srGAP2 Arginine Methylation Regulates Cell Migration and Cell Spreading through Promoting Dimerization*

The Slit-Robo GTPase-activating proteins (srGAPs) are critical for neuronal migration through inactivation of Rho GTPases Cdc42, Rac1, and RhoA. Here we report that srGAP2 physically interacts with protein arginine methyltransferase 5 (PRMT5). srGAP2 localizes to the cytoplasm and plasma membrane protrusion. srGAP2 knockdown reduces cell adhesion spreading and increases cell migration, but has no effect on cell proliferation. PRMT5 binds to the N terminus of srGAP2 (225–538 aa) and methylates its C-terminal arginine residue Arg-927. The methyl mutation mutant srGAP2-R927A fails to rescue the cell spreading rate, is unable to localize to the plasma membrane leading edge, and perturbs srGAP2 homodimer formation mediated by the F-BAR domain. These results suggest that srGAP2 arginine methylation plays important roles in cell spreading and cell migration through influencing membrane protrusion.

Cell migration is essential for a wide variety of physiological and pathological processes such as embryonic development, wound tissue repair, and tumor metastasis (1–2). Classically, the major driving force for migration in mammalian cells is thought to be provided by actin polymerization at the cell protrusion of the front edge and F-actin depolymerization to resect cell body at the rear (3). These coordinated processes are regulated by small Rho GTPases, especially Cdc42, Rac1, and RhoA (4–5). Similar to other GTPases, Rho GTPases act as intracellular molecular switches, cycling between a GDP-bound inactive form and a GTP-bound active form. The two interconvertible forms are controlled by two classes of proteins, guanine nucleotide exchange factors (GEFs), which promote the exchange of GDP for GTP, and GTPase-activating proteins (GAPs), which increase the GTPase activity of Rho GTPases through converting GTP to GDP, thereby closing the switch (6–7).

srGAP is a family of RhoGAP proteins, identified in the SlitRobo signal pathway. The srGAP family consists of three members, srGAP1, -2, and -3. All of them possess three conserved domains, F-BAR, RhoGAP, and SH3 (8). The function of RhoGAP domain of srGAP family protein is extensively studied because of its role in the negative regulation of Rho GTPase activities important for cytoskeleton rearrangement (8–9). In addition to the RhoGAP domain, studies have found that the F-BAR domain-containing proteins function as key regulators in membrane remodeling in eukaryotes. Each of these proteins shares a similar quaternary “banana-like” structure, which forms elongated dimers mediated by the anti-parallel association of α helices in each monomer (10–11). Moreover, positive charges within the “banana-like” structure are aligned to interact with negative charges of the membrane via electrostatic interactions (12). Therefore, the F-BAR domains act as membrane-associated scaffolds and deform cell membrane independently of its F-actin bundling activity (13–14).

Based on structural characteristics and phylogenetic analysis, the BAR domain super family includes “classical” BAR domain, F-BAR domain (FCH domain, followed by a coiled-coil region) (15–17) and I-BAR (Inverse-BAR) domain (11). Classical BAR and F-BAR domains promote the formation of plasma membrane invaginations in endocytosis (14–16). In contrast, I-BAR domains induce plasma membrane protrusions to generate filopodia in vitro and in living cells (16, 18–22). Interestingly, unlike canonical F-BAR, the F-BAR domain of srGAP2 deforms membrane like an I-BAR domain to induce filopodia formation. F-BAR is necessary and sufficient for srGAP2 to directly regulate membrane deformation observed in COS7 cells and in neuronal migration and morphogenesis (23). However, the detail mechanism by which the auto-inhibited srGAP2 monomers are transformed into the functional homodimers via the F-BAR domain remains somewhat unclear.

S-Adenosylhomocysteine hydrolase (SAHH) has been shown to enrich at the leading edge of motile Dictostelium amoebae and human neutrophils (24). SAHH promotes SAM-dependent methylation in eukaryotes (25). Although in general, SAM-dependent methylation functions at the front of migrating cells, the key methyltransferase(s) and critical protein substrate(s) remain unknown. Our previous study has shown that srGAP2 co-exists in a complex with the protein arginine methyltransferase PRMT5 (26). PRMT5 is critical for cell proliferation (27–28) and differentiation (29–31) through modification of histones (14, 32–33) and RNA splicing factors (34). We find recently that PRMT5 dominantly localizes to cytoplasm in somatic cells, and methylates golgin GM130 and ribosomal protein Rps10 (26, 35). In this report, we show that srGAP2...
homodimerization depends on its arginine methylation catalyzed by PRMT5. The formation of srGAP2 homodimers promotes its binding and deformation of cell membrane, leading to accelerated cell membrane protrusion and cell spreading.

