Hydrolysis of Bound GTP by ARF Protein Triggers Uncoating of Golgi-derived COP-coated Vesicles

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Abstract. The cycle of nucleotide exchange and hydrolysis by a small GTP-binding protein, ADP-ribosylation factor (ARF), helps to provide vectoriality to vesicle transport. Coat assembly is triggered when ARF binds GTP, initiating transport vesicle budding, and coat disassembly is triggered when ARF hydrolyzes GTP, allowing the uncoated vesicle to fuse.

THE secretory pathway consists of sequential vesicle budding and fusion events that result in the vectorial movement of cargo proteins through a series of membrane bound compartments until their final destination is reached (Palade, 1975). It is now known that transport requires components from the cytosol (Rothman and Orci, 1992; Pryer et al., 1992), raising fundamental questions concerning vectoriality such as how the assembly and disassembly of vesicle coats is spatially controlled.

The budding of Golgi-derived, COP-coated vesicles has been reconstituted in vitro, and in addition to Golgi membranes, requires only two coat proteins, coatomer and ADP-ribosylation factor (ARF)[1] (Waters et al., 1991; Serafini et al., 1991*a*), from cytosol (Orci et al., 1993*a*). Coatomer binding to Golgi membranes requires prior binding of ARF (Donaldson et al., 1992*b*; Palmer et al., 1993). The Golgi cisternal membranes remain flat when only ARF has bound, but when coatomer binds, coated regions of membrane are deformed into the shape of a vesicle (Orci et al., 1993*a*). ARF can only bind Golgi membranes in its N-myristoylated (mARF) and GTP-bound form (Donaldson et al., 1992*b*; Helms and Rothman, 1992; Haun et al., 1993; Helms et al., 1993), and this activated ARF is generated when cytosolic mARF[GDP] encounters a Golgi-bound nucleotide exchange factor in the presence of free GTP. Brefeldin A (BFA) blocks nucleotide exchange (Donaldson et al., 1992*a*; Helms and Rothman, 1992), thereby preventing ARF binding and subsequent ARF-dependent assembly of coated vesicles (Orci et al., 1991). This explains why BFA blocks secretion (Klausner et al., 1992) and underscores the physiological significance of the coated vesicle pathway, whose in vivo importance is also made clear by the fact that both ARF and Sec21p, the γ-COP homolog, are essential in yeast (Stearns et al., 1990; Stenbeck et al., 1992; Hosobuchi et al., 1992) and that β-COP antibodies block transport from the ER (Pepperkok et al., 1993; Peter et al., 1993). ARF binds to the lipid bilayer and additionally to distinct receptor sites in the Golgi membrane. The latter are likely to be relevant to coated vesicle assembly because ARF-dependent coatomer binding is a saturable process (Donaldson et al., 1992*b*; Palmer et al., 1993).

In this report, we test the hypothesis that uncoating occurs when ARF in coated vesicles hydrolyze their bound GTP. We employ a mutant crippled in GTP hydrolysis according to the following rationale. GTPγS inhibits intercisternal transport reconstituted in a cell-free system of Golgi membranes incubated with cytosol and ATP, and concurrently COP-coated vesicles accumulate (Melançon et al., 1987). A cytosolic component needed for this inhibition has been purified and found to be ARF (Taylor et al., 1992), and ARF is now known to be a subunit of the coat (Serafini et al., 1991*a*) required for vesicle assembly (Donaldson et al., 1992*a*; Palmer et al., 1993; Orci et al., 1993*a*). The simplest explanation of these facts would be that GTP hydrolysis by ARF, which cannot occur when ARF is bound to GTPγS, triggers coat disassembly. This mutant is equivalent to activated Ras mutants which cause oncogenic transformation; activated ARF stimulates the formation of COP-coated vesicles in the context of Golgi transport.

Materials and Methods

Preparation of ARF

A DNA sequence corresponding to human ARF1 was synthesized by PCR and ligated to the expression vector pET11d between the NcoI and BamHI sites (Novagen, Madison, WI; Dubendorff and Studier, 1991). The Q71L mutation was introduced into the wild-type sequence by site-directed muta-

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1. *Abbreviations used in this paper*: ARF, ADP-ribosylation factor; BFA, brefeldin A; COP, coatomer.

genesis (Higuchi et al., 1988). DNA sequences were confirmed with Sequenase 2.0 (Un. States Biochemical, Cleveland, OH). Myristoylated proteins were produced in *E. coli* strain BL21(DE3) (Novagen) by coexpressing yeast N-myristoyltransferase (pBB131 in Duronio et al., 1990) and pET11d-ARF and purified by DEAE-Sephacel (Pharmacia LKB Biotechnology, Piscataway, NJ) and Ultrogel AcA54 (Sepracor, Marlborough, MA) chromatographies (Weiss et al., 1989; Helms et al., 1993). Protein concentrations were determined using the bicinchoninic acid assay and a standard curve of varying amounts of BSA (Pierce, Rockford, IL).

