SMAP, an Smg GDS-associating Protein Having Arm Repeats and Phosphorylated by Src Tyrosine Kinase*

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Kazuya Shimizu‡, Hiroshi Kawabe‡, Seigo Minami‡, Tomoyuki Honda‡, Kenji Takaishi‡, Hiromichi Shiratani‡, and Yoshimi Takaï‡§§

From the ‡Department of Molecular Biology and Biochemistry, Osaka University Medical School, Suite 565 and the §Department of Cell Physiology, National Institute for Physiological Sciences, Okazaki 444, Japan

Smg GDS is a regulator having two activities on a group of small G proteins including the Rho and Rap1 family members and Ki-Ras; one is to stimulate their GDP/GTP exchange reactions, and the other is to inhibit their interactions with membranes. Structurally, it has 11 Arm repeats, a protein interaction motif, found in the Drosophila Armadillo protein, a homolog of mammalian β-catenin. We have isolated here an Smg GDS-interacting protein from a human brain cDNA library by use of the yeast two-hybrid method and named it SMAP (Smg GDS-associated protein). SMAP was a protein with a M, of 91,189 and 792 amino acids. SMAP had 9 Arm repeats. Recombinant SMAP interacted with recombinant Smg GDS but did not affect the two activities of Smg GDS on RhoA. SMAP was tyrosine phosphorylated by v-Src, and this phosphorylation reduced the affinity of SMAP for Smg GDS. Tissue and subcellular distribution analyses indicated that SMAP was ubiquitously expressed and highly concentrated at the endoplasmic reticulum area. Searches for sequence homology to SMAP revealed that SMAP was significantly homologous to sea urchin SpKAP115, suggesting that SMAP is a mammalian counterpart of SpKAP115 or its related protein. SpKAP115 is an accessory subunit of sea urchin kinesin II, an ATPase motor that transports vesicles along microtubules. These results suggest that SMAP serves as an adaptor for both Smg GDS and kinesin II or its related protein and links them with both the Smg GDS-regulated small G protein and Src tyrosine kinase signalings.

Smg GDS has originally been isolated as a regulator that stimulates the GDP/GTP exchange reaction of Rap1 small G protein (1, 2). Subsequently, Smg GDS has been shown to be active on a group of small G proteins including the Rho and Rap1 family members and Ki-Ras and to require the post-translational lipid modifications of these substrate small G proteins for its action (3, 4). Moreover, Smg GDS has been shown to inhibit the interactions of these lipid-modified substrate small G proteins with membranes (5, 6). Ki-Ras is established to regulate gene expression through the mitogen-activated protein kinase cascade (for a review see Ref. 7), and the Rho family members have been shown to regulate reorganization of actin filaments (for reviews see Refs. 8 and 9), but the physiological function of Smg GDS still remains to be clarified.

Armadillo is the Drosophila segment polarity gene product (10), a homolog of mammalian β-catenin (for a review see Ref. 11). Armadillo has 12 repeated unique domains, named Arm repeats, each consisting of about 42 amino acids (10). Arm repeats are also found in β-catenin (12), plakoglobin (13), APC1 (14, 15), SRP1/importin (16), and p120 (17). Smg GDS has also 11 Arm repeats (for a review see Ref. 18). The role of Arm repeats has not fully been understood, but APC has been shown to be associated with β-catenin through the Arm domain of β-catenin and the non-Arm domain of APC (19, 20), and Arm repeat have been implicated in protein-protein interactions (18). In the present study, therefore, we have attempted to isolate an Smg GDS-interacting protein from a human brain cDNA library by use of the yeast two-hybrid method.

