The Interaction of RGSZ1 with SCG10 Attenuates the Ability of SCG10 to Promote Microtubule Disassembly

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RGS proteins (regulators of G protein signaling) are a diverse family of proteins that act to negatively regulate signaling by heterotrimeric G proteins. Initially characterized as GTPase-activating proteins for Gα subunits, recent data have implied additional functions for RGS proteins. We previously identified an RGS protein (termed RGSZ1) whose expression is quite specific to neuronal tissue (Glick, J. L., Meigs, T. E., Miron, A., and Casey, P. J. (1998) J. Biol. Chem. 273, 26008–26013). In a continuing effort to understand the role of RGSZ1 in cellular signaling, the yeast two-hybrid system was employed to identify potential effector proteins of RGSZ1. The microtubule-destabilizing protein SCG10 (superior cervical ganglia, neural specific 10) was found to directly interact with RGSZ1 in the yeast system, and this interaction was further verified using direct binding assays. Treatment of PC12 cells with nerve growth factor resulted in Golgi-specific distribution of SCG10. A green fluorescent protein-tagged variant of RGSZ1 translocated to the Golgi complex upon treatment of PC12 cells with nerve growth factor, providing evidence that RGSZ1 and SCG10 interact in cells as well as in vitro. Analysis of in vitro microtubule polymerization/depolymerization showed that binding of RGSZ1 to SCG10 effectively blocked the ability of SCG10 to induce microtubule disassembly as determined by both turbidimetric and microscopy-based assays. These results identify a novel connection between RGS proteins and the cytoskeletal network that points to a broader role than previously envisioned for RGS proteins in regulating biological processes.

The mechanisms by which G proteins transduce signals from the external milieu to internal targets are complex, and multiple control points exist where this transduction process may be regulated (1, 2). In resting cells, G proteins associate with guanine nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs) and are maintained in an inactive state. Upon binding ligand, receptors induce G protein-coupled receptors to catalyze the exchange of GDP for GTP on Gα subunits, activating the Gα subunit which then forms a heterotrimer with Gβγ subunits. The α subunit then binds to effectors, in most cases GTPase-activating proteins (GAPs), to catalyze the hydrolysis of GTP to GDP and subsequent inactivation of the Gα subunit. However, GAPs have been shown to regulate many other cellular processes, including microtubule dynamics, cell migration, and cell division. The interaction of RGSZ1 with SCG10 is therefore likely to have implications for microtubule dynamics and other cellular processes.

The hypothesis that RGS proteins can bind cellular proteins in addition to Gα subunits has been widely investigated. Recent findings indicate that some RGS proteins do in fact contain a variety of motifs that are important for protein-protein interactions as well as membrane attachment. For example, the R7 family of RGS proteins (consisting of RGS6, RGS7, RGS9, and RGS11) contains a DEP (dishevelled/Egl-10/pleckstrin) domain and a novel GGL (G protein γ subunit-like) domain. The DEP domain is found in a variety of proteins and has been hypothesized to play a role in targeting DEP-domain-containing proteins to G protein-coupled receptors. GGL domains have been shown to be responsible for selective binding to Gβγ, a Gβγ isoform quite divergent from Gβ1-γ, RGS7 and RGS11.

The abbreviations used are: RGS, regulators of G protein signaling; GAP, GTPase-activating protein; DEP, dishevelled/Egl-10/pleckstrin; GGL, G protein γ subunit-like; SCG10, superior cervical ganglia, neural specific 10; MAP, microtubule-associated protein; PIPES, 1,4-piperazinediethanesulfonic acid; GST, glutathione S-transferase; DTT, dithiothreitol; GFP, green fluorescent protein; NGF, nerve growth factor.

