Functional characterization of novel human ARFGAP3

Xiaoqin Liu[a,b], Chenggang Zhang[a], Guichun Xing[a], Qingtang Chen[b], Fuchu He[a,*]

[a] *Department of Genomics and Proteomics, Beijing Institute of Radiation Medicine, Chinese National Human Genome Center at Beijing, 27 Taiping Road, Beijing 100850, PR China*
[b] *Department of Neurology, First Teaching Hospital, Beijing University, Beijing 100034, PR China*

Received 10 October 2000; revised 3 January 2001; accepted 10 January 2001

First published online 22 January 2001

Edited by Felix Wieland

Abstract ADP ribosylation factors (ARFs) are critical in the vesicular trafficking pathway. ARF activity is controlled by GTPase-activating proteins (GAPs). We have identified recently a novel tentative ARF GAP derived from human fetal liver, ARFGAP3 (originally named as ARFGAP1). In the present study, we demonstrated that ARFGAP3 had GAP activity in vitro and remarked that the GAP activity of ARFGAP3 was regulated by phospholipids, i.e. phosphatidylinositol 4,5-diphosphate as agonist and phosphatidylcholine as antagonist. ARFGAP3 is a predominantly cytosolic protein, and concentrated in the perinuclear region. Its transient ectopic overexpression in cultured mammalian cells reduced the constitutive secretion of secreted alkaline phosphatase, indicating that ectopic overexpression of ARFGAP3 inhibits the early secretory pathway of proteins in vivo. These results demonstrated that ARFGAP3 is a novel GAP for ARF1 and might be involved in intracellular traffic of proteins and vesicular transport as predicted.

Key words: Vesicular transport; ADP ribosylation factor; GTPase-activating protein; Secreted alkaline phosphatase

1. Introduction

In eukaryotic cells, the transport of materials between intracellular compartments is mediated by transporting vesicles that bud from a donor membrane and subsequently fuse with an acceptor compartment [1]. ADP ribosylation factors (ARFs), members of the Ras superfamily of GTP-binding proteins, play critical roles in vesicular trafficking [2–4]. Like Ras, ARFs are active in their GTP-bound form, and their duration of activity is controlled by GTPase-activating proteins (GAPs). GTP hydrolysis, in which an ARF GAP is involved, is required for the dissociation of coat proteins from Golgi-derived membranes and vesicles [5,6], and the dissociation of coat proteins is a prerequisite for the vesicle's fusion with target compartment. Some proteins with ARF GAP activity or ARFGAP-like motif have been identified, including *Rattus norvegicus* ARF1 GAP [7,8], *Saccharomyces cerevisiae* Gcs1 [9], *Mus musculus* ASAP1 [10], *R. norvegicus* GIT1 [11], *R. norvegicus* Pap [12], and *S. cerevisiae* Glo3 [13]. Moreover, the crystal structural and functional analysis of the complex of human ARF1 with rat ARF1 GAP was reported recently [14]. However, the knowledge about the complexity and importance of ARF GAP-directed vesicle transport, especially those from human, is still limited.

Recently, we have identified a novel human ARFGAP, ARFGAP3 (originally named as ARFGAP1) [15]. ARFGAP3 encodes a polypeptide of 516 amino acids with an ARF GAP domain that is required for ARF1 GAP catalytic functions [16,17]. Like other members of ARF GAP family, ARFGAP3 contains a distinctive GATA-1-type zinc finger motif ($CX_2CX_{16}CX_2C$). It is highly expressed in endocrine glands and testis. Although ARFGAP3 might play a role in regulation of cellular secretion, its function was not characterized yet. Here we report that ARFGAP3 did have ARFGAP activity in vitro. Remarkably, the GAP activity of ARFGAP3 could be regulated by phospholipids. We also provide evidence that ARFGAP3 inhibits the constitutive secretion of secreted alkaline phosphatase (SEAP) in vivo.

