The small GTP-binding protein ADP-ribosylation factor-1 (ARF1) regulates intracellular transport by modulating the interaction of coat proteins with the Golgi complex. Coat protein association with Golgi membranes requires activated, GTP-bound ARF1, whereas GTP hydrolysis catalyzed by an ARF1-directed GTPase-activating protein (GAP) deactivates ARF1 and results in coat protein dissociation. We have recently cloned a Golgi-associated ARF GAP. Overexpression of GAP was found to result in a phenotype that reflects ARF1 deactivation (Aoe, T., Cukierman, E., Lee, A., Cassel, D., Peters, P. J., and Hsu, V. W. (1997) EMBO J. 16, 7305–7316). In this study, we used this phenotype to define domains in GAP that are required for its function in vivo. As expected, mutations in the amino-terminal part of GAP that were previously found to abolish ARF GAP catalytic activity in vitro abrogated ARF1 deactivation in vivo. Significantly, truncations at the carboxyl-terminal part of GAP that did not affect GAP catalytic activity in vitro also diminished ARF1 deactivation. Thus, a noncatalytic domain is required for GAP activity in vivo. This domain may be involved in the targeting of GAP to the Golgi membrane.

The initial stage of transport vesicle formation, termed budding, is driven by the association of cytoplasmic coat proteins with the cytoplasmic face of the donor organelle membrane. The interaction of coat proteins with membranes is regulated by members of the ARF1 subfamily of small GTP-binding proteins in multiple cellular compartments (1–3). In the early secretory pathway, which includes the ER and the Golgi complex, the ARF1 protein regulates the interaction of a coat protein complex termed COPI with organelle membrane (4, 5). The conversion of ARF1 to the active (GTP-bound) form by a guanine nucleotide exchange protein initiates binding of coat proteins to membranes (6–11). Subsequently a coated vesicle is generated that must be uncoated before fusion with the acceptor compartment. Uncoating requires GTP hydrolysis on ARF1 (12–14), and this process depends on the interaction of ARF1 with a GTPase-activating protein. Thus, the GTPase cycle of ARF1 drives vesicular transport through the regulation of vesicle budding and vesicle fusion.

The uncoating of transport vesicles must be controlled to prevent the premature dissociation of coat protein before the completion of vesicle budding. To gain an understanding of the molecular basis of the uncoating process, we have purified and cloned an ARF-directed GAP from rat liver (15–16). The GAP catalytic domain is required for stimulating the GTPase cycle of ARF1 (18–20), our findings indicate that GAP fulfills its expected role as a negative regulator of ARF1 at the Golgi.

In this study we carried out a structure-function analysis of the GAP molecule to identify domains that are required for its function in vivo. Because measurement of the GTPase cycle of ARF1 in vivo is complicated by the unstable nature its GTP-bound form (21, 22), we used the previously defined phenotype of ARF1 deactivation for our structure-function analysis. The findings reveal that the in vivo activity of GAP requires only the catalytic domain that is responsible for stimulating GTP hydrolysis on ARF1 but also a noncatalytic domain. As the substrate of GAP (GTP-bound ARF1) is membrane-associated, one function of the noncatalytic domain may be the targeting of cytosolic GAP to specific sites at the Golgi membrane.

**EXPERIMENTAL PROCEDURES**

Materials—[α-32P]GTP (800 Ci/mmole) was from New England Nuclear. Recombinant, myristoylated ARF1 was prepared from bacteria coexpressing ARF1 and N-myristoyltransferase as described in (21), except that induction of protein expression was carried out at 27°C to increase the efficiency of protein myristoylation (22). Vent DNA polymerase was from New England Biolabs (Beverly, MA).

Cells and Antibodies—COS7 and HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and 40 μg/ml gentamycin under a 5% CO2 atmosphere.

The following antibodies were used: rabbit anti-GAP antibody was prepared as described (16); mouse monoclonal antibody 7G7 against Tac antigen (kindly provided by H.P. Hauri); mouse monoclonal antibody 9E10 against the myc epitope; mouse monoclonal antibody 6× His (CLONTECH, Palo Alto, CA); fluorescein-conjugated donkey anti-rabbit IgG and rhodamine or Cy3-conjugated donkey anti-mouse antibodies (Jackson Laboratories, West Grove, PA).

*This work was supported by grants from the Israel Science Foundation, the Council for Tobacco Research-U. S. A., and the Fund for Promotion of Research at the Technion (to D. C.) and by a grant from the National Institutes of Health (to V. W. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Contributed equally.

To whom correspondence should be addressed. Tel.: 972-4-829 3408; Fax: 972-4-822 5153; E-mail: danc@techunix.technion.ac.il.