EXPERIMENTAL PROCEDURES

DNA Constructs and Antibodies—Human srGAP2 full-length cDNA clone (KIAA0456) was generously provided by Riken. PRMT5 (BC025979) was purchased from Open Biosystems and its subcloning as described (26, 35). Reps1N (1–373 aa), Reps1C (367–743 aa), srGAP2, srGAP2N (2–538 aa), srGAP2N1 (2–538 aa), srGAP2N2 (2–118 aa), srGAP2N3 (225–538 aa), srGAP2C (485–1068 aa), srGAP2C2 (673–1068 aa), srGAP2C3 (783–1068 aa), and srGAP2C4 (783–854 aa) were subcloned into pET-28b (Novagen), pGEX4T-1/3 (GE Healthcare), pCMV-Tag 2B (Stratagene), pEGFPN3/C1 (Clontech), and pcDNA3×HA-B (a gift of Dr. Zhiheng Xu) in-frame by PCR (as specified in each experiment). The U6 promoter-driven shRNA expression vector pDsU6 and control shRNA (siLuc) were previously described (36). Two short hairpin-based shRNA silencing vectors were generated, which were specific for target srGAP2 mRNA codon sequence. The target sites in the srGAP2 coding region were 466–485 and 721–741 (srGAP2 mRNA codon sequence). The target sites in the srGAP2 previously described (36). Two short hairpin-based shRNA silencing vectors were generated, which were specific for target srGAP2 mRNA codon sequence. The target sites in the srGAP2 coding region were 466–485 and 721–741 (+1 being the A residue in the initiating codon) and were verified in the human genome sequence data base (NCBI) as unique sequences. Two high-efficiency vectors pDs466 and pDs721 were named as U1 and U2, respectively. The shRNA in U1 was subcloned into a GFP-containing shRNA expression vector, and the construct was named GFP-U1. srGAP2 shRNA-resistant mutant (srGAP2-R-GFP) construct was prepared by introducing two silent mutations within the U1-targeting sequence using site-directed mutagenesis with the sequence 5’-GTCCTGAACGAaCTgTACTCG-3’. srGAP2 methylation mutant (R927A, abbreviated with m) was generated with site-directed mutagenesis, and srGAP2-dm-GFP was constructed from the plasmids of srGAP2-R-GFP and srGAP2-m-GFP. HA-Rac1V12 construct was a generous gift from Dr. Kozo Kabcu (Nagoya University), and GST-PBD was a kind gift from Dr. Xueliang Zhu (SIBS). All constructs were verified by double-stranded DNA sequencing. The polyclonal antibody against PRMT5 was purchased from Upstate. The monoclonal antibodies against Actin, GST, Flag, and HA were products of Sigma. Monoclonal antibody against GFP was purchased from Clontech. Anti-Rac1 antibody was from Millipore. The polyclonal and monoclonal antibodies raised against the C terminus of srGAP2 (783–1068) were made by Animal Center of IGDB.

Cell Culture, Transfection, and Immunofluorescence Microscopy—HCT116, HEK293, CHO, HeLa, and U2OS cells were maintained at 37 °C, and 5% CO2 in Dulbecco’s modified Eagle’s medium (Invitrogen) plus 10% fetal bovine serum (Invitrogen) and 1% penicillin-streptomycin (Invitrogen). Cells were transfected using Lipofectamine 2000 (Invitrogen) following manufacturer’s recommendations. For immunofluorescence staining, cells grown on coverslips were either fixed to stain with indicated antibodies or fluorescent dyes, or transfected with GFP, srGAP2-R-GFP, or srGAP2-dm-GFP, and then fixed to stain with TRITC 545-conjugated phalloidin (Sigma). The stained cells were visualized under the Spectral Imaging Confocal Microscope DIGITAL ECLIPSE C1Si (Nikon, Japan). Images were acquired using a 100× Plan Apochromat VC NA 1.40 oil objective and analyzed using the NIS-Elements AR software provided by Nikon.

