells were trypsinized and replated in a black-well clear-bottom 96-well plate (Costar, catalog number 3603) coated with poly-d-lysine. Cells were incubated in serum-free medium with or without 100 ng/ml pertussin toxin for 16–24 h. Prior to imaging, growth medium was replaced with pre-warmed (37 °C) imaging buffer (125 mM NaCl, 5 mM KCl, 1.5 mM MgCl2, 1.5 mM CaCl2, 10 mM d-glucose, 20 mM HEPES (pH 7.4)) and assayed immediately using a Synergy H4 Hybrid Reader (BioTek) equipped with injection pumps for adding forskolin and WIN 55,212-2. Cells were maintained at 37 °C throughout the recording period. FRET donor was excited using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter. Donor emission and acceptor emission were detected every 10 s using a xenon flash lamp and a 420/20 bandpass filter.

$$\Delta F = \frac{\text{FRET ratio} - \text{baseline FRET ratio}}{\text{baseline FRET ratio}}$$

where FRET ratio = (480 nm<sub>emission</sub>/540 nm<sub>emission</sub>) at a given time point and the baseline FRET ratio = average (480 nm<sub>emission</sub>/540 nm<sub>emission</sub>) prior to forskolin stimulation. Triplicate wells were assayed per condition in each independent experiment.

**Author contributions**—S. L. S. designed and performed experiments, analyzed data, and wrote the paper. M. D. C. developed the split-luciferase system used in Figs. 3 and 6. K. M. K. designed, generated, and maintained SF-R7BP transgenic mice and performed experiments shown in Fig. 6. S. M. K. designed and performed experiments shown in Fig. 5, E and F. K. J. B. conceived and directed the study and wrote the paper. All authors approved the final version of the manuscript.

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**References**


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Regulation of neurite morphogenesis by interaction between R7 regulator of G protein signaling complexes and G protein subunit Gα13

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