Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 3063–3066, April 1994
Cell Biology

Identification of a brefeldin A-insensitive guanine nucleotide-exchange protein for ADP-ribosylation factor in bovine brain

(cholera toxin/Golgi apparatus)

SU-CHEN TSAI*, RONALD ADAMIK, JOEL MOSS, AND MARTHA VAUGHAN

Laboratory of Cellular Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Contributed by Martha Vaughan, December 23, 1993

ABSTRACT **ADP-ribosylation factors (ARFs) are ≈20-kDa guanine nucleotide-binding proteins that participate in vesicular transport in the Golgi and other intracellular compartments and stimulate cholera toxin ADP-ribosyltransferase activity. ARFs are active in the GTP-bound form; hydrolysis of bound GTP to GDP, possibly with the assistance of a GTP hydrolysis (GTPase)-activating protein results in inactivation. Exchange of GDP for GTP and reactivation were shown by other workers to be enhanced by Golgi membranes in a brefeldin A-sensitive reaction, leading to the proposal that the guanine nucleotide-exchange protein (GEP) was a target of brefeldin A. In the studies reported here, a soluble GEP was partially purified from bovine brain. Exchange of nucleotide on ARFs 1 and 3, based on increased ARF activity in a toxin assay and stimulation of binding of guanosine 5′-[γ-[^{35}S]thio]triphosphate, was dependent on phospholipids, with phosphatidylserine being more effective than cardiolipin. GEP appeared to increase the rate of nucleotide exchange but did not affect the affinity of ARF for GTP. Whereas the crude GEP had a size of ≈700 kDa, the partially purified GEP behaved on Ultrogel AcA 54 as a protein of 60 kDa. With purification, the GEP activity became insensitive to brefeldin A, consistent with the conclusion that, in contrast to earlier inferences, the exchange protein is not itself the target of brefeldin A.**

ADP-ribosylation factors (ARFs) are ≈20-kDa guanine nucleotide-binding proteins initially discovered as GTP-dependent activators of cholera toxin-catalyzed ADP-ribosylation of the α subunit of the adenylate cyclase-stimulatory G protein ($G_{s\alpha}$), other proteins, and simple guanidino compounds such as agmatine (1). More recently, ARFs have been recognized as participants in intracellular vesicular transport. In the Golgi apparatus, ARFs, in concert with a larger soluble protein complex termed coatomer, are required to initiate vesicle budding (2, 3). ARFs have also been implicated in vesicular trafficking between endoplasmic reticulum and the Golgi compartment, in endocytosis, and in nuclear membrane assembly (4–7). They are dependent on GTP for activity. Only ARF·GTP binds to cellular membranes or to phospholipids (8–10). Conversion of ARF·GDP to ARF·GTP can be accelerated by a guanine nucleotide-exchange protein (GEP). Several groups have reported evidence of GEP activity in Golgi membranes from CHO cells and rat liver. In these studies, brefeldin A (BFA) inhibited ARF GEP and consequently blocked ARF binding to Golgi membranes (11–13). A better characterized GEP is SEC12, which in yeast catalyzes GTP–GDP exchange on SAR1, thereby enhancing its binding to endoplasmic reticulum membrane (14).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

To form transport vesicles, rat brain Golgi membranes required cytosolic components, later identified as ARF and coatomer (2, 15). Reconstituting Golgi membranes with ARFs 1, 3, and 5, and SAPs (soluble accessory proteins lacking ARFs), it was found that SAPs increased association of ARF 1 or 3, but not ARF 5, with Golgi membranes (16). We report here the partial purification from SAP of a GEP that enhanced ARF activity and apparently accelerated binding of guanosine 5′-[γ-[^{35}S]thio]triphosphate (GTP[γ-^{35}S]) to purified ARF 1 and ARF 3.† During purification, GEP susceptibility to inhibition by BFA was lost.

MATERIALS AND METHODS

Reagents. BFA was purchased from Epicentre Technologies (Madison, WI); Sepharose CL-6B (Pharmacia), phosphatidylserine, and cardiolipin were from Sigma. Sources of other materials are noted in earlier publications (8, 16, 18).