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Taken together, these data suggest that portions outside the heterotrimeric G protein signaling and the cytoskeleton that RGSZ1 was transformed into yeast strain PJ69 (superior cervical ganglia, neural specific 10), as a novel bind-
identify a microtubule-destabilizing protein, termed SCG10
G preparations were stored in 80 mM PIPES (pH 6.9), 0.5 mM EGTA, and /H9004
minal truncation mutant (denoted
thought to play a role in membrane attachment (16
preparations were stored in 80 mM PIPES (pH 6.9), 0.5 mM EGTA, and /H9004
°
proteins. Additionally, the RZ family members (RGSZ1,
were purified as His-tagged proteins as previously described (17). The cDNA encoding GAIP was obtained from
°
50 mM glutathione. Eluted GST-SCG10, glutathione-Sepharose beads (Amersham Biosciences)
°
H9004
preferred as a His-tagged protein following a protocol similar to that described for the purification of RGSZ1. Purified RGS proteins were analyzed for GAP activity for Gr (RGSZ1) and Gr (GAIP and RGS10) and in all cases were found to be functionally active (data not shown).

**Protein-Protein Interaction Studies**—For pull-down experiments using purified proteins, His-tagged RGS proteins (5 μg) were incubated with GST or GST-tagged proteins (GST-SCG10 or GST-SCG10) each at 5 μg in 50 mM Tris-HCl (pH 7.7), 1 mM EDTA, 1 mM DTT, 150 mM NaCl, 10 mM imidazole, and 0.1% Lubrol for 4 h at 4 °C with shaking. Glutathione-Sepharose beads were added, and the suspension was allowed to incubate for an additional 1 h. Bound proteins were precipitated by centrifugation and washed three times with the same buffer. The precipitated protein was then isolated, resuspended in 10 mM Tris-HCl buffer (pH 7.7), 10 mM imidazole, and 0.025% Lubrol for 30 and 5 minutes, respectively, in 30-μl reactions containing 50 mM HEPPS (pH 7.7), 1 mM EDTA, 1 mM DTT, 1.7 mM MgCl2, and 330 mM GTP; additional conditions are indicated in the figure legends. Reactions were terminated by addition of 770 μl of Norit A charcoal slurry (5% (w/v) in 50 mM NaH2PO4), and released phosphate was quantified by liquid scintillation counting.

**Turbidimetric Evaluation of Microtubule Assembly and Disassembly**—The assembly/disassembly of tubulin was monitored using a light scattering assay as previously described (31). For the microtubule assembly assays, MAP-rich tubulin (3.25 mg/ml final concentration) in PEM buffer containing 1 mM GTP at 4 °C was mixed with SCG10, RGSZ1, or a combination of these proteins. Microtubule assembly was initiated by raising the temperature to 37 °C, and the absorbance at 350 nm was monitored over 15 min in a Hewlett-Packard Model 8453 spectrophotometer. The preparation of microtubules was as described above, and the temperature was maintained at 37 °C. SCG10, RGSZ1, or a combination of the two proteins was added, and the absorbance at 350 nm was monitored over 10 min. As a test for microtubule assembly experiments, in which the effects of SCG10 and RGSZ1 together were tested, the two proteins were preincubated at 4 °C for 1 h prior to use.
Fluorescence Analysis of Rhodamine-labeled Tubulin Disassembly—MAP-free tubulin and rhodamine-labeled tubulin (Cytokeleton Inc., Denver, CO) were premixed at a ratio of 4:1 (unlabeled/labeled) to a final concentration of 3.25 mg/ml in PEM buffer containing 2 mM GTP at 37 °C for 30 min to form microtubules. GST-SCG10 (5 μM) alone, GST-SCG10 (5 μM) and RGS10 (10 μM), or buffer was then added to the mixture and incubated at 37 °C for 10 min. Reactions (10 μl) were diluted in 50 μl of 60% glycerol and gently mixed. A 4-μl aliquot was then placed on a glass slide with a coverslip, and microtubule patterns were observed by fluorescence microscopy using a Nikon Eclipse TE300 inverted microscope.