Colony Formation Assay and Stable Cell Lines Setup—HCT116 cells were plated on 60-mm dishes (6 × 104 cells/dish), cultured for 24 h at 37 °C, and then transfected with 3 μg of siLuc, U1 and U2, respectively. Cells were reseeded on 100-mm dishes 24 h after transfection (3 × 105 cells/dish), and cultured in 10% FBS of DMEM with 800 μg/ml G418 (Calbiochem). After a 2-week selection, the cell colonies were photographed using an inverted microscope for colony formation assay. For stable cell lines setup, the colonies above were picked into 24-well plate, cultured in 10% FBS of DMEM with 200 μg/ml G418, and then screened with Western blot. The positive cell lines were freeze or used for other specific experiments.
cells in the cell-free area had been repaired after culturing for 12 h, the same wounded areas were photographed using an inverted microscope (Nikon Eclipse TE2000-S) equipped with a digital camera (Nikon DXM1200C) with 10× objective at the intersection of the marked line and wound edge at 0 h and at desired time intervals, and the areas of wound closure were analyzed using the NIH Image J software. Each experiment was carried out three times in duplicate.

**Cell Spreading Assay**—Cells of ~80% confluence were detached using EDTA-trypsin (Invitrogen), and the DMEM with 10% FBS was added to terminate trypsinization immediately. The cells were then spun down, resuspended in DMEM containing 10% FBS, and seeded on coverslips in 24-well dishes to culture in the incubator. At various time points, cells were fixed in 4% paraformaldehyde, permeabilized in 0.5% Triton X-100/phosphate-buffered saline (PBS), blocked in 5% BSA/PBS, and then stained with TRITC 545-conjugated phalloidin (Sigma) and DAPI (Vector Laboratories). Fluorescent images from multiple fields in each coverslip were captured with a CCD camera. To obtain quantitative data of the extent of cell spreading, the surface areas of cells were measured with the NIH Image J software, and cells were scored blindly as “spreading” (cell area >250 μm²). At least 100 cells were analyzed per sample. Results were from three independent experiments. Data shown are mean ± S.D./Data are mean ± S.E.

**Live Cell Imaging**—U2OS cells grown to 20–30% confluence on chambered coverglass (Thermo Scientific Nunc) were transfected with Lipofectamine™ 2000 and imaged at 37 °C, with 5% CO₂ 24 h later. Time-lapse fluorescence images were acquired with the Spectral Imaging Confocal Microscope DIGITAL ECLIPSE C1Si equipped with a T-PFS Perfect Focus Unit and a 20× Plan Apochromat VC NA 0.75 DIC Dry objective. Images were captured with the EZ-C1 Spectral software (Nikon). Single-channel images were acquired at 8-min intervals. Images were exported as TIFF files using NIS-element AR (Nikon).

**Protein Purification and Methylation Analysis**—GST, GST-fused proteins, and His-fused proteins expression, purification, and methylation assays were carried out as previously described (37).

**Immunoprecipitation, in Vitro Binding Assay, and Western Immunoblotting**—For reciprocal immunoprecipitation, 800 μg of cell (transfected with foreign DNA or not) extract protein
was incubated overnight with 2 μg of antibody and protein A/G-agarose beads (OncogeneScience) at 4 °C, or incubated with monoclonal anti-HA-agarose (Sigma) for 2 h. For in vitro binding assay, a series of GST-tagged peptides were incubated with Flag-PRMT5 protein purified from CHO cell lysates with Flag beads at 4 °C for 1 h. After extensive washing, all associated proteins were eluted and subjected to Western immunoblotting (as specific experiment). Cell lysis buffer I (50 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10 μg/ml leupeptin, and 10 μg/ml aprotinin, Roche) was used for srGAP2-PRMT5 reciprocal immunoprecipitation, cell lysis buffer II (20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 0.1% Triton X-100, 10 μg/ml leupeptin, and 10 μg/ml aprotinin,) was for srGAP2-Rac1 reciprocal immunoprecipitation, cell lysis buffer III (50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 15 mM EGTA, 0.1% Triton X-100, 1 mM DTT, 1 mM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) was for srGAP2 dimer formation assay, and cell lysis buffer IV (50 mM Tris-HCl, 150 mM NaCl, 0.5% Triton X-100, pH 7.4, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) was for in vitro binding assay.