Preparation of 40% Ammonium Sulfate Fraction (E_o^{40}) from Bovine Brain Cytosol. Bovine brain cortex (160 g) was homogenized (Polytron) in 4 volumes (vol/wt) of buffer A (0.25 M sucrose/1 mM dithiothreitol/1 mM NaN_3/1 mM EDTA/20 mM Tris Cl, pH 8.0) with protease inhibitors, leupeptin, aprotinin, and soybean and lima bean trypsin inhibitors, each 1 μg/ml (PIs), and 0.5 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 12,000 × *g* for 60 min. The supernatant was then centrifuged at 175,000 × *g* for 75 min (Beckman SW 41 rotor, 37,000 rpm). Solid ammonium sulfate was added to the supernatant to 40% saturation (pH maintained at ≈7.5). After 45 min at 4°C, precipitated protein was collected by centrifugation at 12,000 × *g* for 60 min, dissolved in buffer A, and dialyzed overnight against the same buffer with a final concentration of 0.5 M sucrose. The dialyzed solution was centrifuged at 23,000 × *g* for 60 min and the supernatant was layered on top of 1.4 M sucrose in buffer B (10 mM Hepes buffer, pH 7.5, with 1 mM $MgCl_2$, 1 mM dithiothreitol) containing PIs. After centrifugation at 175,000 × *g* for 90 min (SW 41 rotor, 37,000 rpm), a floating lipid layer was removed and the clear fraction above the interface was carefully collected for use (E_o^{40}), excluding a white suspension that collected at the interface.

Purification of GEP. To the E_o^{40} fraction (600–700 mg of protein), $MgCl_2$ was added (2 mM). The fraction was then applied to a column of DEAE-Sephacel (4 × 24 cm, 300 ml) equilibrated with and then washed with 300 ml of buffer A

Abbreviations: ARF, ADP-ribosylation factor; GEP, guanine nucleotide-exchange protein; SAP, soluble accessory protein(s); CTA, A subunit of cholera toxin; BFA, brefeldin A; GTP[γ-S], guanosine 5′-[γ-thio]triphosphate; PIs, protease inhibitors (leupeptin, aprotinin, and soybean and lima bean trypsin inhibitors, each 1 μg/ml).

*To whom reprint requests should be addressed at: National Institutes of Health, Building 10, Room 5N-307, Bethesda, MD 20892.

†Soluble ARFs from bovine brain which were previously referred to as sARF I and sARF II (17).

with 2 mM $MgCl_2$ at pH 8.0. Material bound to the column was eluted with 300 ml of 15 mM NaCl in buffer A with 2 mM $MgCl_2$ followed by a gradient of NaCl (15–250 mM, 600 ml each) in the same buffer. Fractions containing GEP activity (eluted between 95 and 130 mM NaCl) were pooled, concentrated to ≈30 ml (Amicon Centriprep 10 or Amicon YM10), and applied to a column (15-ml volume) of hydroxylapatite equilibrated with buffer A containing 2 mM $MgCl_2$. After the column was washed with 1 volume of the same buffer, and bound material was eluted with 1 volume of 15 mM potassium phosphate, pH 8.0 (in the same buffer), followed by a gradient of potassium phosphate (15–200 mM, 45 ml each). Fractions containing GEP activity (eluted between 90 and 115 mM phosphate) were pooled, concentrated by Centriprep 10, and applied to a column of Ultrogel AcA 54 (1.2 × 117 cm) which was equilibrated and eluted with buffer A containing 5 mM $MgCl_2$, 100 mM NaCl, PIs, and 0.5 mM phenylmethylsulfonyl fluoride. Fractions (1 ml) were collected and assayed for GEP activity, which emerged between bovine serum albumin and ovalbumin, corresponding to a protein of ≈60 kDa.

The marked instability of GEP, especially after the hydroxylapatite step, continues to be an obstacle to further purification. Attempts to stabilize or restore activity have been thus far unsuccessful. Even at −20°C, activity decreased during storage, although protein degradation could not be detected.

Assays of GEP Activity. GEP assays were based on (*i*) enhancing ARF activation of cholera toxin A-subunit (CTA)-catalyzed ADP-ribosylagmatine synthesis or (*ii*) increasing GTP[γ-^{35}S] binding to ARF.