Coatomer Binding Assay

CHO Golgi (86 μg/ml protein) was incubated with 28 mM Hepes-KOH (pH 7.2), 20 mM NaCl, 35 mM KCl, 2.5 mM $Mg(OAc)_2$, 20 μM guanine nucleotide, 0.2 M sucrose, 107 μg/ml bovine liver coatomer, and 100 μg/ml recombinant mARF at 37°C for 10 min in a volume of 50 μl (Palmer et al., 1993). Golgi membranes were pelleted through a 15% (wt/wt) sucrose cushion, protein was fractionated on a 12% SDS-PAGE gel under reducing conditions (Laemmli, 1970), and coatomer was detected by Western blotting (50 ng/ml of affinity purified antiserum to ε-COP, the 36-kD coatomer subunit) with ECL reagent (Amersham Corp., Arlington Heights, IL).

Nucleotide Exchange Assay

Recombinant mARF (78 μg/ml) was incubated with 25 mM Hepes-KOH (pH 7.2), 20 mM KCl, 2.5 mM $Mg(OAc)_2$, 0.5 mM sodium pyrophosphate, 1.6 mg/ml ovalbumin, CHO Golgi (60 μg/ml protein), 5 μM [γ-^{32}P] GTP (18,400 cpm/pmol, Amersham), and 0.2 M sucrose at 37°C for 15 min in a volume of 0.1 ml (Helms et al., 1993).

In Vitro Transport Assay

Cis- to *medial*-Golgi transport of vesicular stomatitis virus G protein (Balch et al., 1984; Balch and Rothman, 1985) was assayed in reactions containing 25 mM Hepes-KOH (pH 7.2), 10 mM NaCl, 21 mM KCl, 2.5 mM $Mg(OAc)_2$, 3.1 mg/ml bovine brain cytosolic proteins, 50 μM ATP, 250 μM UTP, 5 mM creatine phosphate, 8 IU/ml creatine phosphate kinase, 5 μl acceptor CHO Golgi, 5 μl donor CHO Golgi, 0.2 M sucrose, and 0.4 μM [^{3}H] UDP-GlcNAc (0.5 μCi, Du Pont, New England Nuclear, Boston, MA) in a total volume of 50 μl. After incubation at 37°C for 1 h, VSV-G protein was immunoprecipitated and counted. Brefeldin A was from GIBCO BRL (Gaithersburg, MD) and GTPγS was from Sigma Chem. Co. (St. Louis, MO).

Assembly of Coated Vesicles on the Golgi

CHO Golgi (86 μg/ml protein) was incubated with 25 mM Hepes-KOH (pH 7.2), 10 mM NaCl, 18 mM KCl, 2.5 mM $Mg(OAc)_2$, 1 mg/ml soy bean trypsin inhibitor, 37.5 μg/ml bovine liver coatomer, 52 μg/ml recombinant mARF, either 20 μM GTPγS or 1 mM GTP, and 0.2 M sucrose at 37°C for 10 min in a volume of 0.5 ml (Orci et al., 1993*a*).

Electron Microscopy and Quantitation

Membranes were collected by centrifugation for 15 min in a horizontal microfuge and supernatants were aspirated. Pellets were fixed overnight in the coldroom with 1% glutaraldehyde (EM grade, Polysciences, Warrington, PA) and 50 mM phosphate buffer (pH 7.0), contrasted with tannic acid, embedded in Epon, and quantitated (Orci et al., 1986, 1991). All morphological measurements were done in a double-blind fashion using a code that was not broken until quantitation was completed.

Results

An ARF Mutant That Does Not Hydrolyze GTP

We have focused on the ARF1 protein, which mediates coatomer binding and coated vesicle assembly in vitro (Donaldson et al., 1992*a*; Palmer et al., 1993; Orci et al., 1993*a*) and is sensitive to BFA inhibition (Donaldson et al., 1992*a*; Helms and Rothman, 1992). As a member of the small GTP-binding protein family (Bourne et al., 1991), ARF1 contains consensus GTP-binding sites in its protein sequence. Intrinsic hydrolysis of GTP by ARF1 is not detectable (Kahn and Gilman, 1986) but we assume that bound GTP is hydrolyzed in the appropriate biological context. In analogy to the Ras-Q61L mutation which inhibits GTP hydrolysis but not GTP binding (Der et al., 1986), we mutated the corresponding codon 71 Gln to Leu. Soluble mARF1 (Fig. 1, lane *1*) and mARF1-Q71L (lane *2*) proteins were purified from *E. coli* coexpressing N-myristoyltransferase. Each ARF protein was isolated with stoichiometric amounts of bound GDP as determined by anion exchange chromatography (data not shown).