Detection of GTP-bound Rac1 by Use of GST-PBD—The cells were lysed in binding buffer (50 mM Tris, pH 7.2, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 500 mM NaCl, 10 mM MgCl2, 10 μg/ml leupeptin, and aprotinin, and 1 mM PMSF). The cleared lysates were then transferred quickly into tubes with GST-PBD (20 μg) bound to glutathione-Sepharose 4B beads and rotated at 4 °C for 45 min. The beads were washed four times with washing buffer containing 1% Triton X-100, 50 mM NaCl, 10 mM MgCl2, 10 μg/ml leupeptin, and aprotinin, and 1 mM PMSF) and analyzed for bound protein by Western immunoblotting with anti-Rac1 antibody.

RESULTS
Loss of srGAP2 Increases Cell Migration—To examine the physiological function of srGAP2 in cells, we generated two short hairpin-based interfering RNA (shRNA) constructs named U1 and U2, which knock down srGAP2 expression efficiently (Fig. 1A) and the shRNA in U1 was subcloned into a GFP-containing shRNA expression vector (GFP-
To gain further insights into srGAP2 in cell spreading, we tested F-actin distribution as an indicator of cell spreading at different time intervals after plating the control and srGAP2-depleted cells. We found that only about 1.9, 2.8, and 16% of srGAP2-depleted cells (U1A5) spread at 20, 24, 33 h after plating, respectively. In contrast, about 16, 30, and 80% of control cells spread completely at the respective time points (Fig. 3, A and B). At longer incubation time such as 40 h after plating, the difference of cell spreading rate between srGAP2 lesion and control cells was much less, suggesting that loss of srGAP2 slows down cell spreading process significantly but does not abolish it. To exclude the possibility of the result of nonspecific activation of the RNAi machinery or off-target effects, we performed rescue experiments. The srGAP2-depleted cells (U1A5) transfected with a shRNA-resistant form of srGAP2 (srGAP2-R-GFP) complemented the defects of cell spreading (Fig. 3, C and D).

Adherent eukaryotic cells round up during prophase and metaphase and reacquire their extended and flattened shape during cytokinesis (38–39). Consistently, time-lapse confocal microscopy analysis confirmed that cells transfected with GFP-U1 spread much slower than the untransfected cells (Fig. 3E). This observation suggests that srGAP2 influences cell migration through regulating lamelipodia-like membrane protrusions.

**Interaction of srGAP2 and PRMT5**—Our previous study has shown that srGAP2 is a component of the PRMT5 protein complex (26). To further verify potential interactions between the endogenous srGAP2 and PRMT5 proteins, we performed coimmunoprecipitation with anti-srGAP2 and -PRMT5 antibodies. As shown in Fig. 4A, reciprocal interactions were confirmed between srGAP2 and PRMT5.

To localize which region of srGAP2 is required for its interaction with PRMT5, in vitro protein binding analysis was carried out. To avoid the influence of anti-Flag antibody cross reaction with endogenous PRMT5 in human cells, Flag-tagged PRMT5 was purified from Chinese hamster ovarian (CHO) cells with stable expression of Flag-PRMT5 and then incubated with recombinant GST-tagged srGAP2N (1–538 aa), srGAP2C (484–1068 aa), and two GST-tagged fragments (Reps1N (1–373 aa) and Reps1C (367–743 aa)) of Reps1, another component of the PRMT5 complex. As shown in Fig. 4A, B–D, Flag-PRMT5 could pull down GST-srGAP2N, but not GST-srGAP2C or GST-Reps1. The PRMT5-srGAP2 interaction domain was further defined to the 225–538 aa N-terminal