ARF activation of CTA-catalyzed ADP-ribosylagmatine formation was assayed in two steps. In the first, 30–60 μg of bovine serum albumin, 10 mM dithiothreitol, 20 μg of phosphatidylserine, GEP fraction (up to 30 μl) with or without ARF (1 μg of bovine ARF fraction or 0.1 μg of purified ARF 1 or ARF 3), and 10–20 μM GTP[γ-S] (total volume, 50 μl) were incubated at 37°C for 40 min, then placed in an ice bath. For the second step, assay of CTA-catalyzed ADP-ribosylation of agmatine (9, 17, 18), the following additions were made: 50 μl containing 30 μg of ovalbumin, 120 μM Cibachrome blue, and 20 μg of phosphatidylserine; 50 μl containing 2 μg of CTA in buffer A; 100 μl of buffer A; and 50 μl of solution containing 30 mM $MgCl_2$, 3 mM ATP, 120 mM dithiothreitol, 60 mM agmatine, 1.2 mM [^{14}C]NAD (10^5 cpm), and 300 mM potassium phosphate buffer, pH 7.5. Incubation was at 30°C for 1 hr. CTA activity with or without GEP fraction was subtracted to assess ARF activity.

For assay of GTP[γ-^{35}S] binding to ARF, mixtures (100 μl) containing 30–60 μg of bovine serum albumin, PIs, 20 μg of phosphatidylserine, 10 mM dithiothreitol, ≈4 μg of bovine ARF fraction or 0.5 μg of ARF 1 or ARF 3, GEP fraction (up to 60 μl), and 5–10 μM GTP[γ-^{35}S] (2–4 × 10^6 cpm) were incubated at 37°C for 40 min. Protein with bound GTP[γ-^{35}S] was collected on nitrocellulose filters for radioassay. Data are reported as pmol of GTP[γ-S] bound to ARF (i.e., binding by GEP fraction alone, which was not time dependent, has been subtracted).

Preparation of Phosphatidylserine or Other Lipid. Chloroform was evaporated under N_2 from a solution of phosphatidylserine. Buffer B was added to phosphatidylserine and the mixture was incubated at 37°C with intermittent vortexing and sonification (final phosphatidylserine concentration, 4 mg/ml).

Purification of Bovine ARF 1 and ARF 3. ARF 1 and ARF 3 were purified as described (17). For some experiments, a mixed ARF fraction separated from other bovine brain cytosolic proteins on Ultrogel AcA 54 was used (8, 16).

Table 1. Effect of phosphatidylserine (PS) on GEP activation of ARF

Addition(s)	ADP-ribosylagmatine formed, nmol/hr		
	No PL	PS	CL
Without PS			
ARF	2.0	5.5	20
SAP	4.0	2.5	0
ARF/SAP	4.5	12.5	20.5
With PS			
ARF	2	5.5	24
SAP	3.5	1.5	0
ARF/SAP	16.5	32	47

Bovine ARFs (15 μg), SAP (60 μg), 50 μM GTP[γ-S], 50 μg of ovalbumin, with or without 20 μg of PS and other additions (total volume, 50 μl) were incubated at 37°C for 40 min before dilution to a final 250-μl volume with buffer B containing PIs. Duplicate 10-μl samples from each (containing 10 μM GTP[γS]) were assayed without phospholipid (PL), with 20 μg of PS, or with 144 μg of cardiolipin (CL) for ARF activity with 1 μg of CTA, 200 μM [^{14}C]NAD, and other additions in a total volume of 150 μl. CTA activity in the absence of additions has been subtracted. The experiment was replicated three times.

RESULTS AND DISCUSSION

We have reported that ARFs 1, 3, and 5 differ in the characteristics of their association with Golgi membranes (8,