Purified mARF1-Q71L is native because it satisfies the ARF requirement for coatomer binding to Golgi membranes (Fig. 1, lanes *3–5*) and this process requires GTP. The binding of coatomer to Golgi membranes mediated by wild-type mARF1 is enhanced by nonhydrolyzable GTPγS as compared to GTP (cf. lanes *7* and *8*). However, the same amount of coatomer is bound via mutant mARF1-Q71L regardless of whether GTP or nonhydrolyzable GTPγS is used (cf. lanes *4* and *5*). This strongly suggests that mARF1-Q71L binds to membranes but does not hydrolyze GTP, in contrast to wild-type mARF1.

To directly examine membrane-dependent GTP hydrolysis by wild-type and mutant ARF proteins, [γ-^{32}P] GTP was incubated in a nucleotide exchange reaction with mARF and Golgi, membranes and bound proteins were pelleted through a sucrose cushion, and bound nucleotide retained on nitrocellulose filters was counted (Fig. 2). In accordance

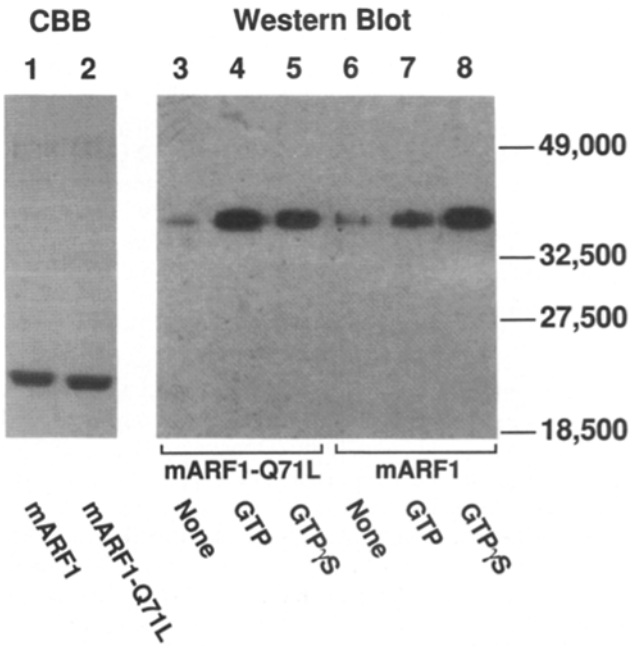

Figure 1. mARF1-Q71L promotes coatomer binding to Golgi membranes. 1 μg of mARF1 (lane *1*) or mARF1-Q71L (lane *2*) was run on a 15% PAGE-SDS gel under reducing conditions and stained with Coomassie blue (Laemmli, 1970). Molecular weight standards are shown. Coatomer binding assays (Palmer et al., 1993) included either recombinant mARF1-Q71L (lanes *3–5*) or mARF1 (lanes *6–8*). No additional guanine nucleotide (lanes *3* and *6*), 20 μM GTP (lanes *4* and *7*), or 20 μM GTPγS (lanes *5* and *8*) was included in the binding reaction. ε-COP bound to Golgi membranes was detected by Western blotting.

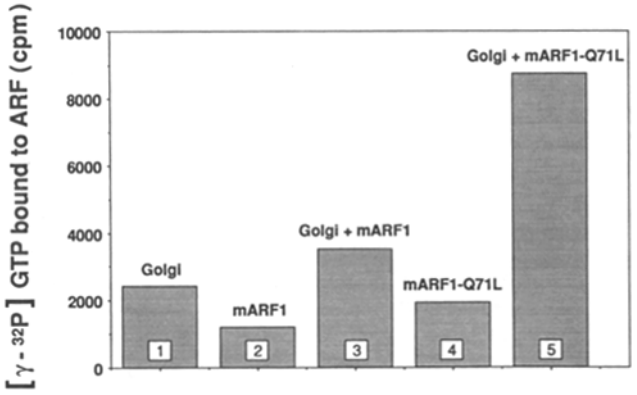

Figure 2. Q71L mutation inhibits GTP hydrolysis by mARF1. Golgi-catalyzed nucleotide exchange of ARF using [γ-^{32}P] GTP was performed as described in Materials and Methods (Helms et al., 1993). Golgi membranes and bound ARF protein were pelleted through a cushion of 25% (wt/vol) sucrose in 25 mM Hepes-KOH (pH 7.2), 20 mM KCl, and 2.5 mM $Mg(OAc)_2$; solubilized with 0.5% Triton X-100 in PBS; and radioactivity bound to protein was determined by the nitrocellulose filter trapping assay (Northup et al., 1982).