Cell Culture and Immunofluorescence Analysis—PC12 cells were cultured as previously described (32). Transient transfections of PC12 cells were performed using LipofectAMINE® reagent (Invitrogen) according to the manufacturer’s recommendations. Cells destined for transfection with GFP constructs were plated on chambered coverslips coated with poly-d-lysine. Cells were treated with nerve growth factor (NGF) (Promega) for the indicated times and placed in phosphate-buffered saline, and live cells were viewed using the Nikon Eclipse TE300 inverted microscope. Cells destined for immunofluorescence analyses were plated on poly-d-lysine-coated glass coverslips and treated with NGF as described above prior to processing.

For immunofluorescence analysis, cells were fixed in 4% paraformaldehyde in phosphate-buffered saline for 20 min and rinsed two times with phosphate-buffered saline. Cells were blocked in phosphate-buffered saline containing 10% goat serum, 2% bovine serum albumin, and 0.3% Tween 20 for 30 min and subsequently incubated with anti-SCG10 primary antibodies overnight at 4 °C in the same buffer. After three rinses with the same buffer, the cells were incubated with Cy3-conjugated goat anti-rabbit secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 1 h at 25 °C. Cells were mounted using Prolong® antifade mounting medium (Molecular Probes, Inc., Eugene, OR) and viewed using the Nikon Eclipse TE300 inverted microscope.

RESULTS

Yeast Two-hybrid Screen of RGSZ1—In our ongoing efforts to elucidate the roles of RGSZ1 in cellular signaling, a yeast two-hybrid screen was performed. We initially attempted to conduct screens using full-length RGSZ1 as the bait, but it alone acted as a transcriptional activator in our system, rendering it unsuitable for screening purposes (data not shown). However, we were successful using the C-terminal 130 residues of RGSZ1 (ΔRGSZ1) as bait. This truncation mutant lacked the cysteine string and contained only the RGS domain of RGSZ1. After construction of the bait plasmid, yeast strain PJ69–2a was transformed and evaluated for bait protein expression, toxic effects of the protein, and transcriptional activation in the absence of a binding partner. These initial control studies suggested that ΔRGSZ1 would be an effective bait molecule in the screen (data not shown). The actual screen employed a pre-transformed cDNA library derived from fetal human brain, as RGSZ1 is predominantly found in brain (17, 18). The library contained a total of 3.5 × 108 clones, and the screen yielded ∼5.8 × 103 total transformants, representing a 1.7-fold over-screen of the library.

The yeast two-hybrid screen yielded a total of 415 positives, the inserts of which were isolated and sequenced. Evaluation of the cDNA sequences revealed that 47 inserts encoded the same protein, all of which act to destabilize microtubules. Neither null bait (empty vector) nor false bait (the N-terminal region of protein farnesyltransferase) demonstrated any binding to RGSZ1, suggesting that the interaction between ΔSCG10 and ΔRGSZ1 is largely nonspecific. Taken together, these data indicate that the N-terminal domain of SCG10 is important for the interaction with RGSZ1.

To examine specificity of the RGSZ1-SCG10 interaction, RGS10 (a member outside the RZ family of RGS proteins) and GAIP (a member within the RZ family) were tested for their abilities to bind SCG10. Pull-down experiments similar to those used to show RGSZ1 binding revealed that GAIP also bound to SCG10, whereas RGS10 did not (Fig. 1B). Both

![Fig. 1. Binding of RGS proteins to SCG10 in vitro.](http://www.jbc.org/)

A

B

GST

GST-ΔSCG10

GST-SCG10

RGSZ1

ΔRGSZ1

GST

GST-SCG10

GST-ΔSCG10

RGS10

ΔRGS10

SCG10

ΔSCG10

GAIP

ΔGAIP

49

36

25

n

m

p

q

r

s

RGSZ1 Interaction with SCG10

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RGSZ1 and GAIP contain a region rich in cysteines referred to as a cysteine string. This region serves as a site for palmitoylation, post-translational modification involved in membrane anchoring, trafficking, and protein-protein interactions (16). Although the analysis of RGS proteins was not totally inclusive, it appears that SCG10 may specifically interact with the RZ family of RGS proteins.