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**FIGURE 4. srGAP2 interacts with PRMT5.** A, endogenous PRMT5 and srGAP2 interact with each other. HEK293 cell lysates were subjected to immunoprecipitation with anti-srGAP2 or preimmuniserum (Pre-I) and anti-PRMT5 or IgG antibodies as indicated. The immunocomplexes were separately and probed for PRMT5 and srGAP2. B, diagrams of different fragment of srGAP2. C, pull-down assay to test interaction of Flag-tagged PRMT5 with GST-tagged N (1–538 aa)/C (484–1068 aa)-terminal srGAP2 and N (1–373 aa)/C (367–743 aa)-terminal Reps1. Western blot analysis was performed with GST and Flag antibodies. D, pull-down assay to test the interaction of Flag-tagged PRMT5 with different fragments of GST-tagged srGAP2-N-terminal. Each protein is indicated with an arrow. Western blot analysis was performed with GST and Flag antibodies.
region of srGAP2, whereas other regions of N-terminal srGAP2 did not interact with Flag-PRMT5.

**PRMT5 Methylates srGAP2**—Because srGAP2 interacts with PRMT5, it is reasonable to consider whether srGAP2 is a substrate of PRMT5. We subsequently examined whether PRMT5 can methylate arginine residues of srGAP2. A series of recombinant fragments in srGAP2 purified from *Escherichia coli* and incubated with 1 μg of Flag-PRMT5, purified from CHO cells. Incorporation of 3H-labeled methyl groups (from the SAM donor) was visualized by autoradiography. Each protein is indicated with an arrow. A, 30.4 kDa methylated polypeptide band (srGAP2-C3) in Fig. 5A was fragmented and analyzed by mass spectrometry, and the result indicated the molecular weight of srGAP2 fragments and assignment of the methylated arginine. Note that the mass shifts reflect the tritium molecular weight. * represents 3H-labeled -CH3. arginine residues in srGAP2 C and C3 fragments were mutated to alanine (R927A). 2–5 μg of His-tagged C, C2, C3, C4, and the R927A mutants in C (Cm) and C3 (C3m) were purified from *E. coli*, and the methylation assay as in A. Each protein is indicated with an arrow. D, amino acid sequence alignment of human Arg-927 site of srGAP2, marked with *, in some eukaryotic species was performed using DNAMAN version 4.0 software. Conserved residues are highlighted with the following color type: black (≥100%) and gray (≥75%).

the C terminus of srGAP2 was methylated by PRMT5, and the signal from the srGAP2C3 fragment (783-1068 aa) was the most prominent, whereas no methylation was observed with GST or the srGAP2N-terminal GST fusion (GST-srGAP2N, N1, N2, and N3). Thus, it appeared that PRMT5 most likely methylates arginine residue(s) in the srGAP2C3 region (Fig. 5A). Consistently, mass spectrometry analysis of the 30.4 kDa methylated srGAP2C3 polypeptide identified Arg-927 in srGAP2 as the residue methylated by PRMT5 (Fig. 5B).
To confirm the mass spectrometry results, we generated Arg-927 → Ala substitution (R927A) in srGAP2. In vitro methylation assay showed that the methylation signals drastically reduced in the R927A srGAP2 C or C3 mutants (Fig. 5C). This result suggests that Arg-927 in srGAP2 is the primary target site of PRMT5. Amino acid sequence alignment of vertebrate srGAP2 suggests that Arg-927 is highly conserved in evolution (Fig. 5D). It raises the possibility that the function of Arg-927 methylation in srGAP2 is significant.

**srGAP2 Arginine Methylation Affects Cell Spreading**—To test whether the function of srGAP2 methylation by PRMT5 is involved in cell spreading, we first generated a GFP-tagged srGAP2 R927A mutant (srGAP2-dm-GFP), which is also resistant to srGAP2 shRNA (Fig. 6A). Cell spreading assay indicated that srGAP2-R-GFP rescued the cell spreading defect in srGAP2-depleted cells (Fig. 6B), while the srGAP2 methylation mutant failed to do so (Fig. 6, C and D). Furthermore, we found the R927A methylation mutant could not be localized at membrane protrusions, compared with wild-type srGAP2, which is found far away from F-actin in the membrane protrusions (Fig. 6, B, C, E, F). Therefore, srGAP2 methylation at arginine 927 is required for cell spreading and srGAP2 localization in the cell membrane protrusion.