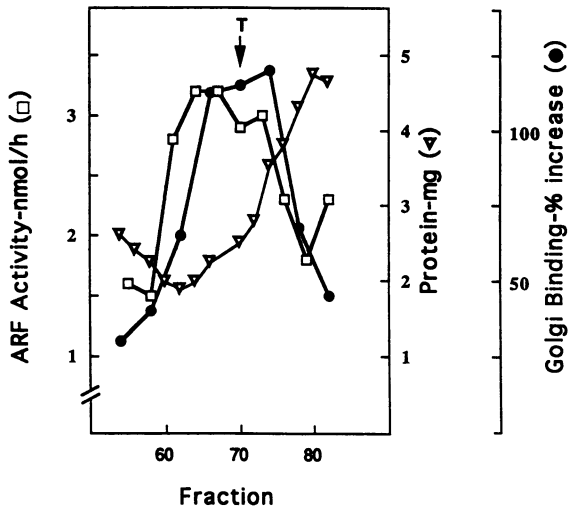

FIG. 1. Identification of GEP activity in fractions from Sepharose CL-6B chromatography of E_o^{40} fraction. E_o^{40} (100–160 mg of protein) was applied to a column of Sepharose CL-6B (2 × 91 cm, 286 ml) equilibrated and eluted with buffer A containing 100 mM NaCl and 5 mM $MgCl_2$. Fractions (2.5 ml) were collected for assay of GEP activity. Enhancement of ARF activity was assayed with 0.9 μg of bovine ARFs, 20 μl of column fraction, 20 μM GTP[γ-S], and additional components (see *Materials and Methods*) in a total volume of 50 μl incubated at 37°C for 40 min and then assayed for ARF activity with 2 μg of CTA, 200 μM [^{14}C]NAD, and other additions in 300 μl. Basal CTA activity has been subtracted. The experiment was replicated numerous times. To assess the effect of GEP on ARF binding to Golgi membranes, mixtures containing Golgi membranes (12.5 μg of protein), bovine ARFs (11 μg), sample of column fraction (8 μg of protein), and 10 μM GTP[γ-S] in 100 μl were incubated at 37°C for 40 min before membranes were pelleted by centrifugation and assayed for ARF activity (16, 18). The observations were replicated with five different preparations. □, ARF activity; ●, ARF binding to Golgi is reported as percentage increase over that without column fraction; ▽, protein (mg per fraction). Elution position of thyroglobulin (664 kDa) is indicated (T). It should be noted that ARF activity is representative of total activity, whereas Golgi binding parallels specific activity.

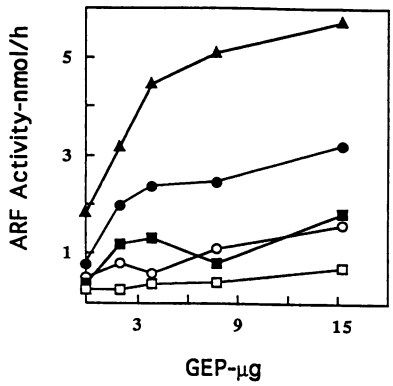

FIG. 2. Effect of GEP on ARF activity. Bovine ARFs (0.64, 1.3, or 2.6 μg), GEP fraction (partially purified somewhat differently than described in *Materials and Methods*, using Ultrogel AcA 34, DEAE-Sephacel, and Sepharose CL-6B), 10 μM GTP[γ-S], and 20 μg of phosphatidylserine in 60 μl were incubated at 37°C (■, ●, ▲) or 4°C (□, ○) for 40 min before assay of samples for ARF activity with 2 μg of CTA, 200 μM [^{14}C]NAD, and other additions in 300 μl. Activity of CTA alone has been subtracted. ARF, 0.64 μg (□, ■), 1.3 μg (○, ●), or 2.6 μg (▲). The experiment was replicated twice.

16). Binding of ARFs 1 and 3 was enhanced by the presence of a SAP, but that of ARF 5 was unaffected. The presence of SAP also resulted in much higher total recovery of ARF activity in those experiments. To begin to identify the protein activator in the SAP fraction we included phosphatidylserine during the incubation of ARF, with or without SAP, and then assayed ARF activation of CTA without phosphatidylserine or with phosphatidylserine or cardiolipin (Table 1). The presence of phosphatidylserine during incubation of ARF alone at 37°C with 50 μM GTP[γ-S] had no effect on activity, although ARF activity was clearly enhanced by addition of phosphatidylserine and, to a greater extent, cardiolipin. The presence of SAP during incubation of ARF and GTP[γ-S] increased by ≈100% ARF activity as assayed with phosphatidylserine. When phosphatidylserine was present during incubation of SAP, ARF, and GTP[γ-S], increased ARF activity was observed under all assay conditions (Table 1). The action of SAP (presumably of a GEP-like protein) in stimulation of ARF was clearly facilitated by phosphatidylserine.