We also examined whether the binding of SCG10 affects the ability of RGSZ1 to accelerate GTP hydrolysis of Goα. To date, the only known biological function of RGSZ1 is to act as a GAP for Goα, so it seemed plausible that the interaction with SCG10 may have some effect on the ability of RGSZ1 to act as a GAP. However, even using concentrations of SCG10 that were 10-fold greater than those of RGSZ1, no effect on the GAP activity of RGSZ1 was observed (data not shown). This result suggests that the binding of these two proteins occurs in such a way that the critical residues responsible for the ability of RGSZ1 to act as a GAP are unaffected, thus allowing unperturbed interaction with the Goα protein.

Localization of GFP-RGSZ1 and SCG10 in PC12 Cells—As noted in the Introduction, expression of SCG10, like that of RGSZ1, is predominantly confined to neuronal tissue. Expression of SCG10 has also been detected in certain neuron-like cell lines such as PC12 cells. In addition, it has been shown that treatment of PC12 cells with NGF induces the expression of SCG10 (20). Using antibodies directed against SCG10 in immunoblot and immunofluorescence protocols, we confirmed that expression of the protein is indeed up-regulated by NGF treatment (Fig. 2, A–C). Consistent with previous reports, endogenous SCG10 was localized to the Golgi region of the cells as well as to the growth cones of the extending neurites (Fig. 2, B and C). Taking advantage of these findings, we transfected PC12 cells with either GFP-RGSZ1 or GFP-ΔRGSZ1 and examined their localization before and after NGF stimulation. Although RGSZ1 has been reported to localize to the Golgi complex in certain cells (18), expression of the GFP-RGSZ1 fusion protein in PC12 cells resulted in a predominantly diffuse cytosolic expression pattern. GFP-ΔRGSZ1, which lacks the cysteine string motif thought to direct the membrane localization of RGSZ1, also exhibited cytosolic localization in untreated PC12 cells. However, treatment of PC12 cells with NGF resulted in a striking redistribution of GFP-RGSZ1 to a punctate staining pattern that closely resembles Golgi staining patterns in PC12 cells (Fig. 2G). It must be noted that the transfection efficiency of PC12 cells was extremely low, normally achieving only 3–5% of the total cell population. However, it was observed that in all experiments performed, 50–75% of the cells that expressed GFP-RGSZ1 exhibited the redistribution pattern presented in Fig. 2G. Importantly, although GFP-RGSZ1 translocated in response to NGF treatment, the localization of GFP-ΔRGSZ1, which interacted only poorly with SCG10, was unaffected by NGF treatment (Fig. 2, D and E). Taken together, these results suggest that production of Golgi-associated SCG10 causes GFP-RGSZ1 to translocate from the cytosol to the Golgi because of an interaction between the two proteins. Influence of RGSZ1 Binding on the Ability of SCG10 to Modulate Microtubule Assembly and Disassembly—A well-characterized activity of SCG10 is its ability to prevent microtubule assembly (34). To evaluate the effect of RGSZ1 binding on this activity of SCG10, an in vitro assay of microtubule assembly was employed. Incubation of soluble tubulin at 37 °C in the presence of GTP results in its polymerization into microtubules, and this process can be followed using a turbidimetric assay (31). Consistent with previous results (34), addition of SCG10 resulted in a dose-dependent decrease in tubulin polymerization under the assay conditions (Fig. 3A). To evaluate the effect of RGSZ1 in this system, an intermediate concentration of SCG10 (5 μM) was chosen, and the assays were repeated under varying concentrations of added RGSZ1. However, even at concentrations as high as 40 μM, RGSZ1 had no discernible effect on the ability of SCG10 to attenuate microtubule assembly (Fig. 3B).

In addition to its ability to prevent microtubule assembly, SCG10 is also capable of initiating microtubule disassembly both in vitro (34) and in intact cells (26). Although the precise mechanism by which SCG10 triggers microtubule disassembly is not clear, this process can also be monitored using the turbidimetric assay described above. Under conditions in which PC12 cells were transfected with GFP-ΔRGSZ1 (D and E) or GFP-RGSZ1 (F and G) were either left untreated (D and F) or treated with 50 ng/ml NGF for 48 h (E and G). Live cells were subsequently viewed using a Nikon Eclipse TE300 inverted microscope, and results are from a single experiment that is representative of three separate experiments.