2. Materials and methods

2.1. Reagents

pEGFP-C1, mouse monoclonal anti-GFP antibody, and Great EscAPe™ SEAP chemiluminescence detection kit were purchased from Clontech; the pBAD/TOPO® Thio-Fusion™ expression system, Xpress™ system protein purification kit, EnterokinaseMax™ kit, EK-away kit, pcDNA3.1/myc-His A, mouse monoclonal anti-myc-HRP antibody from Invitrogen; arabinose, L-α-dimyristoyl phosphatidylcholine (DMPC), sodium cholate, creatine phosphokinase, phosphatidylcholine (PC), phosphatidylinositol 4,5-diphosphate (PIP_2), and polyethyleneimine–cellulose plates from Sigma; DMEM, and fetal bovine serum from Life Technologies; restriction enzymes, unless otherwise indicated, from Promega. Recombinant, myristoylated ARF1 was prepared from bacteria co-expressing ARF1 and *N*-myristoltransferase, which was kindly provided by Dr. Dan Cassel [8,18]. pGEM-SEAP was kindly provided by Dr. Monier [19].

2.2. Construction of expression vector of ARFGAP3

For mammalian fusion expression with GFP, the coding region of ARFGAP3 was generated by PCR from pGEM-T-ARFGAP3 [15] with the forward primer 5′-CG*GAATTC*GATGGGGGACCCCAGCAAGCA-3′ (italicized sequence, *Eco*RI site) and reverse primer 5′-CG*GGATCC*AGAACCGTAGCGATCCTGAA-3′ (italicized sequence, *Bam*HI site). The PCR product after digestion with *Eco*RI and *Bam*HI was extracted and purified from low-melting (LM) agarose gel, and ligated in-frame to the *Eco*RI- and *Bam*HI-digested pEGFP-C1 expression vector. The subcloned sequence of the ARFGAP3 was confirmed by automated sequencing on an ABI Prism 377-XL DNA sequencer (Perkin-Elmer) in both forward and reverse direction using EGFP-C1 sequencing primers (Clontech).

For expression of the recombinant ARFGAP3 as a fusion protein

*Corresponding author. Fax: (86)-10-68214653.
E-mail: hefc@nic.bmi.ac.cn

Abbreviations: ARF, ADP ribosylation factor; DMPC, L-α-dimyristoyl phosphatidylcholine; GAP, GTPase-activating protein; PC, phosphatidylcholine; PIP_2, phosphatidylinositol 4,5-diphosphate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SEAP, secreted alkaline phosphatase

0014-5793/01/$20.00
PII: S0014-5793(01)02134-2

with thioredoxin in *Escherichia coli*, the coding region of ARFGAP3 was generated by PCR from pGEM-T-ARFGAP3 [15] with the forward primer 5′-G*GGATCC*CGATGGGGGACCCCAGC-3′ (italicized sequence, *Bam*HI site) and reverse primer 5′-*CTCGAG*AGAACCGTAGCGATCCTG-3′ (italicized sequence, *Xho*I site). The PCR product was extracted and purified from LM agarose gel, and ligated in-frame to the pBAD/Thio-TOPO bacterial fusion expression vector with thioredoxin and a 6×His tag sequence.

For mammalian expression, the coding region of ARFGAP3 was obtained by digesting pBAD/thio–ARFGAP3 with *Bam*HI and *Xho*I, extracted and purified from LM agarose gel, and ligated in-frame to the *Bam*HI- and *Xho*I-digested pcDNA3.1/myc-hisA expression vector (Invitrogen).

2.3. Expression and purification of the recombinant ARFGAP3 in bacteria

The thioredoxin–ARFGAP3 fusion protein was expressed in *E. coli* TOP10 induced by arabinose, and purified by affinity chromatography on a nickel-chelating column (Invitrogen) and cleaved by enterokinase (Invitrogen). Enterokinase was excluded by using EKaway kit (Invitrogen), and then ARFGAP3 was purified again on a nickel-chelating column according to the manufacturer's instructions.

2.4. Cell culture and transient transfection

COS-7 cells were plated into 6-well trays (6×35 mm) at a density of 2×10^5 cells per well and grown in complete medium that consisted of Dulbecco's modified Eagle medium (DMEM) with 10% fetal calf serum, 2 mM glutamine at 37°C in a 5% CO_2 incubator. The cells were transfected at the next day with 5 μg of plasmid DNAs together with 10 μl lipofectamine (Life Technologies) according to the manufacturer's instructions. Plasmid DNAs (5 μg) and lipofectamine (10 μl) were diluted separately with DMEM without serum and antibiotics, and combined (1 ml in final volume) just before applying to cells (80% confluent). After 6∼12 h of incubation at 37°C, 2 ml of culture medium with fetal bovine serum was added. Expression of ARFGAP3 was assessed by Western blotting analysis 48 h after transfection, except in the time-course experiment.