The abbreviations used are: ARF, ADP-ribosylation factor; ER, endoplasmic reticulum; GAP, GTPase-activating protein.
peroxidase-conjugated goat anti-rabbit IgG (Sigma); peroxidase-conjugated rabbit anti-mouse IgG (Bio Yeda, Rehovot, Israel).

**Plasmids and Transient Transfection**—cDNAs encoding GAP and its mutants were amplified by polymerase chain reaction using primers with extensions containing HindIII and XbaI restriction sites for sense and antisense primers, respectively. The products were cloned into the respective sites of the mammalian expression vector pECE. Translation of all constructs is initiated from the ATG of the GAP cDNA and is terminated by either primer or vector-introduced stop codons. To introduce an amino-terminal polyhistidine track, cDNAs were cloned between the BamHI and KpnI sites of the pQE30 vector (Qiagen products, Valencia, CA) and then transferred to pECE. These vectors introduce an amino-terminal extension of Arg-Gly-Ser-His6, GAP with a carboxyterminal extension of myc/His6 tags was expressed in the pcDNA 3.1+/-Mye-His vector (Invitrogen Corp., San Diego, CA) after cloning between the EcoRI and BamHI sites of the vector.

Transfection of COS7 cells was done by electroporation using a modification of the method of Chu et al. (23). Cells were trypsinized and resuspended in growth medium to give 2-5 × 10^6 cells/mL. To 400 μL of cell suspension in a 4-mm electroporation cuvette was added 10 μg of DNA. Electroporation was carried out by a pulse at 960 microfarads, 260 V, and infinite internal resistance. The cells were immediately plated in a 10-cm-diameter culture dish. The medium was changed after 16 h, and the cells were used for experiments 40 h after transfection. HeLa cells were transfected by the standard calcium phosphate method.

**Immunofluorescence Microscopy**—Cells were grown to 60–70% confluency on glass coverslips. Cells were fixed in 2% formaldehyde in phosphate-buffered saline for 20 min at room temperature, then treated with cold methanol for 1 min. Cells were incubated with anti-GAP and anti-giantin antibodies (1:2,000 and 1:1,000 dilutions, respectively) in PSS (phosphate-buffered saline containing 10% fetal calf serum, 0.2% saponin, and 15 mM sodium azide) for 1 h, then washed and incubated with fluorescein isothiocyanate and either rhodamine or Cy3-conjugated secondary antibodies (respectively) in PSS for 1 h. Coverslips were mounted in Fluoromount G. Microscopy was performed with 60× oil lenses in a Bio-Rad MCR-1024 confocal microscope.

**Immunoprecipitation**—Proteins were separated by SDS-polyacrylamide gel electrophoresis (10% gels) and transferred to nitrocellulose paper. The blots were incubated with polyclonal anti-GAP antibodies (1:10,000 dilution) followed by incubation with peroxidase-conjugated secondary antibody and were developed by ECL.

**Immunoprecipitation**—Transfected cells were lysed in buffer containing 1% Triton X-100, 50 mM Tris, pH 7.4, 300 mM NaCl, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 0.5 mM phenylmethylsulfonyl fluoride. Lysates were precleared with protein A-Sepharose beads and incubated with antibody-coupled protein A-Sepharose for 1 h at 4°C. The beads were washed and treated with sample buffer, and proteins were separated by SDS-polyacrylamide gel electrophoresis and subjected to immunoblotting.

**Golgi-specific Glycosylation of Tac-E19**—This assay was done as described previously (17). Briefly, HeLa cells were cotransfected with the Tac-E19 and GAP constructs, labeled 24 h later with 100 μCi/mI [35S]methionine for a 2 h pulse, then chased for 16 h. Cells were lysed, immunoprecipitated with anti-Tac antibody coupled to protein A, treated (or mock-treated) with endoglycosidase H, and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography.

**Purification of Overexpressed Proteins from COS Cells**—Proteins were purified from cells that were transfected with GAP cDNAs encoding an amino-terminal hexahistidine extension. Cells grown in a 10-cm diameter plate in 10 ml of lysis buffer containing 50 mM sodium phosphate, pH 8, 200 mM NaCl, 1% hydrogenated Triton X-100, 0.1 mM dithiothreitol, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 0.25 mM phenylmethylsulfonyl fluoride. The lysate was cleared by centrifugation in the microfuge for 10 min. The NaCl concentration in the lysates was increased to 0.5 M, and imidazole was added to give 10 mM. The combined lysate from two 10-cm plates was loaded on a 1 ml of lysis buffer containing 50 mM sodium phosphate, pH 8.0, 5 mM NaCl, 0.5% hydrogenated Triton X-100, and 10 mM imidazole, then washed twice with 50 mM sodium phosphate, pH 6.0, 0.5 mM NaCl, 0.05% hydrogenated Triton X-100, 10% glycerol, and 10 mM imidazole. Protein was eluted by increasing the imidazole concentration to 250 mM. The eluate was supplemented with 5 mM dithiothreitol, and the pH was increased to 8.0 by the addition of 1 M Tris, pH 8.5.