![Figure 2: Localization of SCG10 and GFP-RGSZ1 in PC12 cells](http://www.jbc.org/)

**Fig. 2. Localization of SCG10 and GFP-RGSZ1 in PC12 cells.** A, analysis of SCG10 expression in PC12 cells. Control PC12 cells (−NGF) or PC12 cells treated with 50 ng/ml NGF (+NGF) were harvested and processed by SDS-PAGE and immunoblot analysis using anti-SCG10 antibody as described under “Experimental Procedures.” B and C, immunolocalization of SCG10 in PC12 cells. PC12 cells were plated on poly-D-lysine-coated coverslips and either left untreated (B) or treated with 50 ng/ml NGF (C) for 48 h. Cells were fixed in 4% paraformaldehyde and stained for SCG10 using a mouse monoclonal antibody generated against SCG10. D–G, localization of GFP-RGSZ1 in PC12 cells. PC12 cells transfected with GFP-ΔRGSZ1 (D and E) or GFP-RGSZ1 (F and G) were either left untreated (D and F) or treated with 50 ng/ml NGF for 48 h (E and G). Live cells were subsequently viewed using a Nikon Eclipse TE300 inverted microscope, and results are from a single experiment that is representative of three separate experiments.

**Localization of SCG10 and GFP-RGSZ1 in PC12 cells**—As noted in the Introduction, expression of SCG10, like that of RGSZ1, is predominantly confined to neuronal tissue. Expression of SCG10 has also been detected in certain neuron-like cell lines such as PC12 cells. In addition, it has been shown that treatment of PC12 cells with NGF induces the expression of SCG10 (20). Using antibodies directed against SCG10 in immunoblot and immunofluorescence protocols, we confirmed that expression of the protein is indeed up-regulated by NGF treatment (Fig. 2, A–C). Consistent with previous reports, endogenous SCG10 was localized to the Golgi region of the cells as well as to the growth cones of the extending neurites (Fig. 2, B and C). Taking advantage of these findings, we transfected PC12 cells with either GFP-RGSZ1 or GFP-ΔRGSZ1 and examined their localization before and after NGF stimulation. Although RGSZ1 has been reported to localize to the Golgi complex in certain cells (18), expression of the GFP-RGSZ1 fusion protein in PC12 cells resulted in a predominantly diffuse cytosolic expression pattern. GFP-ΔRGSZ1, which lacks the cysteine string motif thought to direct the membrane localization of RGSZ1, also exhibited cytosolic localization in untreated PC12 cells. However, treatment of PC12 cells with NGF resulted in a striking redistribution of GFP-RGSZ1 to a punctate staining pattern that closely resembles Golgi staining patterns in PC12 cells (Fig. 2G). It must be noted that the transfection efficiency of PC12 cells was extremely low, normally achieving only 3–5% of the total cell population. However, it was observed that in all experiments performed, 50–75% of the cells that expressed GFP-RGSZ1 exhibited the redistribution pattern presented in Fig. 2G. Importantly, although GFP-RGSZ1 translocated in response to NGF treatment, the localization of GFP-ΔRGSZ1, which interacted only poorly with SCG10, was unaffected by NGF treatment (Fig. 2, D and E). Taken together, these results suggest that production of Golgi-associated SCG10 causes GFP-RGSZ1 to translocate from the cytosol to the Golgi because of an interaction between the two proteins.
tubulin was initially polymerized and subsequently treated with SCG10, it was observed that SCG10 promoted the disassembly of assembled microtubules in a concentration-dependent manner (Fig. 4A). Strikingly, addition of RGSZ1 blocked SCG10-induced microtubule disassembly in a dose-dependent manner (Fig. 4B). In fact, microtubule disassembly induced by 5 μM SCG10 was almost totally ablated upon addition of 10 μM RGSZ1. Similar experiments were also conducted using ΔSCG10. Although ΔSCG10 was also capable of initiating microtubule disassembly, RGSZ1 had no effect on this response (data not shown). This finding corresponds well to the in vitro binding data indicating that RGSZ1 interacts only with full-length SCG10.