**EXPERIMENTAL PROCEDURES**

**Materials and Chemicals—**SMAP fused to MBP (MBP-SMAP) and Smg GDS fused to GST (GST-Smg GDS) were purified from overexpressing Escherichia coli (21, 22). A rabbit anti-SMAP polyclonal antibody was generated against MBP-SMAP, passed through a MBP-coupled affinity column (23). The antibody reacted with SMAP that was cleaved from MBP-SMAP by factor Xa (See Fig. 4B, a). A baculovirus expressing v-Src was provided by F. McCormick (Onyx Pharmaceuticals, Richmond, CA). Mouse anti-v-Src, anti-phosphotyrosine, and anti-HA monoclonal antibodies were obtained from Oncogene Science Inc. (Cambridge, MA), Upstate Biotechnology Inc. (Lake Placid, NY), and Berkeley Antibody Co. (Richmond, CA), respectively.

**Screening for an Smg GDS-interacting Protein by the Yeast Two-Hybrid Method—**Yeast strain L40 carrying pBTM116-HA-Smg GDS was transformed with a human brain cDNA library made in pGAD10 (Clontech Laboratories Inc., Palo Alto, CA). The screening was carried out (24). The full-length human brain SMAP cDNA was determined by use of the human brain 5′-RACE Ready® cDNA Kit (Clontech).

**Plasmid Construction for the Yeast Two-Hybrid Method—**A DNA fragment encoding the HA (YPYDVPDYA) epitope was inserted into the cloning site of pB7TM116 (25) to express the LexA-HA-tagged fusion protein. Plasmids used in the yeast two-hybrid method were as follows: pBTM116-HA-Smg GDS containing LexA-HA-Smg GDS fusion gene, pACTII-HK-Smg GDS containing GAD-HA-Smg GDS fusion gene (26); pACTII-HK-SMAP (593–792 amino acids); pACTII-HK-m-importin;

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) U59919.

‡To whom correspondence should be addressed: Dept. of Molecular Biology and Biochemistry, Osaka University Medical School, 2-3 Yamada-oka, Suita 565, Japan. Tel.: 81-6-879-3410; Fax: 81-6-879-3419; E-mail: ytakei@molbio.med.osaka-u.ac.jp.

§§To whom correspondence should be addressed: Dept. of Molecular Biology and Biochemistry, Osaka University Medical School, 2-3 Yamada-oka, Suita 565, Japan. Tel.: 81-6-879-3410; Fax: 81-6-879-3419; E-mail: ytakei@molbio.med.osaka-u.ac.jp.

The abbreviations used are: APC, adenomatous polyposis coli tumor suppressor protein; MBP, maltose-binding protein; GST, glutathione S-transferase; HA, influenza hemagglutinin; GAD, GAL4 transcriptional activating domain; PAGE, polyacrylamide gel electrophoresis; ER, endoplasmic reticulum; DTI, dithiothreitol; GTP, guanosine 5′-3-O-thiotriphosphate; kb, kilobase.
pGAD424-β-catenin containing GAD-β-catenin fusion gene; and GBT9-APC containing GAL4 DNA-binding domain (GBT9-APC (1212–1727 amino acids) fusion gene, containing β-catenin-binding domain. The m-importin cDNA was from Y. Yoneda (Osaka University, Osaka, Japan); pGAD424-β-catenin was from S. Tkaika (Kyoto University, Kyoto, Japan); GBT9-APC was from T. Akiyama (Osaka University, Osaka, Japan); and pBTM161-Ras(G12V) and pVP16-Raf were from K. Matsumoto (Nagoya University, Nagoya, Japan) (24). The yeast strains used were L40 (MATa trp1 leu2 his3 ade2 lys2::GAL-HIS3 URA3::lexa-z) for pGAD424 and THG (MATb trp1 leu2 his3 ade2 lys2::GAL-HIS3 URA3::lexa-z) for pBTM161-HA-Smg GDS and pBTM161-Ras(G12V) and Y190 (MATa gal4 gal80 his3 trp1 ade2 ura3 leu2 lys2::GAL-HIS3 URA3::lexa-z cyh) for GBT9-APC. Western blotting using the anti-HA antibody confirmed that yeast strains carrying pBTM161-HA-Smg GDS, pACTII-HK-Smg GDS, pACTII-HK-SMAP, and pACTII-HK-m-importin expressed proteins of about 70, 70, 50, and 80 kDa, the expected sizes of Smg GDS, Smap, and m-importin fused to Lexa-HA or GAD-HA, respectively.