**RESULTS**

The Amino-terminal Catalytic Domain of ARF GAP Is Required to Deactivate ARF1 in Vivo—Previous structure-function analysis of GAP suggested that its catalytic domain is localized at the amino-terminal portion that includes an essential Cys4 zinc finger structure encompassing amino acids 22–45 (16). To investigate the role of the catalytic domain of GAP in vivo, we tested the effect of mutations in this domain on the ability of overexpressed GAP to induce a phenotype of ARF1 deactivation (17). Immunofluorescence microscopy was used to monitor the integrity of the Golgi complex using the Golgi membrane protein giantin as a marker (24). As shown in Fig. 1, cells overexpressing wild-type GAP did not display the characteristic perinuclear staining of the Golgi complex. By contrast, cells overexpressing GAP mutants in which there was either a deletion of the entire zinc finger (GAP64–415) or the zinc finger was inactivated by a mutation in one of its scaffold cysteines (C22A), the Golgi complex appeared intact in most cells. These findings indicate that these mutants are inactive in vivo, as might be anticipated from their inability to stimulate GTP hydrolysis on ARF1 in vitro (16). This conclusion was substantiated by quantitative analysis of the expression and activity of GAP and its mutants. As shown in Fig. 2A, approximately 90% of cells overexpressing wild-type GAP did not show a Golgi structure by immunofluorescence. The GAP64–415 mutant was completely inactive, whereas the C22A mutant caused an apparent disassembly of the Golgi in a minor fraction of the cell population. Western blot analysis (Fig. 2B) showed that GAP and its mutants displayed the expected size and were expressed at comparable levels. Thus the difference in the abilities of wild-type and mutant GAP to induce Golgi
disassembly is unlikely to result from differences in levels of protein expression.

A Carboxyl-terminal Domain of GAP Is Also Required for Its Activity in Vivo—To screen for a potential role of other parts of GAP in its activity in vivo, we generated a series of truncated proteins and tested their ability to induce Golgi disassembly (Fig. 3). In a previous study we found that a polypeptide that includes the first 146 amino acids (of 415 in the complete protein) shows GAP catalytic activity in vitro, whereas a polypeptide that includes the first 257 amino acids is as active as tissue-purified protein (16). Significantly, however, this truncated GAP (GAP1–257) did not effectively induce a phenotype of ARF1 deactivation, as assessed by Golgi disassembly (Fig. 3A). In comparing mutant GAPs that are serially truncated from the carboxyl terminus, overexpression of a mutant with a relatively small truncation (containing the first 359 of 415 residues of GAP) induced Golgi disassembly nearly to the same extent as wild type GAP. Further truncations of GAP from the carboxyl terminus, generating mutant GAPs that contained 335 amino acids or less, resulted in a significantly reduced ability of GAP to induce Golgi disassembly (Fig. 3A). Western blot analysis (Fig. 3B) showed that the expression level of the truncated mutants was 1.5–2.5-fold lower than the expression level of wild-type protein. However, when corrected for transfection efficiency, which varied between 8 and 18%, the expression level per cell was similar for all mutants (data not shown), suggesting that the difference in the ability of different GAP mutants to induce Golgi disassembly was not due to differences in the level of expressed proteins. Thus, together with the findings on amino-terminal mutants of GAP (Figs. 1 and 2), the results on carboxy truncation mutants suggested that GAP function in vivo required not only its catalytic domain but also part of the noncatalytic domain.

Carboxyl-terminal Mutants Do Not Cause a Massive Redistribution of Golgi to ER—In view of the rather unexpected finding that carboxyl-terminal truncation mutants retaining the GAP catalytic domain are ineffective in vivo, we sought to confirm the findings by a different approach. As Golgi redistribution to the ER is the most specific manifestation of the phenotype of ARF1 deactivation, we had previously established a biochemical assay to measure this parameter in a quantitative fashion (17). This assay measures the glycosylation pattern of the ER glycoprotein Tac-E19, which becomes endoglycosidase H-resistant after mixing of Golgi with ER due to its processing by Golgi glycosidases. Using this approach, we found that both wild-type GAP and the mutant containing the first 359 amino acids induced endoglycosidase H resistance of Tac-E19 to a similar extent. However, the shorter carboxyl-terminal truncation mutants showed similarly reduced activity in this assay (Fig. 4, A and B). Western blotting revealed that all mutants were expressed at least as well as the wild-type form (Fig. 4C). Thus, these results were similar to those obtained by assessing Golgi disassembly through a morphologic approach that examined the fate of the Golgi marker giantin by immunofluorescence microscopy.