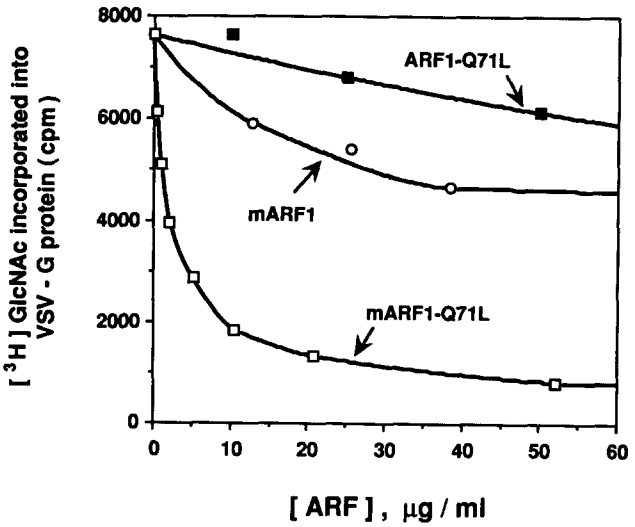

Figure 3. mARF1-Q71L inhibits Golgi protein transport. Recombinant ARF (ARF1-Q71L, *filled squares*; mARF1, *open circles*; and mARF1-Q71L, *open squares*) was titrated into transport assays (Balch et al., 1984; Balch and Rothman, 1985). All reactions included the same concentrations of salts.

with the previous observation that mARF1 quickly hydrolyzes GTP after membrane-catalyzed nucleotide exchange (Helms et al., 1993), incubating wild-type mARF1 and Golgi together (column 3) results in no additional counts over Golgi or mARF1 alone (columns 1 and 2) because when the GTP is hydrolyzed, the γ-^{32}P label is released. A control with [α-^{32}P] GTP demonstrated that the derived GDP remains bound to mARF1 (data not shown). In contrast, mARF1-Q71L retains the γ phosphate of GTP (cf. column 5 with the sum of columns 1 and 4). Altogether, these experiments show that mARF1-Q71L binds GTP but hydrolyzes it more slowly than does wild-type protein.

The ARF GTPase Mutant Assembles Coated Vesicles and Inhibits Cell-Free Transport

A clear prediction from the above is that mutant mARF1-Q71L should retain the capacity to assemble coated vesicles. Wild-type or mutant mARF1 proteins were incubated with CHO Golgi membranes, coatomer, and either GTP or GTPγS; the production of COP-coated vesicles was assayed by quantitative electron microscopy (Table I). Coated buds and coated vesicles were formed with mARF1-Q71L and either GTP or GTPγS.

mARF1-Q71L is a potent inhibitor in the *cis* to *medial* Golgi transport assay (Fig. 3). Inhibition by the GTP hydrolysis mutant means that it is dominant over endogenous mARF in bovine brain cytosol, as would be predicted if the mutant substitutes functionally for wild-type protein and allows the transport pathway to proceed up to the point at which GTP hydrolysis is required. The concentration of mutant mARF1 that inhibits the transport assay by 50% (IC_{50} of 2 μg/ml) is lower than that of ARF purified by Taylor et al. (1992) (IC_{50} of 12–32 μg/ml) and may reflect the difference in transport conditions used in the two assays. Wild-type mARF1 does not inhibit in the absence of GTPγS over the range of concentrations used and N-myristoylation of the GTPase mutant is required for inhibitory activity. Transport intermediates resistant to mARF1-Q71L are generated at the same rate as GTPγS-resistant intermediates (Fig. 4), as would be expected if inhibition by GTPγS and mARF1-Q71L affect the same step in the transport reaction.

To confirm that inhibition by mARF1-Q71L requires exchange of GTP for bound GDP, we used BFA. Addition of mARF1-Q71L after BFA does not completely inhibit VSV-G protein glycosylation (Fig. 5, column 3) and implies that the mutant must be activated by nucleotide exchange to inhibit transport. Adding BFA after mARF1-Q71L does not reverse

Table I. Wild-Type mARF1 and Mutant mARF1-Q71L Initiate Coat Assembly

	A Uncoated vesicles (*no. per µm²*)	B Coated buds and coated vesicles (*no. per µm²*)	C Percentage of buds or vesicles with a complete coat
(1) mARF1+GTPγS	0.2 ± 0.2	55.4 ± 2.5	100%
(2) mARF1-Q71L+GTP	ND	41.3 ± 3.3	100
(3) mARF1-Q71L+GTPγS	ND	71.2 ± 3.8	100

Coated buds and coated vesicles were assembled on Golgi membranes in cytosol-free reactions containing guanine nucleotide, recombinant mARF, and coatomer (see Materials and Methods). Values are means ± standard error based on analyzing ten Golgi areas for each pellet. *ND*, none detected.