Biochemical Assay for Interaction of Smg GDS with Smap—GST or recombinant GST-Smg GDS (1.5 nmol) was applied to a glutathione-Sepharose column (100 µl) equilibrated with 2 ml of Buffer A (20 mM Tris/Cl, pH 7.5, 0.5 mM EDTA, and 1 mM DTT). After the column was washed with Buffer B (20 mM Tris/Cl, pH 7.5, 1 mM EDTA, 5 mM MgCl2, and 1 mM DTT), 100 µl of recombinant MBP-SMAP (2.8 µg) was applied to the column. After the column was washed with 3 ml of Buffer B, the bound protein was eluted with GST or GST-Smg GDS by 0.4 ml of Buffer B containing 20 mM glutathione. 120 or 20 µl of the eluate was subjected to SDS-PAGE followed by silver staining or Western blotting, respectively.

Tyrosine Phosphorylation of Smap by v-Src—The phosphorylation of Smap by v-Src was performed in both cell-free and intact cell systems. In a cell-free system, the cytosol fraction of v-Src-expressing SF9 cells was obtained (4). 200 µl of the cytosol fraction (2 mg of protein) was incubated with the anti-v-Src antibody (10 µl) for 1.5 h at 4 °C. After 10 µl of protein A-Sepharose beads (Pharmacia Biotech Inc.) and 0.5 ml of Buffer C (50 mM Tris/Cl, pH 7.5, 1 mM EDTA, 5 mM MgCl2, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) were added, the immunocomplex was precipitated and washed sequentially with Buffer C and Buffer D (20 mM Tris/Cl, pH 8.0, 5 mM MgCl2, and 1 mM DTT). Reaction was started by the addition of 30 µl of Buffer D containing 0.2 mM [γ-32P]ATP (2,000 cpm/pmol) and 0.2 µM MBP-SMAP to the precipitate. The reaction was terminated by the addition of 15 µl of Laemmli’s sample buffer and subjected to SDS-PAGE, followed by autoradiography and Western blotting.

In an intact cell system, COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum at 37 °C. Cells were plated at a density of 6 × 10^5/60-mm dish. After 16 h, cells were transfected with 2 µg of pEF-BOS-HA-tagged Smap and 6 µg of pED-v-Src (provided by M. Seiki, Kanazawa University, Kanazawa, Japan) by the DEAE-dextran method, and cultured in Dulbecco’s modified Eagle’s medium for 48 h (27). HA-tagged Smap was immunoprecipitated from lysates with the anti-HA antibody coupled to protein A-Sepharose beads (28). The immunoprecipitate was subjected to SDS-PAGE followed by Western blotting.

RESULTS

We attempted to isolate an Smap GDS-interacting protein by use of the yeast two-hybrid method with Smg GDS as a bait from a human brain cDNA library. Among the selected clones, one clone encoded a novel amino acid sequence that had Arm repeats. We isolated the entire coding sequence of the clone. One clone encoded a novel amino acid sequence that had Arm repeats. We isolated the entire coding sequence of the clone.

By use of the yeast two-hybrid method Smg GDS interacted with Smap. Ras(G12V) and Raf, known to interact in the yeast two-hybrid method, were used as positive controls (24). However, Smap GDS did not interact with β-catenin, APC, or importin (Fig. 2A). Under these conditions, APC interacted with β-catenin, when yeast strain Y190 expressing APC was transformed with the plasmid encoding β-catenin instead of Smap GDS (data not shown). The direct interaction of Smap GDS with Smap was confirmed in a cell-free system using the recombinant proteins. MBP-SMAP was first applied to a GST-coupled or GST-Smap GDS-coupled glutathione-Sepharose column, and the bound protein was eluted with GST or GST-Smap GDS by glutathione. MBP-SMAP was coeluted with GST-Smap GDS but not with GST (Fig. 2B). However, when MBP was applied to a GST-Smap GDS-coupled glutathione-Sepharose column, MBP was not coeluted with GST-Smap GDS (data not shown). Under these conditions, about 15% of MBP-SMAP applied bound to GST-Smap GDS. This result indicates that Smap GDS directly interacts with Smap.