2.5. Subcellular localization of ARFGAP3 by fluorescence labeling

Live cells for epifluorescence microscopy were plated into 6-well trays (6×35 mm) at a density of 2×10^5 cells per well and transfected with plasmid pEGFP-C1-ARFGAP3 at the next day. Cells were examined 12–48 h after transfection with a Zeiss Axioscope epifluorescence microscope equipped with a Princeton Instruments cooled CCD camera with MetaMorph digital imaging software (Universal Imaging). The expression of pEGFP-C1-ARFGAP3 was verified by immunoblotting analysis.

2.6. Immunoblotting analysis

For immunoblotting analysis, cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed in 50 mM HEPES (pH 7.2), 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1% Triton X-100, 1 mM sodium orthovanadate, 40 mM β-glycerophosphate, 10 mM sodium pyrophosphate, 1 mM phenylmethyl sulfonyl fluoride (PMSF), 10 μg/ml of leupeptin, and 10 μg/ml of aprotinin (lysis buffer). Total cell lysates from transfected COS-7 cells were pre-cleared by centrifugation, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose by conventional techniques. Filters were blocked overnight with TBS buffer containing 5% dry milk at 4°C and incubated with the mixture of antibodies and TBS buffer containing 5% dry milk; then washed by TBS buffer containing 0.05% Tween 20 and stained by ECL$^+$ Western blotting detection system according to the manufacturer's instructions.

2.7. GAP activity assay of ARFGAP3

ARF GAP activity was determined by an in vitro assay that measures a single round of GTP hydrolysis on recombinant ARF1 [20,21]. ARF1 was first loaded with [α-^{32}P]GTP as described [22]. DMPC and cholate was used to facilitate the loading of ARF1 with GTP [8]. The loading reaction mixture contained ARF1 (0.5 mg/ml), [α-^{32}P]GTP (0.2 mCi/ml, 0.25 μM, 5 mM $MgCl_2$, 1 mM DTT, 1 mM ATP plus ATP/GTP regeneration system (5 mM phosphocreatine and 50 μg/ml creatine phosphokinase), 25 mM MOPS buffer (pH 7.5), 150 mM KCl, and a mixture of DMPC and sodium cholate added last from a 10×stock to give 3 mM and 1 mg/ml respectively. Loading was carried out for 90 min at 30°C. Then GAP activity was assayed in a final volume of 10μl in the presence of 5 mM $MgCl_2$, 1mM DTT, 1 mM ATP plus the above mentioned ATP/GTP regeneration system, 25 mM MOPS buffer (pH 7.5), 0.5 mM unlabeled GTP, 0.1 unit/ml guanylate kinase, and 1 μl of [α-^{32}P]GTP-loaded ARF. Considering that filter binding assays [23] showed that about 30–40% of the [α-^{32}P]GTP becomes associated with recombinant ARF1, free GTP was removed from the reaction system after GAP3 digestion, i.e. following incubation for 15 min at 30°C, unbound GTP was removed by centrifugation filtration through nitrocellulose and rinsed five times with 25 mM MOPS buffer (pH 7.5). The protein-bound nucleotides were trapped on nitrocellulose filters and then eluted from the filters in 2 M formic acid. ARF-bound GTP and GDP were separated by thin layer chromatography on polyethyleneimine–cellulose plates in 1 M formic acid/1 M LiCl. [α-^{32}P]GDP formation was determined by autoradiography at −80°C for 18–28 h. Data were presented as the percentage of ARF-bound [α-^{32}P]GTP that is converted to ARF-bound [α-^{32}P]GDP. GAP activity was assessed by serial dilutions of the sample.

2.8. Secretion assay of SEAP

COS-7 cells grown in 6-well plates were co-transfected with pGEM-SEAP and pcDNA3.1/myc-His-ARFGAP3 by the lipofectamine-mediated method. At 12 h after transfection, the medium was changed. 24, 36, and 48 h later, the culture supernatants were taken, and the cells were lysed in ice-cold growth medium supplemented with 1% Triton X-100 and 1 mM PMSF, 10 μl/ml of leupeptin, and 10 μl/ml of aprotinin. SEAP activity in culture supernatants and in cell lysates was determined by using Great EscAPe™ SEAP chemiluminescence detection kit (Clontech). Secretion was expressed as the ratio of SEAP activity in culture supernatants to the sum of SEAP activity in both culture supernatants and cell lysates. All experiments were performed in triplicate. The expression of pcDNA3.1/myc-His-ARFGAP3 constructs was verified by immunoblotting analysis.