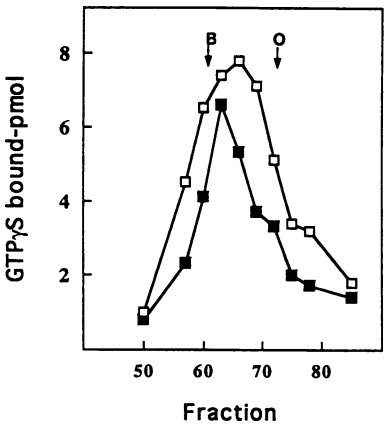

FIG. 3. Effect of GEP in fractions from Ultrogel AcA 54 on GTP[γ-^{35}S] binding to ARFs 1 and 3. ARF 1 (□) or ARF 3 (■) (each 0.3 μg), 30 μl of column fraction (1–2 μg of protein), 10 μM GTP[γ-^{35}S] (2000 cpm/pmol), and other components were incubated in 60 μl at 37°C for 40 min. GTP[γ-^{35}S] bound by fraction alone (pmol) has been subtracted. The experiment was replicated numerous times. ARF 1 and ARF 3 bound 1.0 and 0.8 pmol, respectively, of GTP[γ-^{35}S]. Elution of bovine serum albumin (B) and ovalbumin (O) is indicated.

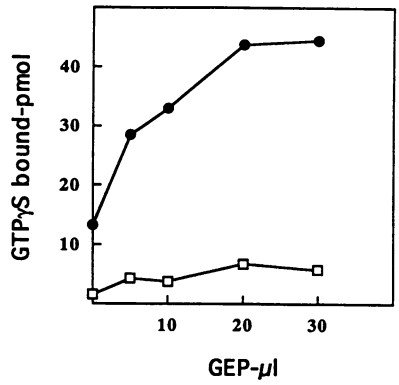

FIG. 4. Effect of GEP on binding of GTP[γ-^{35}S] to ARF 1. ARF 1, 0.3 (□) or 1.5 (●) μg, GEP from Ultrogel AcA 54 (≈1 μg of protein per 30 μl), 10 μM GTP[γ-^{35}S] (1225 cpm/pmol), and other components were incubated in 60 μl at 37°C for 40 min. GTP[γ-^{35}S] bound (0.2–0.5 pmol) to different amounts of GEP protein has been subtracted.The experiment was replicated many times.

GEP activity in the ammonium sulfate fraction E_o^{40}, which represents essentially 100% of that in SAP, was eluted from Sepharose CL-6B as a macromolecule of >700 kDa (Fig. 1), which activated partially purified mixed bovine ARFs (Fig. 1), as well as purified ARF 1 and ARF 3 (data not shown). GEP activity assessed by increased association of ARF with Golgi membranes was roughly coincident in column fractions with enhancement of ARF activity (Fig. 1). In addition, GEP increased total recovery of ARF activity—i.e., activity of Golgi pellet plus supernatant (data not shown). Since GEP activity at this stage behaved as a molecule of the size of a coatomer (≈700 kDa), GEP and coatomer in the same fractions may have participated consecutively in the ARF–Golgi interaction.

Increasing GEP concentration markedly increased ARF activity (Fig. 2). Each amount of ARF reached an essentially maximal activity with a sufficient amount of GEP, and with more ARF more GEP was required to reach that level during the usual 40-min incubation at 37°C. When incubation was carried out at 4°C, ARF activity was much lower but still increased with increasing GEP concentration. Activation did not, however, reach the maximum, even in the presence of an amount of GEP that had a maximal effect at 37°C. (ARF assay was for 1 hr at 30°C during which there was probably some GEP activity.)

After sequential purification steps, GEP behaved on Ultrogel AcA 54 as a protein of ≈60 kDa (Fig. 3) that promoted binding of GTP[γ-^{35}S] to ARF 1 and ARF 3. Accumulated evidence was consistent with the conclusion that the same GEP catalyzed guanine nucleotide exchange on both ARF 1 and ARF 3, although these activities may, of course, separate with further purification.