**srGAP2 Arginine Methylation Is Not Associated with Its RhoGAP Activity**—srGAP2 is a Rac1-specific activating protein. Rac1 activates the Arp2/3 complex via WAVE to initiate F-actin polymerization and promote lamellipodia formation (4, 40). Thus, it is possible that arginine methylation of srGAP2 regulates cell spreading by affecting its RhoGAP activity. To verify this notion, we first tested whether srGAP2 methylation mutant could interact with constitutively active form of Rac1. Immunoprecipitation assay was performed using HEK293 cells co-transfected with HA-tagged constitutively active form of Rac1 (HA-Rac1) and GFP-tagged srGAP2 wt (srGAP2-R-GFP) or srGAP2 methylation mutant (srGAP2-dm-GFP). The IP results showed that both srGAP2 wt and methylation mutant maintained interaction with the constitutively active form of Rac1 (Fig. 7A), suggesting that the RhoGAP activity was unaltered by the R927A mutation.

To further confirm that srGAP2 methylation mutant does not affect the RhoGAP activity, we applied GST pull-down assay to test the level of GTP-bound form Rac1 between srGAP2-depleted cells transfected with srGAP2-R-GFP and srGAP2-dm-GFP, using a GST fusion protein of the p21 binding domain of in PAK1 (GST-PBD) (41–42). The binding activity of srGAP2-dm-GFP was the same with 35139
srGAP2-R-GFP-transfected cells. Both decreased the level of active form Rac1, compared with srGAP2-depleted cells (Fig. 7B). Collectively, these data ruled out that srGAP2 methylation mediated by PRMT5 is associated with its RhoGAP activity.

**srGAP2 Arginine Methylation Promotes the Dimerization of srGAP2**—Light scattering analysis indicated that srGAP2 forms homodimer through its F-BAR domains in solution (23). The homodimer mediated by F-BAR domain possesses a quaternary “banana-like” structure, which can deform the cell membrane (10, 12, 14, 43). F-BAR domain of srGAP2 induces filopodia in COS7 cells (23). We therefore tested whether srGAP2 R927 methylation was required for the formation of a homodimer. HA or GFP-tagged srGAP2 wild type or its arginine methylation mutant were co-expressed in cells. Immunoprecipitation showed that HA-srGAP2 binds srGAP2-GFP with high affinity as expected. However, the association between HA-srGAP2 and srGAP2-m-GFP or HA-srGAP2-m and srGAP2-m-GFP was reduced drastically (Fig. 7, C and D), suggesting that srGAP2 Arg-927 methylation is required for its homodimer formation.

**DISCUSSION**

Results from this report suggest that srGAP2 is a novel substrate of PRMT5, and arginine methylation of srGAP2 regulates membrane protrusion, cell spreading, and migration. Our findings that srGAP2 depletion in cultured cells led to promotion of cell migration are consistent with a recent report that srGAP2 negatively regulates neuronal migration (23).

It has been proposed that the F-BAR domain of srGAP2, resembling I-BAR domains, induces filopodia-like membrane protrusion, and this process is dependent on srGAP2 homodimerization. srGAP2 might normally be in an auto-inhibited conformation through interaction between the N-terminal F-BAR domain and the C-terminal regions (including the SH3 domain). Given that the SH3 domain mutant completely abrogated the srGAP2 ability to induce filopodia formation and that deletion of the C terminus induces filopodia, it is expected that the C terminus of srGAP2 harbors a regulatory domain(s) critical for its function (23). In this work, we find that the srGAP2-N3 region (225 to 538 aa) interacts with PRMT5 and srGAP2 is methylated by PRMT5 at R927 (Fig. 4D). The methylation site and the interacting region of srGAP2 are distant in the primary structure. srGAP2-N3 contains the CC motif of F-BAR domain, and Arg-927 site is in the C terminus outside the SH3 domain (Fig. 4B), this is congruent with the notion that there is an auto-inhibitory conformation through structural interaction between the N-terminal F-BAR domain and the C-terminal region (23). However, it remains unclear as to how the auto-inhibitory conformation transforms into the active form of the homodimer. Our findings show that the srGAP2 methylation mutant fails to bind to the cell membrane or spread the cell (Fig. 6) or form the dimer (Fig. 7, C and D). Therefore, we propose a model (Fig. 8) for the role of srGAP2 methylation. In this model, the F-BAR domain of srGAP2 is restricted by the C-terminal region when Arg-927 is unmodified, constituting steric hindrance for the F-BAR region. Upon PRMT5-mediated Arg-927 methylation, the C-terminal region is disengaged from the F-BAR domain, allowing dimerization to take place, with the result that the active form of srGAP2 translocates into...
the plasma membrane and enacts membrane deformation and protrusion necessary for cell spreading.