3. Results

3.1. Subcellular localization of ARFGAP3

To determine the subcellular localization of ARFGAP3 in eukaryotic cells, we tagged the N-terminus of ARFGAP3 with GFP, expressed the fusion protein in cultured cells by transfection, and examined the transfected cells under a epifluorescence microscope. The expression of GFP–ARFGAP3 was verified by Western blotting analysis using mouse monoclonal anti-GFP antibodies (Fig. 1A).

12 h after transfection, weak and diffuse fluorescence was observed in the whole cytoplasm of COS-7 cells transfected with pEGFP-C1-ARFGAP3 (Fig. 1B-b). 24 h after transfection, the fluorescence was restricted to the perinuclear region. In some cells, the most prominent feature was an intense, compact, well-delineated area of fluorescence adjacent to the nucleus (Fig. 1B-c). 48 h after transfection, the fluorescence was further concentrated in the perinuclear region (Fig. 4B-d). In comparison, only weak and diffuse fluorescence was observed in the whole COS-7 cells transfected with control plasmid pEGFP-C1 (Fig. 1B-a) at the same time. These results showed that ARFGAP3 is a cytosolic protein and concentrated mainly in the perinuclear region.

3.2. Expression and purification of recombinant ARFGAP3 protein

The Thioredoxin–ARFGAP3 fusion protein was expressed in TOP10 cells induced by arabinose (Fig. 2, lane 4), and purified by affinity chromatography on a nickel-chelating column (Fig. 2, lane 5) and cleaved by Enterokinase (Fig. 2, lane 6), then purified again on a nickel-chelating column (Fig. 2, lane7). At last, the target protein ARFGAP3 was obtained at

a purity of 90 percent with an identical molecular weight as expected from its cDNA.

3.3. ARFGAP3 protein exhibits ARF GAP activity in vitro

The purified recombinant ARFGAP3 protein was tested in vitro for ARF GAP activity by using ARF1 as substrate. As shown in Fig. 3A, ARFGAP3 was unable to hydrolyze free [α-^{32}P]GTP, but led to hydrolysis of ARF-bound GTP in a concentration-dependent manner (Fig. 3B), indicating that ARFGAP3 acts as a GAP for ARF1. Furthermore, we also determined the effect of phospholipids upon GAP activity exhibited by the recombinant ARFGAP3 and indicated that the GAP activity of ARFGAP3 was enhanced by PIP_2 (Fig. 3C), but decreased by PC (Fig. 3D).

3.4. Ectopic expression of ARFGAP3 reduces constitutive secretion of protein in vivo

To determine whether ARFGAP3 regulates cellular secretion in vivo we ectopically expressed ARFGAP3 in COS-7 cells. The expression of recombinant ARFGAP3 in COS-7 cells was confirmed by Western blotting analysis. The myc-tagged ARFGAP3 was evidenced in lysates from COS-7 cells transfected with expression vectors pcDNA3.1/myc-His-ARFGAP3 using mouse monoclonal anti-myc antibodies (Fig. 4A). Then, as shown in Fig. 4B, approximately 90% of the total SEAP synthesized in mock-transfected cells was released into the medium after 24 and 36 h. In contrast, ARFGAP3 significantly decreased SEAP secretion at 24 and 36 h after co-transfection. At 48 h after co-transfection, the inhibitory activity disappeared. As a control, co-transfection of exogenous Lac Z with SEAP had no effect on SEAP secretion of transfected cells, suggesting that the inhibition effect of ARFGAP3 on SEAP secretion may not due to the overexpression of non-specific exogenous protein(s). These results indicate that ARFGAP3 reduced constitutive secretion of target cells, suggesting that ARFGAP3 may play a role in protein secretion of host cells.