In addition to the rather indirect method of monitoring microtubule formation using light scattering, methods have been developed to visualize this process directly using fluorescence microscopy. Using rhodamine-labeled tubulin in in vitro assembly systems, microtubule formation can be viewed directly by fluorescence microscopy (21). Incubation of rhodamine-labeled tubulin with GTP at 37 °C induced the assembly of microtubules (Fig. 5A), whereas incubation in the absence of GTP at 4 °C prevented microtubule assembly, as indicated by the diffuse staining pattern (Fig. 5D). Subsequent addition of 5 μM SCG10 resulted in almost complete loss of microtubule structure, which coincided with an increase in the more homogeneous fluorescence pattern of soluble tubulin (Fig. 5B). However, preincubation of SCG10 with a 2-fold excess of RGSZ1 markedly attenuated the ability of SCG10 to induce microtubule disassembly (Fig. 5C). Together with the data obtained from the turbidimetric studies, these data confirm that the binding of RGSZ1 blocks the ability of SCG10 to induce microtubule disassembly. It is interesting to note that there appears to be an increased number of high density microtubule areas upon treatment with both SCG10 and RGSZ1. At present, it is unclear why this occurs, but it may be that the two proteins act together as a nucleating point for microtubule formation.

**DISCUSSION**

Our search for novel interactors of RGSZ1 led to the identification of SCG10 as directly interacting with RGSZ1. Although recent evidence points to RGS proteins as playing roles in addition to serving as GAPs for heterotrimeric G proteins (for
Microtubules were incubated with buffer (A), 5 μM GST-SGD10 (B), or a combination of 5 μM GST-SGD10 and 10 μM RGSZ1 (C) for 10 min at 37 °C. Microtubule disassembly was monitored by fluorescence microscopy using the same conditions for each image. The data are from a single experiment that is representative of four separate experiments. As a control (D), rhodamine-labeled tubulin (3.25 mg/ml) was incubated at 4 °C without GTP for 30 min and viewed as described above.

review, see Refs. 4 and 6), this seems to be the first indication that an RGS protein may directly regulate cytoskeletal organization.

SCG10 binds quite selectively to full-length RGSZ1 compared with the truncated form ΔRGSZ1, which lacks the N terminus of the protein. This is somewhat surprising because the two-hybrid screen was conducted using only the core domain (ΔRGSZ1) as bait. However, the ability of this system to detect very weak interactions provides a possible explanation as to why this was observed (35, 36). The N terminus of SCG10 was also found to be essential for RGSZ1 binding. This region contains two cysteine residues (positions 22 and 24) that are key for directing SCG10 to Golgi membranes (27, 37). Deletion of this region totally ablated the ability of RGSZ1 to interact with SCG10, suggesting either that this region directly interacts with RGSZ1 or that its removal causes a change in the overall conformation of SCG10 and thus prevents RGSZ1 interaction.

Once the interaction was established in vitro, we explored the functional consequences resulting from this interaction. As indicated under “Results,” we were unable to show that SCG10 could block the ability of RGSZ1 to accelerate the GTP hydrolysis of Gαi1. Previous studies have indicated that certain RGS complexes do indeed maintain their GAP functionality. For instance, the R7 family of RGS proteins contains a GGL domain that resides outside the RGS core domain and is responsible for binding Gαi1. Recent work has shown that both RGS6 and RGS7 (38) as well as RGS11 (13) contain this GGL domain and bind Gαi1, yet still maintain significant GAP activity selectively for Gαi1. Other studies suggest that interacting proteins that bind to RGS proteins within their core domain can directly block GAP activity. Benzing et al. (39) demonstrated that the binding of 14-3-3 to phosphorylated RGS7 inhibits its GAP activity for Gαi1. The importance of RGS interactors binding either outside or within the core domain with regard to modulating GAP activity remains to be determined.