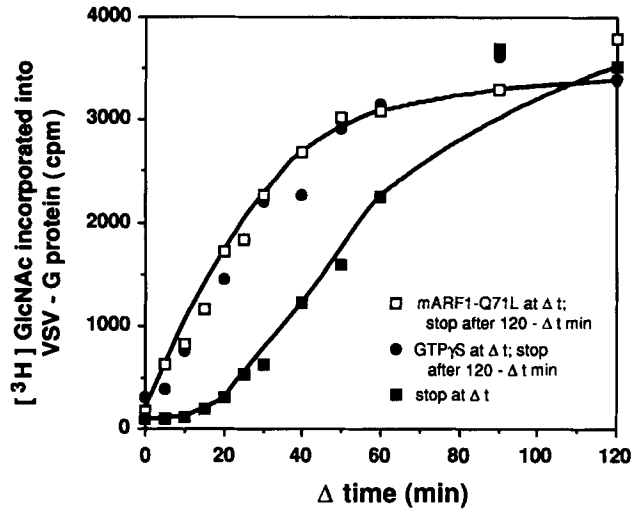

Figure 4. mARF1-Q71L and GTPγS inhibit at the same stage of Golgi protein transport. A standard time course (*filled squares*) was generated by stopping transport assays at different times Δt (Melançon et al., 1987). GTPγS-resistant transport intermediates (*filled circles*) were assayed by adding GTPγS to 20 μM at the indicated times and incubated at 37°C for a total of 2 h. Intermediates resistant to 52 μg/ml mARF1-Q71L (*open squares*) were assayed in the same manner.

inhibition (column 4), as is also the case when adding BFA after GTPγS (Orci et al., 1991).

Inability to Hydrolyze GTP Prevents Uncoating

Because the GTPase mutant inhibits transport (Fig. 3) but does not inhibit the production of coated vesicles (Table I), the simplest possibility would be that mARF1-Q71L blocks transport by preventing the consumption of coated transport vesicle intermediates. If so, most transport intermediates should accumulate at the specific stage of the reaction at which hydrolysis of GTP by ARF is first required, and also accumulate at earlier stages.

Addition of recombinant wild-type mARF1 (Fig. 6 *a* and Table II, row 3) or coatomer (row 4) to the reaction each increases the total number of vesicles in a cell-free transport reaction (cf. row 1), both with and without the characteristic electron-dense coat. However, the GTPase mutant of ARF, mARF1-Q71L (Fig. 6 *b* and Table II, row 5) markedly increases the number of coated vesicles about 3.5-fold relative to the control with wild-type mARF1 and correspondingly reduces the level of their uncoated products, similar to the effect of GTPγS (row 2). An increase of ~1.3-fold in the number of coated buds is also observed.

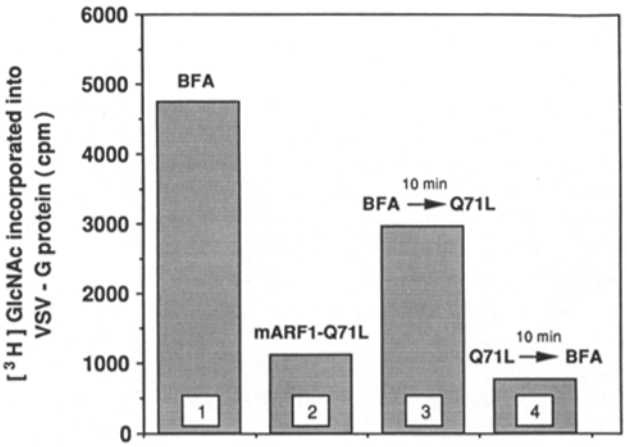

Figure 5. mARF1-Q71L inhibition is blocked by brefeldin A. Transport was assayed in 50 μl reactions with 200 μM BFA (column *1*), 5.2 μg mARF1-Q71L (column 2), 200 μM BFA followed by 5.2 μg mARF1-Q71L 10 min later (column *3*), or 5.2 μg mARF1-Q71L followed by 200 μM BFA 10 min later (column *4*). All reactions were incubated at 37°C for a total of 70 min and included 1 μl methanol, the solvent of BFA.