Of the substrate small G proteins for Smap GDS, RhoA is the...
best substrate (4). It was examined whether SMAP affects the two activities of Smg GDS to stimulate the GDP/GTP exchange reaction of RhoA and to inhibit the interaction of RhoA with erythrocyte membranes.

MBP-SMAP did not affect the activity of Smg GDS to stimulate the \( {\left[ {\text{32P}} \right]} \text{GTP}\gamma \text{S} \) binding to RhoA (data not shown). Neither did it affect the activity of Smg GDS to inhibit the interaction of RhoA with erythrocyte membranes (data not shown).

p120 and \( \beta \)-catenin have been shown to be tyrosine phosphorylated by v-Src (31, 32). SMAP has a C-terminal tyrosine-rich region that includes consensus sequences for tyrosine phosphorylation. Therefore, it was examined whether SMAP is tyrosine phosphorylated by v-Src. MBP-SMAP was tyrosine phosphorylated by recombinant v-Src in a cell-free system (Fig. 3A). When the stoichiometry of this phosphorylation was examined in the same way as described (33), 2 mol of phosphate were maximally incorporated into 1 mol of MBP-SMAP. To confirm that SMAP is tyrosine phosphorylated by v-Src in intact cells, HA-tagged SMAP was expressed alone or coexpressed with v-Src in cultured COS-7 cells. HA-tagged SMAP was tyrosine phosphorylated only in the v-Src-expressing COS-7 cells (Fig. 3B). These results indicate that SMAP is tyrosine phosphorylated by v-Src in both cell-free and intact cell systems.

The effect of the v-Src-catalyzed phosphorylation of SMAP on its interaction with Smg GDS was next examined. The amount of the phosphorylated form of SMAP bound to Smg GDS was about one-third of that of the unphosphorylated form (Table 1). This result indicates that the v-Src-catalyzed phosphorylation of SMAP reduces its affinity for Smg GDS.

In the last set of experiments, the tissue and subcellular distributions of SMAP were examined. Northern blotting indicated that a 4.1-kb band was detected strongly in brain and weakly in heart, lung, skeletal muscle, kidney, and testis (Fig. 4A). The message size was enough long to encode a 90-kDa protein. The tissue distribution of the SMAP mRNA was similar to that of the Smg GDS mRNA (2). The anti-SMAP antibody by Western blotting of rat brain recognized only a single band of 90 kDa, which was similar to a \( M_1 \) value calculated from the open reading frame (Fig. 4B, a). However, the position of the band was slightly lower than that of SMAP, which was cleaved from MBP-SMAP by factor Xa on SDS-PAGE. This difference might be due to the difference in species. This band disappeared when the antibody was preincubated with an excess amount of SMAP (Fig. 4B, a). Western blotting of various rat tissues showed the similar ubiquitous distribution of SMAP with its high expression in brain and testis (data not shown).

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Immunostaining of cultured PC12 cells with the anti-SMAP antibody but not with preimmune rabbit IgG showed an ER pattern estimated with DiOC6 (3) (Fig. 4B, b–d). The area strongly stained with wheat germ agglutinin and p58, Golgi complex markers, was not stained with the anti-SMAP antibody (data not shown). These results suggest that SMAP is highly concentrated at the ER area.

**DISCUSSION**

We have isolated here an Smg GDS-interacting protein and determined its primary structure. Arm repeats are defined as sequences with \( \geq 10 \) of 48 matches to an individual protein.