Analysis of microtubule polymerization and depolymerization revealed a clear functional consequence of the RGSZ1-SCG10 interaction. Although RGSZ1 had no effect on the ability of SCG10 to block microtubule assembly, there was a striking effect on SCG10-induced disassembly. The mechanism for SCG10-mediated destabilization of microtubules is not yet clear, and there has been considerable controversy in the field concerning this activity of stathmin proteins. Initially, Belmont and Mitchison (21) reported that stathmin interacts with tubulin dimers and increases the catastrophe frequency of microtubules by 3–6-fold in vitro. They concluded that direct stimulation of the catastrophe frequency is the mechanism by which stathmin operates. Subsequently, Curmi et al. (40) failed to reproduce the catastrophe promotion by stathmin and demonstrated that one molecule of stathmin can sequester two molecules of tubulin, forming a tight “T2S complex” that can alter the equilibrium conditions leading to depolymerization of microtubules (24). This mechanistic conflict was finally resolved when the discrepancy between these two studies was found to be the result of different pH conditions (41). At pH 7.5, it appears that stathmin interacts with microtubules and specifically induces catastrophes at the plus ends (41), whereas at pH 6.8, stathmin acts primarily by tubulin sequestration. It was further shown that these two distinct activities can be separated by mutational analysis: the N-terminal region was found to be responsible for catastrophe promotion at stathmin plus ends, whereas the C-terminal region was found to be necessary for tubulin sequestration (41).

It is intriguing that RGSZ1 blocks only microtubule disassembly, whereas microtubule assembly remains unaffected. This observation is considered quite remarkable, as it suggests that RGSZ1 binding to SCG10 may provide a mechanism for the separation of the two distinct activities of SCG10, i.e. tubulin sequestration versus initiation of microtubule catastrophes. Perhaps the interaction of RGSZ1 with SCG10 alters the microenvironment of SCG10 such that it more resembles the high pH (7.5) condition than the low pH (6.8) condition. It must be noted that this hypothesis is based on data obtained from studies of stathmin, not SCG10. Although high in sequence homology and closely related (42, 43), differences exist between studies of stathmin, not SCG10. Although high in sequence homology and closely related (42, 43), differences exist between the two that may complicate these assumptions. Further work is needed to clarify whether SCG10 does indeed behave like stathmin with regard to the separation of its microtubule-regulating activities and to clarify the mechanism by which the affinity of SCG10 for tubulin is influenced by RGSZ1.

One of the more interesting questions surrounding the RGSZ1-SCG10 interaction is its relevance in neurons, where distinct populations of both stable and labile microtubules coexist. The growing tips of extending processes contain particularly high levels of very labile polymers, and a number of studies have shown that axonal growth and guidance strongly depend on the highly dynamic behavior of microtubules in these motile structures (44–46). Specific targeting of SCG10 involves the amino terminus, which mediates its association with membranous vesicles that are apparently directed to the growth cones (27, 37). Although this region also contains MAPs that act to stabilize microtubules, the relative contribution of individual proteins controlling stabilization and destabilization in regions of neuronal outgrowth is a matter of debate. For example, tau, a MAP found in developing neurons (47), appears to play significant roles in stabilizing microtubules in vitro (48, 51).
49), but in vivo data suggest that it is not a principal determinant of microtubule stability (50). At present, it is not clear what prevents the depolymerization of microtubules when they need to be stabilized, e.g. in response to extrinsic guidance cues. Possibilities include the presence of stabilizing proteins, the phosphorylation of SCG10 (which has been shown to inhibit its depolymerizing activity) (26), or perhaps the inhibition of SCG10 by RGSZ1, all of which could provide an alternative mechanism for stabilization. The idea that RGSZ1 could contribute to axonal outgrowth is an interesting hypothesis that may provide a link between G protein signaling and neuronal development.

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