Discussion

The discovery that the small GTP-binding protein ARF is a

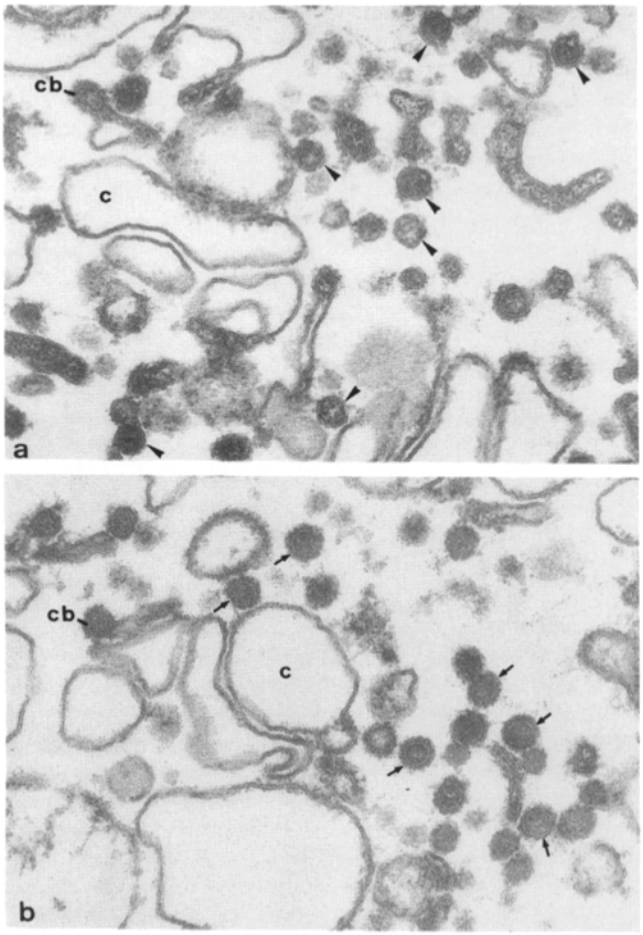

Figure 6. Electron microscopy of Golgi-derived vesicles. (*a*) Standard transport plus wild-type mARF1 or (*b*) standard transport plus mutant mARF1-Q71L. The striking difference is that most vesicles are uncoated in *a* (*arrowheads*), while they remain coated in *b* (*arrows*). Some remnants of coat structure appear on the periphery of uncoated vesicles in *a*. Coated buds (*cb*) are present in both conditions. *c*, cisternal elements. See Table II for quantitation of the respective numbers of vesicles and buds. CHO Golgi membranes (86 μg/ml protein in 0.2 M sucrose) were incubated in transport reactions including 19 μM palmitoyl CoA and 52 μg/ml recombinant mARF (see Materials and Methods) in a total volume of 0.5 ml at 37°C for 10 min. ×59,000, magnification.

Table II. Requirements for Production and Uncoating of COP-coated Vesicles

	A Uncoated vesicles (*no. per μm²*)	B Coated buds (*no. per μm²*)	C Coated vesicles (*no. per μm²*)	D Percentage of buds or vesicles with a complete coat
(1) Standard transport	2.4 ± 0.9	5.2 ± 1.6	2.0 ± 0.8	75%
(2) Standard transport + GTPγS	ND	14.6 ± 2.6	9.5 ± 1.5	100
(3) Standard transport + mARF1	11.0 ± 1.6	9.0 ± 1.6	2.2 ± 0.8	50
(4) Standard transport + coatomer	5.5 ± 2.1	9.1 ± 0.7	2.5 ± 0.9	68
(5) Standard transport + mARF1-Q71L	0.8 ± 0.4	11.8 ± 1.9	7.8 ± 1.0	96

CHO Golgi membranes were incubated in transport reactions containing 19 μM GTPγS, 52 μg/ml wild-type mARF1 or mutant mARF1-Q71L, or 24 μg/ml bovine liver coatomer as described in Fig. 6. Values are means ± standard error based on analyzing ten Golgi areas for each pellet. ND, none detected.

subunit of the coat (Serafini et al., 1991*a*), together with the observation that GTPγS blocks transport and accumulates coated vesicles (Melançon et al., 1987), led to the specific hypothesis that GTP hydrolysis by ARF triggers vesicle uncoating. Evidence that ARF plays a critical role in the assembly of the coat has since accumulated (Donaldson et al., 1992*a*,*b*; Kahn et al., 1992; Taylor et al., 1992; Helms and Rothman, 1992; Haun et al., 1993; Helms et al., 1993; Palmer et al., 1993; Orci et al., 1993*a*), and we now provide a direct test of this hypothesis by employing a mutant of ARF1. This GTPase mutant dominantly interferes with cell-free transport and concomitantly accumulates coated transport vesicles at the expense of their uncoated products. This affords strong confirmation of the role coated vesicles play as transport intermediates in the cell-free system, and in particular provides genetic proof that the hydrolysis of GTP by ARF is needed for removal of the coat and to allow fusion of the enclosed vesicle (as evidenced by the lack of glycosylation of VSV-G protein when the GTPase mutant is used). This suggests that in addition to forming the vesicle (Orci et al., 1993*a*,*b*), the coat plays an important role in preventing inappropriate fusion events.