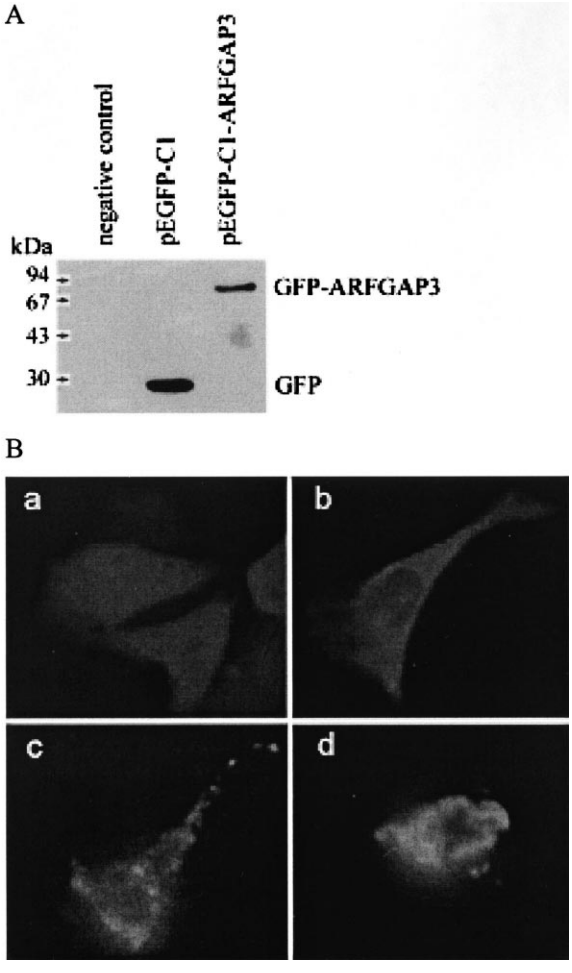

Fig. 1. Subcellular localization of ARFGAP3 tagged with GFP. A: Western blotting analysis of the expressed recombinant fusion protein GFP–ARFGAP3. Lysates from COS-7 cells transfected with pEGFP-C1, or pEGFP-C1-ARFGAP3 were subjected to immunoblotting with anti-GFP antibodies. Lysates from parent COS-7 cells without transfection were used as negative control. B: Subcellular localization of ARFGAP3 tagged with GFP. a: COS-7 cells transfected with pEGFP-C1; b–d: COS-7 cells transfected with pEGFP-C1-ARFGAP3 at 12, 24, or 48 h after transfection.

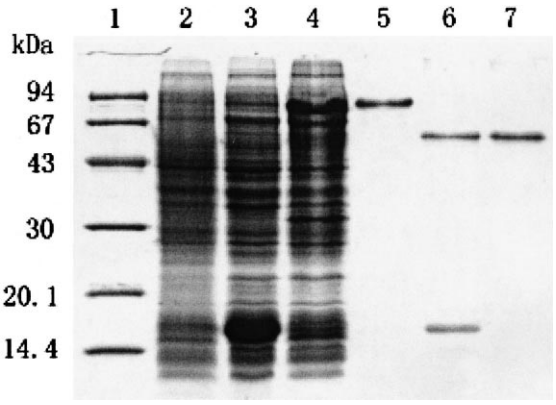

Fig. 2. Expression, purification, and cleavage of thioredoxin–ARFGAP3 protein from *E. coli* cells. (1) Molecular weight markers; (2) negative control: total cell proteins of TOP10 cell; (3) positive control: total cell proteins of TOP10 cell carrying pBAD-Thio induced by arabinose; (4) total cell proteins of induced TOP10 cell carrying pBAD/Thio–ARFGAP3; (5) purified recombinant fusion protein Thio–ARFGAP3 (5 μg); (6) purified recombinant fusion protein cleaved by EKMax™; (7) target protein ARFGAP3 purified again after cleavage by EKMax™ (5 μg). These proteins were analyzed by electrophoresis on SDS–polyacrylamide gels, and stained with Coomassie bright blue.

4. Discussion

ARFs are critical components of vesicular trafficking pathways in eukaryotes, and the duration of their activity is controlled by GAPs [2–4]. Recently, we reported a novel human gene, ARFGAP3, which was deduced to be a new member of ARF GAP family with a potential function in regulation of cellular secretion [15]. Herein, the cellular localization and functional characterization of ARFGAP3 was reported. It was demonstrated that the purified recombinant ARFGAP3 exhibited strong GAP activity towards ARF1 protein. Randazzo and Kahn [20] reported that ARF GAP activity in a partially purified preparation from bovine brain was modulated by phospholipids. Among various phospholipids, PIP_2 strongly stimulated GAP activity of some ARF GAPs; PC caused a small but reproducible inhibition of ARF GAP activity [8,10,12,20,24]. Like all the known ARFGAPs, ARFGAP3 displayed similar phospholipid sensitivity results. Thus, it was evident that ARFGAP3 exhibited a typical activity of ARF GAP.