GTP[γ-^{35}S] binding to ARF 1 (or ARF 3, in unpublished experiments) increased with increasing amounts of partially purified GEP from Ultrogel AcA 54 (Fig. 4). This result and that in Fig. 2, in which GEP was much more impure and was assayed by its effect on ARF activity, led to the same conclusion—i.e., that activation of ARF in 40 min was dependent on the concentration of GEP.

In the presence of GEP, half-maximal binding of GTP[γ-^{35}S] to ARF 1 or ARF 3 occurred at a concentration (EC_{50}) of 1.4 or 1.2 μM, respectively (Fig. 5). In the absence of GEP, the binding was much lower, but EC_{50} values for ARF 1 and ARF 3 were 0.9 and 1.0 μM, respectively. It appeared that GEP promoted nucleotide exchange but did not alter ARF affinity for the nucleotide.

In earlier studies, BFA inhibited the increment in binding of ARF 1 and ARF 3 to Golgi membranes that was produced

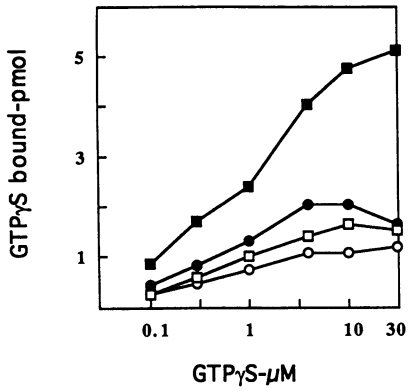

FIG. 5. Effect of GTP[γ-S] concentration on binding to ARF 1 and ARF 3. ARF 1 (□, ■) or ARF 3 (○ ●), (each 0.5 μg), with (●, ■) or without (○, □) GEP from Ultrogel AcA 54 column (1–2 μg of protein), 20 μg of phosphatidylserine, 0.1–30 μM GTP[γ-^{35}S], and other components were incubated in 60 μl at 37°C for 40 min. Nonspecific binding with or without GEP has been subtracted from the data. The experiment was replicated once.

by SAP, but did not inhibit binding in the absence of SAP, or the binding of ARF 5, which was unaffected by SAP (16). Similarly, GEP activity for ARF 1 and ARF 3 in E_o^{40} was inhibited by BFA. Effects of BFA on GEP activity of fractions at successive stages of purification (assessed by GTP[γ-^{35}S] binding) are summarized in Table 2. BFA inhibited GEP activity in E_o^{40} and in fractions from Sepharose CL-6B, but inhibition was not seen after chromatography on DEAE (Table 2).

At the beginning of purification, GEP behaved on Sepharose CL-6B as a macromolecule of >700 kDa, just as it did in E_o^{40} (Fig. 1), which enhanced not only ARF activation of CTA but also the association of ARF with Golgi (data not shown). The partially purified GEP, however, appeared to be a protein of ≈60 kDa (Fig. 3). It seems likely that the loss of BFA inhibition with DEAE chromatography is related to separation of an ≈60 kDa GEP molecule from another protein(s) that is sensitive to BFA, with which it was initially associated in the larger complex. We have not yet succeeded in isolating a BFA-sensitive protein from DEAE column fractions to demonstrate that it can restore BFA inhibition to the partially purified GEP.

Table 2. Effect of BFA on GTP[γ-S] binding to ARF with different preparations of GEP

GEP fraction		GTP[γ-S] bound to ARF, pmol			
Step	μg	No BFA	2 μg of BFA	4 μg of BFA	8 μg of BFA
I. E_o^{40}*	0	1.1	1.2	1.5	1.2
	30	7.4	5.1	5.1	4.4
II. CL-6B†	0	0.61	0.57	—	0.61
	16.8	3.3	2.5	—	1.8
III. DEAE*	0	1.2	1.2	1.2	1.2
	18	6.4	6.6	6.6	7.1
IV. HAP‡	0	3.8	3.7	3.5	3.5
	3.7	11.1	11.2	11.2	10.4
	7.8	11.9	11.8	15.1	12.4
V. AcA 54§	0	0.74	—	0.9¶	—
	1	5.3	—	5.4¶	—
AcA 54*	0	0.49	—	0.55¶	—
	1	1.7	—	2.7¶	—