Indeed, in work to be described elsewhere (Elazar, Z., L. Orci, M. Amherdt, G. Tanigawa, and J. E. Rothman, manuscript in preparation), we will report that removal of either ARF or coatomer from cell-free transport reactions halts vesicular transport but simultaneously unleashes a direct fusion between Golgi stacks of the kind associated with BFA (Orci et al., 1991; Klausner et al., 1992). This essentially nonphysiological fusion reaction, induced by removing components that are normally present, also explains why substantially removing ARF does not halt glycosylation of VSV-G protein in cell-free assays (Taylor et al., 1992). A signal can be produced in these assays when transport is due either to COP-coated vesicles generated in crude cytosol or to direct fusion, uncoupled from budding, with cytosol depleted of coat proteins.

Based on the physical properties of ARF and the requirement for ARF in binding coatomer to membranes, a simple mechanism for uncoating can be proposed (Fig. 7), essentially coating in reverse. The conformational switch that all GTP-binding proteins undergo when alternating between GDP- and GTP-bound forms (Bourne et al., 1991; Wittinghofer, 1992) is used by mARF to control exposure of its N-myristoyl group: mARF[GTP], but not mARF[GDP], binds to artificial phospholipid bilayers (Kahn, 1991; Walker et al., 1992) and the lipid bilayer of Golgi membranes (Haun et al., 1993; Helms et al., 1993) and binding requires the myristoyl modification. Switching into the bilayer-binding GTP form is required for mARF to bind to specific sites in Golgi membranes ("ARF receptors") and to form complexes with coatomer before coated vesicle budding (Donaldson et al., 1992*a*,*b*; Helms and Rothman, 1992; Haun et al., 1993; Helms et al., 1993; Palmer et al., 1993; Orci et al., 1993*a*).

Given these properties, it is predictable that when ARF hydrolyzes its GTP, the protein will switch back to the GDP conformation, "retract" its fatty acid, and dissociate from the membrane of the coated vesicle. Because coatomer binding to membranes is ARF-dependent (Donaldson et al., 1992*b*; Palmer et al., 1993), coatomer release into the cytosol would be expected to follow dissociation of ARF. Consistent with this, neither ARF nor coatomer is present in uncoated vesicles that accumulate when the fusion protein NSF is inactivated, as judged by electron microscopic immunocytochemistry (Serafini et al., 1991*a*,*b*). While our experiments show that GTP hydrolysis by ARF is necessary for uncoating, they do not formally prove that the relevant ARF molecules are those actually in the coated vesicles. The fact that the N-myristoyl group is needed for the GTPase mutant of ARF to block transport implies that the inhibitory ARF protein must be membrane bound, consistent with the idea that mARF1-Q71L inhibits by substituting for endogenous, wild-type mARF1 in forming coated vesicles.

Our present observations, together with earlier ones, provide new insights into the way in which this small GTP-binding protein is used to provide vectoriality in vesicle transport (Fig. 7). Coat assembly is initiated at the donor membrane where ARF can productively interact with its receptor; coat disassembly (required to initiate fusion) results when ARF is triggered to hydrolyze its bound GTP. Is uncoating (e.g., hydrolysis of ARF-bound GTP) triggered before or after contact with the target membrane? When transport is blocked by GTPγS, coated vesicles accumulate bound to Golgi membranes and to each other but not to other membranes (plasma membranes, endosomes, etc.) present in Golgi membrane fractions (Melançon et al., 1987; Orci