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**TABLE I**

| MBP-SMAP | Bound to and co-eluted with GST or GST-Smg GDS | % |
|----------|-----------------------------------------------|---|
| GST + unphosphorylated form | 100 | 0 |
| GST + phosphorylated form | 100 | 0 |
| GST-Smg GDS + unphosphorylated form | 100 | 15 |
| GST-Smg GDS + phosphorylated form | 100 | 5 |

**Fig. 3.** Tyrosine phosphorylation of SMAP by v-Src. A, in a cell-free system. Lanes 1 and 2, autoradiography; lanes 3 and 4, Western blotting with the anti-phosphotyrosine antibody. Lanes 1 and 3, without the anti-v-Src antibody; lanes 2 and 4, with the anti-v-Src antibody. An arrow indicates the position of MBP-SMAP. B, in an intact cell system. Lanes 1–3, Western blotting with the anti-phosphotyrosine antibody; lanes 4–6, Western blotting with the anti-HA antibody. Lanes 1 and 4, pEF-BOS-HA-tagged SMAP alone; lanes 2 and 5, pEF-BOS-HA-tagged SMAP and pcD-v-Src; lanes 3 and 6, pcD-v-Src alone. An arrow indicates the position of HA-tagged SMAP. The results shown are representative of three independent experiments.

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2 A 2.8-kb band was detected in testis. The exact reason for this band is not known, but one explanation is that it results from alternative splicing of the primary transcript of a single gene.
KAP115 is an accessory subunit of sea urchin kinesin II. Sea urchin kinesin II consists of SpKRP85, SpKRP95, and SpKAP115. It has been reported that the murine homologs of SpKRP85 and SpKRP95, namely KIF3A and KIF3B, respectively, form a heterotrimeric complex with a third polypeptide, KAP3 (34), which has not yet been characterized. It seems likely that KAP3 will turn out to be a homolog of SpKAP115 and SMAP. Sea urchin kinesin II is an ATPase motor that may transport vesicles along microtubules from their minus to plus ends (for a review see Ref. 35). SpKAP115 may select the vesicles as an adaptor for sea urchin kinesin II (30). Immunocytochemical studies have demonstrated that kinesin is located at the ER Golgi area (36), but the exact localization of kinesin II is not known. We have shown here that SMAP is ubiquitously expressed and highly concentrated at the ER area in PC12 cells. It is likely that SMAP is associated with kinesin II or its related protein and regulates the ER Golgi vesicle transport as an adaptor for both Smg GDS and kinesin II or its related protein.

The kinesin-bound vesicles could be transported between the ER and Golgi membranes along microtubules (36). Microtubules are generally known not to directly interact with the plasma membranes or the membranes of intracellular organelles. It could be speculated that microtubules between the membranes of intracellular organelles are connected by microfilaments. The Rho family members are known to regulate reorganization of actin filaments (8, 9) and to be located not only at the plasma membranes but also at the ER Golgi area (37). SMAP may determine the positions of both Smg GDS and kinesin II or its related protein, where Smg GDS regulates reorganization of actin filaments through its substrate Rho family members. This reorganization of actin filaments may be involved in the kinesin II- or kinesin II-related protein-mediated vesicle trafficking.

We have shown here that SMAP is tyrosine phosphorylated by v-Src in both cell-free and intact cell systems. We have not determined here the tyrosine phosphorylation sites of SMAP, but SMAP has three consensus tyrosine sites, Tyr698, Tyr787, and Tyr788, for the v-Src phosphorylation, and consistently the incorporation of phosphate was maximally 2 mol/mol for SMAP. The two Tyr residues of the three may be the phosphorylation sites. The physiological function of this phosphorylation remains to be clarified, but we have shown here that this phosphorylation reduces the affinity of SMAP for Smg GDS. Therefore, it is possible that the v-Src-catalyzed phosphorylation of SMAP regulates its interaction with Smg GDS. Src has also been shown to be associated with the perinuclear vesicles, presumably at the ER Golgi area, and to be involved in the vesicle trafficking (38). It could be speculated that this action of Src may be mediated through SMAP.

Note Added in Proof—After submission of this article, Yamazaki et al. (41) reported the primary structure of mouse KAP3A. The sequence of mouse KAP3A shares 97% amino acid sequence identity with human SMAP. This result indicates that human SMAP is a true counterpart of mouse KAP3A.