The indicated GEP preparation with 20 μg of phosphatidylserine and ARF and BFA as indicated (total volume, 90 μl) was incubated for 10 min at 21–22°C. After addition of 10 μl of 50 μM GTP[γ-^{35}S], incubation was continued for 40 min at 37°C. Step I, 40% ammonium sulfate fraction; step II; Sepharose CL-6B; step III, DEAE-Sephacel; step IV, hydroxylapatite (HAP); step V, Ultrogel AcA 54.
*ARF 3, 0.5 μg.
†Bovine ARFs, 4.6 μg.
‡ARF 3, 1.0 μg.
§ARF 1, 0.5 μg.
¶BFA, 5 μg.

It seems quite possible that the earlier reports (11, 12) of membrane-bound GEP which was inhibited by BFA, reflect the presence of a similar component in the Golgi preparations, most likely as part of a protein complex that includes ARF regulatory proteins (e.g., ARF–GEP) and perhaps other proteins with which ARF interacts along with coatomer to initiate vesicle budding (e.g., tubulin). It is not clear just what aspect of the preparation enabled us to obtain GEP activity in the soluble fraction. It is clear, however, that this has facilitated attempts to purify it and, in the process, find that BFA inhibition of ARF–GEP activity depends on the presence of another protein(s) in addition to GEP. The apparent existence of a protein that confers BFA sensitivity to guanine nucleotide exchange on ARF could be related to the ability of BFA to inhibit function of several types of intracellular vesicular transport systems (19).

We thank Carol Kosh for expert secretarial assistance.

1. Serventi, I. M., Moss, J. & Vaughan, M. (1992) *Curr. Top. Microbiol. Immunol.* **175**, 43–67.
2. Donaldson, J. G., Kahn, R. A., Lippincott-Schwartz, J. & Klausner, R. D. (1991) *Science* **254**, 1197–1199.
3. Donaldson, J. G., Cassel, D., Kahn, R. A. & Klausner, R. D. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6408–6412.
4. Balch, W. E., Kahn, R. A. & Schwaninger, R. (1992) *J. Biol. Chem.* **267**, 13053–13061.
5. Boman, A. L., Taylor, T. C., Melançon, P. & Wilson, K. L. (1992) *Nature (London)* **358**, 512–514.
6. Lenhard, J. M., Kahn, R. A. & Stahl, P. D. (1992) *J. Biol. Chem.* **267**, 13047–13052.
7. Zeuzem, S., Feick, P., Zimmermann, P., Haase, W., Kahn, R. A. & Schulz, I. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6619–6623.
8. Tsai, S.-C., Adamik, R., Haun, R. S., Moss, J. & Vaughan, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9272–9276.
9. Walker, M. W., Bobak, D. A., Tsai, S.-C., Moss, J. & Vaughan, M. (1992) *J. Biol. Chem.* **267**, 3230–3235.
10. Stearns, T., Willingham, M. C., Botstein, D. & Kahn, R. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1238–1242.
11. Donaldson, J. G., Finazzi, D. & Klausner, R. D. (1992) *Nature (London)* **360**, 350–352.
12. Helms, J. B. & Rothman, J. E. (1992) *Nature (London)* **360**, 352–354.
13. Randazzo, P. A., Yang, Y. C., Rulka, C. & Kahn, R. A. (1993) *J. Biol. Chem.* **268**, 9555–9563.
14. Barlowe, C. & Schekman, R. (1993) *Nature (London)* **365**, 347–349.
15. Rothman, J. E. & Orci, L. (1990) *FASEB J.* **4**, 1460–1468.
16. Tsai, S.-C., Adamik, R., Haun, R. S., Moss, J. & Vaughan, M. (1993) *J. Biol. Chem.* **268**, 10820–10825.
17. Tsai, S.-C., Noda, M., Adamik, R., Chang, P. P., Chen, H.-C., Moss, J. & Vaughan, M. (1988) *J. Biol. Chem.* **263**, 1768–1772.
18. Haun, R., Tsai, S.-C., Adamik, R., Moss, J. & Vaughan, M. (1993) *J. Biol. Chem.* **268**, 7064–7068.
19. Pelham, H. R. B. (1991) *Cell* **67**, 449–451.