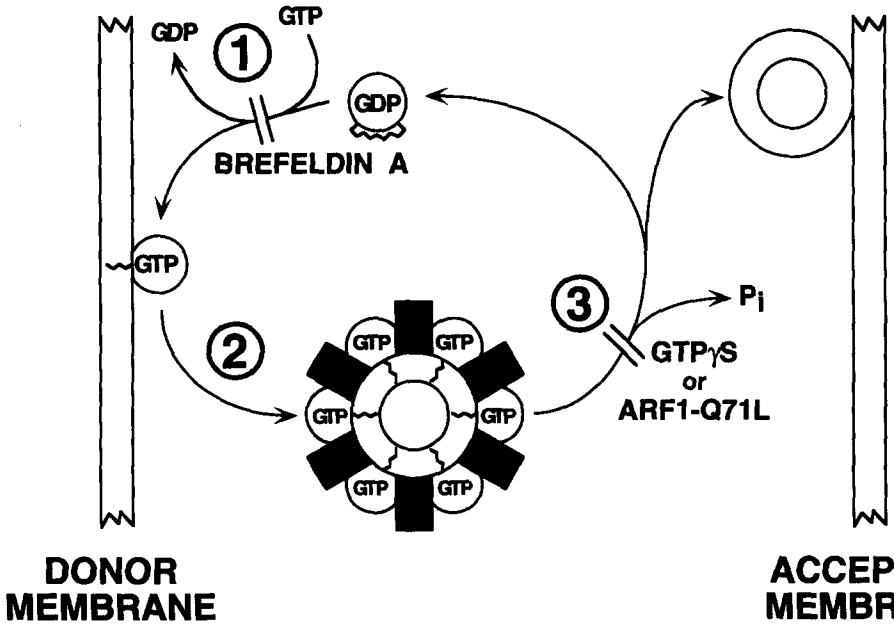

Figure 7. Diagram of vectoriality by GTP exchange and hydrolysis of ARF. Nucleotide exchange of mARF (*circle*) at the donor membrane triggers binding to the Golgi; brefeldin A blocks this process (Donaldson et al., 1992*a*; Helms and Rothman, 1992) (step *1*). Binding of mARF to the donor membrane enables coatomer (*rectangles*) to bind to the Golgi and bud off coated vesicles (Donaldson et al., 1992*b*; Palmer et al., 1993) (step *2*). GTP hydrolysis by mARF in the coat at the acceptor membrane (or possibly before vesicle attachment) triggers uncoating to enable subsequent fusion of the vesicle and Golgi (step *3*).

et al., 1989). This reveals that at least to some extent coated vesicles can select their target while the coat is still intact. Many of these accumulated coated vesicles must have departed from donor Golgi and then become associated with acceptor Golgi because the vesicles contain VSV-G protein but are juxtaposed to Golgi stacks virtually free of VSV-G protein (derived from acceptor). Thus, the simplest interpretation is that uncoating occurs after the coated vesicle initially binds to the target membrane, and when GTP cannot be hydrolyzed, the reaction proceeds no further. However, we can not rule out that docking occurs before GTP is hydrolyzed. Also, the fact that coated vesicles can dock with Golgi selectively does not rule out that additional, higher affinity docking interactions (Söllner et al., 1993) may occur after uncoating takes place. That the same species of ARF can bind coatomer (COP-coated vesicles, Orci et al., 1993*a*) and Golgi-specific adaptor AP-1 (clathrin-coated vesicles, Stamnes and Rothman, 1993) to Golgi membranes illustrates that ARF must decode rather than store vectorial information. This model differs from that proposed by Bourne (1988) which envisioned that small GTP-binding proteins attach vesicles to targets and provide specificity to this process. It now appears that the best candidate for encoding targeting specificity is the SNARE family (Söllner et al., 1993; Brennwald and Novick, 1993; Dunn et al., 1993).

ARF interacts with Golgi membranes in two distinct ways (Helms et al., 1993) and both events require that ARF be myristoylated and be activated by nucleotide exchange to the GTP form. The loosely bound population of mARF interacts directly with the lipid bilayer via the fatty acid, as it is extracted by phospholipid bilayer vesicles, and as it does not exhibit saturable binding with respect to ARF concentration. The tightly bound population of mARF is resistant to liposomes and is saturable with respect to ARF concentration, thereby operationally defining "ARF receptor" sites. Thus the product of nucleotide exchange, activated ARF, can be transferred between membranes in vitro. If this occurs in vivo, then nucleotide exchange enzymes need not be located specifically to the donor compartments. It is thus an open question as to whether compartmentally specific ARF receptors, exchange enzymes, or possibly both define the donor site.

A considerable body of genetic and biochemical evidence has established that small GTP-binding proteins of the Rab family are needed for unknown reasons in many transport or fusion processes in living cells and in cell-free systems (Pfeffer, 1992; Sztul et al., 1992). In fact, antibodies to Rab1 (Plutner et al., 1991) inhibit Golgi transport and this suggests that at least one other small GTP-binding protein is required. Perhaps these proteins resist GTPγS because they act in their GTP-bound forms, or because an adequate supply of the GTP-bound form is present in the crude extracts employed in these assays.

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