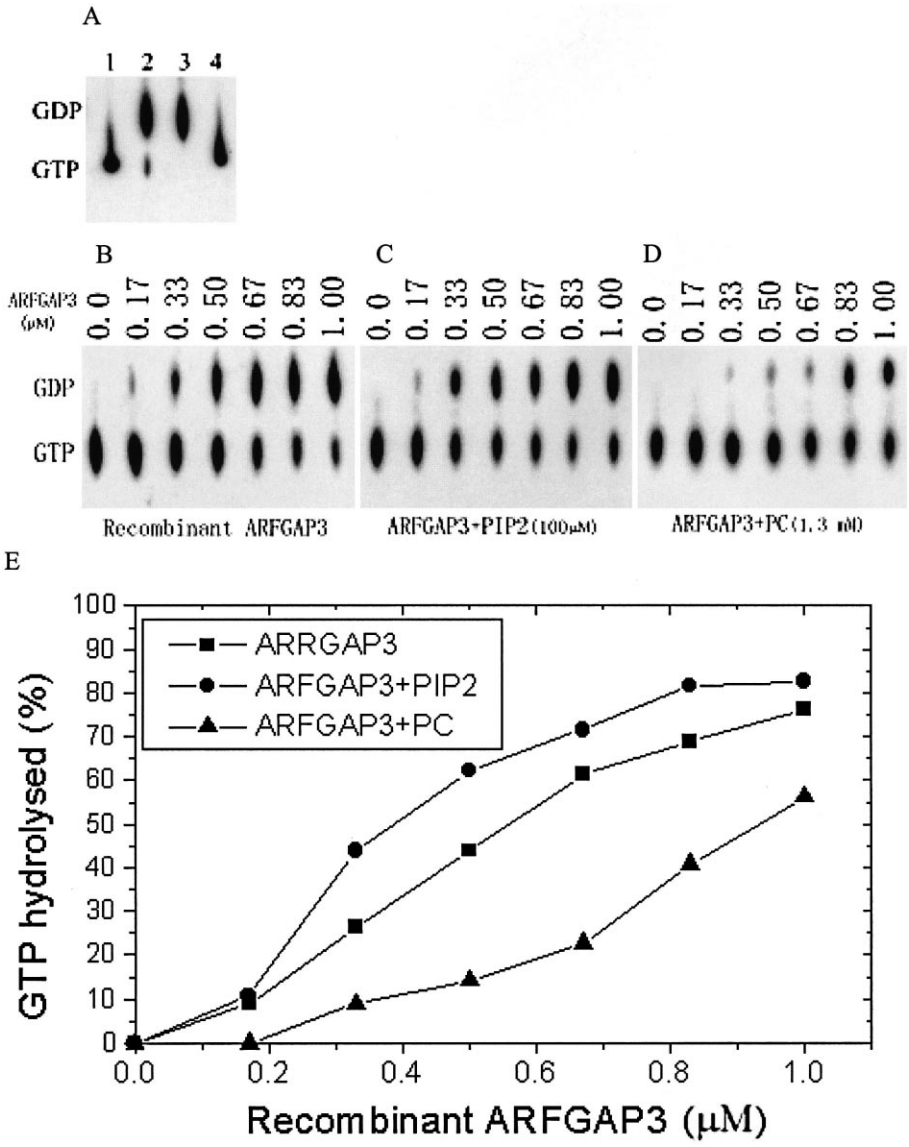

Fig. 3. GAP activity of ARFGAP3 and its regulation by lipids. A: GAP activity of ARFGAP3 with substrate of recombinant ARF1: GAP activity was assayed with 5 μg/ml ARF1 plus 0 μM (1), 1.0 μM (2), 10 μM (3) ARFGAP3, or 10 μM ARFGAP3 with free GTP instead of ARF-GTP (4). B–E: Effect of lipids on the GAP activity of ARFGAP3. GAP activity was assayed in the absence or presence of 100 μM PIP_2 or 1.3 mM PC. The data are the average of two experiments.