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REFERENCES

1. Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T., and Horwitz, A. R. (2003) Science 302, 1704–1709
2. Lauffenburger, D. A., and Horwitz, A. F. (1996) Cell 84, 359–369
3. Webb, D. J., Parsons, J. T., and Horwitz, A. F. (2002) Nat. Cell Biol. 4, E97–100
4. Jaffe, A. B., and Hall, A. (2005) Annu. Rev. Cell Dev. Biol. 21, 247–269
5. Hall, A. (2005) Biochem. Soc. Trans. 33, 891–895
6. Lamarche, N., and Hall, A. (1994) Trends Genet. 10, 436–440
7. Narumiya, S. (1996) J. Biochem. 120, 215–228
8. Wong, K., Ren, X. R., Huang, Y. Z., Xie, Y., Liu, G., Saito, H., Tang, H., Wen, L., Brady-Kalnay, S. M., Mei, L., Wu, J. Y., Xiong, W. C., and Rao, Y. (2001) Cell 107, 209–221
9. Yang, Y., Marcellino, M., Endris, V., Saffrich, R., Fischer, R., Trendelenburg, M. F., Sprengel, R., and Rappold, G. (2006) Exp. Cell Res. 312, 2379–2393
10. Henne, W. M., Kent, H. M., Ford, M. G., Hegde, B. G., Daumke, O., Butler, P. J., Mittal, R., Langen, R., Evans, P. R., and McMahon, H. T. (2007) Structure 15, 839–852
11. Frost, A., De Camilli, P., and Unger, V. M. (2007) Structure 15, 751–753
12. Peter, B. J., Kent, H. M., Mills, I. G., Vallis, Y., Butler, P. J., Evans, P. R., and McMahon, H. T. (2004) Science 303, 495–499
13. Lim, K. B., Bu, W., Goh, W. I., Koh, E., Ong, S. H., Pawson, T., Sudhaharan, T., and Ahmed, S. (2008) J. Biol. Chem. 283, 20454–20472
14. Shimada, A., Niwa, H., Tsujita, K., Suettsugui, S., Nitta, K., Hanawa-Suettsugui, K., Akasaka, R., Nishino, Y., Toyama, M., Chen, L., Liu, Z., Wang, B. C., Yamamoto, M., Terada, T., Miyazawa, A., Tanaka, A., Sugano, S., Shirouzu, M., Nagayama, K., Takenawa, T., and Yokoyama, S. (2007) Cell 129, 761–772
15. Itoh, T., Erdmann, K. S., Roux, A., Habermann, B., Werner, H., and De Camilli, P. (2005) Dev. Cell 9, 791–804
16. Tsujita, K., Suettsugui, S., Sasaki, N., Furutani, M., Oikawa, T., and Takenawa, T. (2006) J. Cell Biol. 172, 269–279
17. Aspenström, P. (1997) Curr. Biol. 7, 479–487
18. Mattila, P. K., Pykäläinen, A., Saarikangas, J., Paasilinna, V. O., Vihinen, H., Jokitalo, E., and Lappalainen, P. (2007) J. Cell Biol. 176, 953–964
19. Suettsugui, S., Murayama, K., Sakamoto, A., Hanawa-Suettsugui, K., Seto, A., Oikawa, T., Mishima, C., Shirouzu, M., Takenawa, T., and Yokoyama, S. (2006) J. Biol. Chem. 281, 35354–35358
20. Mattila, P. K., Salmiminen, M., Yamashiro, T., and Lappalainen, P. (2003) J. Biol. Chem. 278, 8452–8459
21. Yamagishi, A., Masuda, M., Ohki, T., Onishi, H., and Mochizuki, N. (2004) J. Biol. Chem. 279, 14929–14936
22. Woodings, J. A., Sharp, S. J., and Machesky, L. M. (2003) Biochem. J. 371, 463–471
23. Guerrier, S., Coutinho-Budd, J., Sassa, T., Gresset, A., Jordan, N. V., Chen, K., Jin, W. L., Frost, A., and Polleux, F. (2009) Cell 138, 990–1004