REFERENCES

1. Yamamoto, T., Kaibuchi, K., Mizuno, T., Hiroyoshi, M., Shirataki, H., and Takai, Y. (1990) J. Biol. Chem. 265, 16626–16634
2. Kaibuchi, K., Mizuno, T., Fujioka, H., Yamamoto, T., Kishi, K., Fukumoto, Y., Hata, Y., and Takai, Y. (1991) V. Cell. Biol. 14, 2073–2080
3. Hiroyoshi, M., Kaibuchi, K., Kawamura, S., Hata, Y., and Takai, Y. (1991) J. Biol. Chem. 266, 2962–2969
4. Mizuno, T., Kaibuchi, K., Yamamoto, T., Kawamura, M., Sakoda, T., Fujioka, H., Matsuura, Y., and Takai, Y. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6442–6446
5. Kawamura, S., Kaibuchi, K., Hiroyoshi, M., Hata, Y., and Takai, Y. (1991) Biochem. Biophys. Res. Commun. 174, 1085–1102
6. Kawamura, M., Kaibuchi, K., Kishi, K., and Takai, Y. (1993) Biochem. Biophys. Res. Commun. 190, 832–841
7. Blenis, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5889–5892

Fig. 4. Tissue and subcellular distributions of SMAP. A, Northern blotting. A mouse embryo multiple tissue Northern blot (Clontech) was probed for expression of the SMAP gene using randomly primed, 32P-labeled fragments from the 3.4-kb mouse SMAP cDNA, which was isolated from a mouse brain cDNA library. (We screened a mouse cDNA library using the 1.4-kb human SMAP cDNA isolated from the two-hybrid method as a probe and determined the primary structure of mouse SMAP. Mouse SMAP shares 85% amino acid sequence identity with human SMAP.) Lane 1, heart; lane 2, brain; lane 3, lung; lane 4, skeletal muscle; lane 5, kidney; lane 6, testis. B, immunocytochemistry of PC12 cells. a, specificity of the anti-SMAP antibody. The homogenate (100 μg of protein) of rat brain was Western blotted with the anti-SMAP antibody. Lane 1, recombinant human SMAP (50 ng of protein) that was cleaved from MBP-SMAP by factor Xa; lanes 2 and 3, homogenate of rat brain. Lanes 1 and 2, with the anti-SMAP antibody; lane 3, with the anti-SMAP antibody preincubated with an excess amount of SMAP. An arrow indicates the position of recombinant SMAP. b–d, immunostaining. The cells were double stained with the anti-SMAP antibody (4.2 μg/ml of protein) (b) or DiOC6(3) (Molecular Probes Inc., Eugene, OR) (5 μg/ml) as an ER and mitochondrial marker (d) and stained with preimmune rabbit IgG (4.2 μg/ml of protein) (c). Rhodamine-conjugated donkey anti-rabbit IgG (Chemicon International, Temecula, CA) was used as the second antibody. The cells were examined using a confocal laser scanning microscope (27, 39). The results shown are representative of three independent experiments. Bar, 10 μm.

consensus (18). According to this definition, the Smg GDS-interacting protein isolated here is a novel protein having 9 Arm repeats and named SMAP. We have moreover shown by use of the yeast two-hybrid method that Smg GDS does not interact with other proteins having Arm repeats and specifically interacts with SMAP. The direct interaction of Smg GDS with SMAP has moreover been confirmed in a cell-free system using the recombinant proteins. We have not yet determined the domains of Smg GDS and SMAP that interact with each other, but the interaction of Smg GDS with SMAP does not interfere with the two activities of Smg GDS on RhoA, suggesting that Smg GDS has at least two regions, each interacting with RhoA and SMAP.

During the present study, the full sequence of sea urchin SpKAP115 was reported, and it shares 66% amino acid sequence identity with SMAP, although it was not shown that SpKAP115 has Arm repeats (30). SMAP is likely to be a mammalian counterpart of SpKAP115 or its related protein. Sp-
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