To test the subcellular localization of ARFGAP3, we tagged the N-terminus of ARFGAP3 with GFP, expressed the fusion protein in cultured cells by transfection and observed in living cells by epifluorescence microscopy. It was demonstrated that ARFGAP3 is a cytosolic protein and concentrated in the perinuclear region. The perinuclear region contains many different organelles, including Golgi, the microtubule-organizing center, and vesicular structures of the endosome–lysosome system [25]. It has been demonstrated that ARF1 displays a typical Golgi localization, where ARF1 exert its function of control of vesicular transport [26]. Thus, it is reasonable to propose that ARFGAP3 may be associated with the structures above and involved in the regulation of vesicular transport.

ARF1 was implicated in the control of vesicle transport in different intracellular compartments, including the Golgi complex. In this study, transient ectopic expression of ARFGAP3 in cultured mammalian cells caused partial inhibition of the constitutive secretion of SEAP. The result is quantitatively similar with that of Pap, a new Pyk2 binding protein with ARF GAP activity [12]. The inhibition implicated that ARFGAP3, like other known ARFGAPs, may exert ARF GAP activity in these cells, and function in protein secretion or/and vesicle transport.

Taken together, these results suggest that ARFGAP3 is a novel member of ARF GAP family and might be involved in the regulation of cellular secretion. So far, it remains to be determined what is its exact physiological role, e.g. in endocrine glands and testis, and what is its specificity for different ARFs. However, the characterization of biochemical function of ARFGAP3 will advance our understanding of the regulation of the cellular process in which ARF proteins participate, especially in human. In addition, considering that ARFGAP3 was the first known member of ARFGAP family in human beings and strongly expressed in endocrine glands and testis, the knowledge about it might significantly facilitate the under-

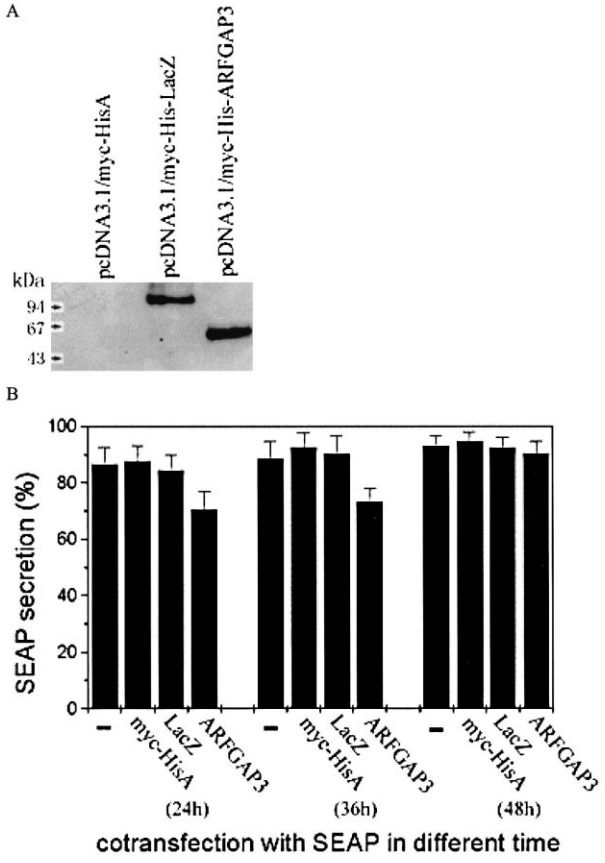

Fig. 4. Inhibition of cellular SEAP secretion by overexpressed ARFGAP3 in vivo. A: Western blotting analysis of the expression of recombinant ARFGAP3 protein in COS-7 cells. Lysates from COS-7 cells transfected with expression vectors pcDNA3.1/myc-His A, pcDNA3.1/myc-His-lacZ, pcDNA3.1/myc-His-ARFGAP3 respectively, were subjected to immunoblotting with anti-myc antibodies. B: Inhibition of cellular SEAP secretion by overexpression of ARFGAP3. Cells were co-transfected with expression vectors for SEAP and for ARFGAP3. The graphs depict the amount of SEAP released into the medium as a percentage of the total SEAP expressed. All experiments were done in triplicate. Error bars represent standard deviation values.

standing of physiology and pathology of endocrine glands and testis and their related diseases.

Acknowledgements: This research was partially supported by Chinese National Natural Sciences Foundation Key Project (39730310, 30070386), Chinese National Key Project of Basic Research, Chinese High-tech Program and Chinese National Distinguished Young Scientist Award (39625014). We are grateful to Drs. Dan Cassel, Lin Mei and S. Monier for kindly providing constructs used in this study and helpful suggestions.