24. Sha, S., Mahadeo, D. C., Liu, X., Liu, W., Parent, C. A., and Korn, E. D. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 19788–19793
25. De La Haba, G., and Cantoni, G. L. (1959) J. Biol. Chem. 234, 603–608
26. Zhou, Z., Sun, X., Zou, Z., Sun, L., Zhang, T., Guo, S., Wen, Y., Liu, L., Wang, Y., Qin, J., Li, L., Gong, W., and Bao, S. (2010) Cell Res. 20, 1023–1033
27. Pal, S., Vishwanath, S. N., Erdjument-Bromage, H., Tempst, P., and Sif, S. (2004) Mol. Cell. Biol. 24, 9630–9645
28. Jansson, M., Durant, S. T., Cho, E. C., Sheahan, S., Edelmann, M., Kessler, B., and La Thangue, N. B. (2008) Nat. Cell Biol. 10, 1431–1439
29. Ancelin, K., Lange, U. C., Hajkova, P., Schneider, R., Bannister, A. J., Kouzarides, T., and Surani, M. A. (2006) Nat. Cell Biol. 8, 623–630
30. Gonsalvez, G. B., Rajendra, T. K., Tian, L., and Mata, A. G. (2006) Curr. Biol. 16, 1077–1089
31. Dacwig, C. S., Bedford, M. T., Sif, S., and Imbalzano, A. N. (2009) Mol. Cell. Biol. 29, 1909–1921
32. Kwik, Y. T., Guo, J., Prapaijat, S., Park, K. I., Surabhi, R. M., Miller, B., Gehrig, P., and Gaynor, R. B. (2003) Mol. Cell 11, 1055–1066
33. Zhao, Q., Rank, G., Tan, Y. T., Li, H., Moritz, R. L., Simpson, R. J., Cerruti, L., Curtis, D. J., Patel, D. J., Allis, C. D., Cunningham, J. M., and Jane, S. M. (2009) Nat. Struct. Mol. Biol. 16, 304–311
34. Chari, A., Golas, M. M., Klingenhäger, M., Freytag, S., Bader, B., Engbrecht, C., Sickmann, A., Stark, H., and Fischer, U. (2008) Cell 135, 497–509
35. Ren, J., Wang, Y., Liang, Y., Zhang, Y., Bao, S., and Xu, Z. (2010) J. Biol. Chem. 285, 12695–12705
36. Bao, S., Lu, T., Wang, X., Zheng, H., Wang, L. E., Wei, Q., Himmel, W. N., and Li, L. (2004) Oncogene 23, 5586–5593
37. Rho, J., Choi, S., Seong, Y. R., Cho, W. K., Kim, S. H., and Im, D. S. (2001) J. Biol. Chem. 276, 11393–11401
38. Bourc'hort, E., and Kirchhausen, T. (2007) Proc. Natl. Acad. Sci. U. S. A. 104, 7939–7944
39. Bourc'hort, E., and Kirchhausen, T. (2008) PLoS One 3, e1477
40. Takenawa, T., and Miki, H. (2001) J. Cell Sci. 114, 1801–1809
41. Benard, V., Bohl, B. P., and Bokoch, G. M. (1999) J. Biol. Chem. 274, 13198–13204
42. Sander, E. E., van Delft, S., ten Klooster, J. P., Reid, T., van der Kammen, R. A., Michiels, F., and Collard, J. G. (1998) J. Cell Biol. 143, 1385–1398
43. Frost, A., Perera, R., Roux, A., Spasov, K., Destaing, O., Egelman, E. H., De Camilli, P., and Unger, V. M. (2008) Cell 132, 807–817

FIGURE 8. Methylation of srGAP2 is required for filopodia protrusions and cell spreading. Hypothetical model of the molecular mechanisms illustrating that methylation of srGAP2 promotes the dimerization of srGAP2, plasma membrane protrusions, and cell spreading. See text for details.

PRMT5-mediated srGAP2 Methylation and Cell Migration