References

[1] Schekman, R. and Orci, L. (1996) Science 271, 1526–1533.
[2] Rothman, J.E. (1994) Nature 372, 55–63.
[3] Moss, J. and Vaughan, M. (1995) J. Biol. Chem. 270, 12327–12330.
[4] Moss, J. and Vaughan, M. (1998) J. Biol. Chem. 273, 21431–21434.
[5] Teal, S.B., Hsu, V.W., Peters, P.J., Klausner, R.D. and Donaldson, J.G. (1994) J. Biol. Chem. 269, 3135–3138.
[6] Tanigawa, G., Orci, L., Amherdt, M., Ravazzola, M., Helms, J.B. and Rothman, J.E. (1993) J. Cell Biol. 123, 1365–1371.
[7] Cukierman, E., Huber, I., Rotman, M. and Cassel, D. (1995) Science 270, 1999–2002.
[8] Makler, V., Cukierman, E., Rotman, M., Admon, A. and Cassel, D. (1995) J. Biol. Chem. 270, 5232–5237.
[9] Poon, P.P., Wang, X., Rotman, M., Huber, I., Cukierman, E., Cassel, D., Singer, R.A. and Johnston, G.C. (1996) Proc. Natl. Acad. Sci. USA 93, 10074–10077.
[10] Brown, M.T., Andrade, J., Radhakrishna, H., Donaldson, J.G., Cooper, J.A. and Randazzo, P.A. (1998) Mol. Cell. Biol. 18, 7038–7051.
[11] Premont, R.T., Claing, A., Vitale, N., Freeman, J.L.R., Pitcher, J.A., Patton, W.A., Moss, J., Vaughan, M. and Lefkowitz, R.J. (1998) Proc. Natl. Acad. Sci. USA 95, 14082–14087.
[12] Andreev, J., Simon, J-P., Sabatini, D.D., Kam, J., Plowman, G., Randazzo, P.A. and Schlessinger, J. (1999) Mol. Cell. Biol. 19, 2338–2350.
[13] Poon, P.P., Cassel, D., Spang, A., Rotman, M., Pick, E., Singer, R.A. and Johnston, G.C. (1999) EMBO J. 18, 555–564.
[14] Goldberg, J. (1999) Cell 96, 893–902.
[15] Zhang, G., Yu, Y., Zhang, S., Liu, M., Xing, G., Wei, H., Bi, J., Liu, X., Zhou, G., Dong, C., Hu, Z., Zhang, Y., Luo, L., Wu, C., Zhao, S. and He, F. (2000) Genomics 63, 400–408.
[16] Hannon, R., Evans, T., Felsenfeld, G. and Gould, H. (1991) Proc. Natl. Acad. Sci. USA 88, 3004–3008.
[17] Vitale, N., Horiba, K., Ferrans, V.J., Moss, J. and Vaughan, M. (1998) Proc. Natl. Acad. Sci. USA 95, 8613–8618.
[18] Franco, M., Chardin, P., Chabre, M. and Paris, S. (1995) J. Biol. Chem. 270, 1337–1341.
[19] Monier, S., Chardin, P., Robineau, S. and Goud, B. (1998) J. Cell Sci. 111, 3427–3436.
[20] Randazzo, P.A. and Kahn, R.A. (1994) J. Biol. Chem. 269, 10758–10763.
[21] Xu, G., Lin, B., Tanaka, K., Dunn, D., Wood, D., Gesteland, R., White, R., Weiss, R. and Tamanoi, F. (1990) Cell 63, 835–841.
[22] Vital, N., Moss, J. and Vaughan, M. (1996) J. Biol. Chem. 272, 3897–3904.
[23] Kahn, R.A. and Gilman, A.G. (1986) J. Biol. Chem. 261, 7906–7911.
[24] Ding, M., Vitale, N., Tsai, S.C., Adamik, R., Moss, J. and Vaughan, M. (1996) J. Biol. Chem. 271, 24005–24009.
[25] Vitale, N., Horiba, K., Ferrans, V.J., Moss, J. and Vaughan, M. (1998) Proc. Natl. Acad. Sci. USA 95, 8613–8618.
[26] Donaldson, J.G., Kahn, R.A., Lippincott-Schwartz, J. and Klausner, R.D. (1991) Science 254, 1197–1199.