Localization of GAP Mutants—To gain further insight into the function of the noncatalytic domain of GAP, we investigated the intracellular localization of carboxyl-terminal GAP mutants. Immunofluorescence examination of such mutants (GAP1–257 and GAP1–277) showed a diffused distribution throughout the cell (Fig. 5). By contrast, the zinc finger mutant C22A that is a full-length protein but is catalytically inactive showed intense perinuclear staining that was colocalized with that of the Golgi marker giantin in most cells. However, for reasons that are unclear at present, the distribution of the C22A mutant in a minority of cells overlapped only partially with that of giantin (an example of each pattern is shown in Fig. 5). These findings suggest that the catalytically inactive mutant is capable of localizing correctly to the Golgi complex, similar to the endogenous GAP (16), whereas the carboxyl-terminal truncation mutants seem to have lost this capacity. This mislocalization could account, at least in part, for the low in vivo activity of the carboxyl-terminal mutants.

Decreased Effectiveness of GAP1–257 in Vivo Is Not Due to...
Reduced Catalytic Activity—Next, we sought to rule out possible competing explanations for our interpretation of the inactivity of carboxyl-terminal mutants in vivo. Even though in vitro assays showed that bacterially expressed GAP 1–257 has similar catalytic activity as that of tissue-purified protein, we first sought to rule out that the reduced activity of GAP 1–257 in vivo reflects a reduced catalytic activity of the protein when overexpressed in the mammalian cell. For this purpose, both wild-type GAP and GAP 1–257 were tagged with a hexahistidine in their amino termini. As assessed by immunofluorescence microscopy, the tag did not seem to affect GAP function in vivo, because tagged wild-type GAP was still able to effectively induce the phenotype of ARF1 deactivation, whereas tagged GAP 1–257 showed reduced activity similar to that of the non-tagged protein (data not shown). Overexpressed proteins were then purified from COS cells by nickel nitrilotriacetic acid chromatography. When assayed for GAP catalytic activity in vitro, both wild-type and truncated proteins showed similar dose dependence for their ability to stimulate GTP hydrolysis on ARF1 (Fig. 6). It is therefore unlikely that the differential activity of wild-type GAP and GAP 1–257 is due to a difference in their catalytic activity. Interestingly, the protein concentration that was required for half-maximal GAP activity of the proteins purified from COS cells (Fig. 6) was approximately 5-fold lower than that previously reported for recombinant or tissue-purified protein (15, 16). An explanation for this difference may be that the preparation of GAP proteins in our previous studies included a denaturation-renaturation step that was unnecessary in the purification of GAP proteins through their epitope tags from mammalian cells.

GAP Does Not Self-oligomerize—As another possibility, the reduced in vivo activity of GAP 1–257 could be due to GAP activity in vivo requiring self-oligomerization, which, in turn, requires the carboxyl-terminal domain. Oligomerization of GAP was suggested by our previous observation that GAP from...
Functional Domains of ARF GAP

**FIG. 7.** Neither wild-type GAP nor GAP$_{1-257}$ self-oligomerizes. Wild-type GAP (WT) and GAP$_{1-257}$ were expressed as nontagged (nt) or epitope-tagged (tg) forms. In panels A and B, the two forms of each protein were coexpressed (co), whereas in panel C, tagged wild-type GAP was coexpressed with nontagged GAP$_{1-257}$. The epitope tags were a carboxyl-terminal addition of a Myc-His$_6$ epitope to wild-type GAP and amino-terminal RGS-His$_6$ addition to GAP$_{1-257}$. Cotransfected cells were lysed with Triton X-100 and subjected to immunoprecipitation with anti-myc and anti-His$_6$ antibodies for tagged wild-type GAP and GAP$_{1-257}$, respectively. Samples from the cell lysates (nt, tg, and co) and from the immunoprecipitates (ip) were subjected to Western blot analysis using the polyclonal anti-GAP antibody. The two bands seen in cells that have been transfected with His$_6$-tagged GAP$_{1-257}$ represent products from two initiation sites; the larger product is initiated from vector-derived ATG and thus contains the epitope tag, and the smaller product is initiated from the ATG of the GAP cDNA and is thus identical to the nontagged protein.

In this study we analyzed two sets of mutations in ARF GAP for their effect on the ability of the protein to deactivate ARF1 in vivo. The first set of mutations affected the amino-terminal part of the GAP. This part of the protein constitutes the GAP catalytic domain that includes an essential Cys$_4$-type zinc finger structure (16). As expected, a truncated protein that lacked the first 63 amino acids including the zinc finger domain, as well as a protein with a C22A mutation that prevents the formation of the finger structure, were both ineffective in causing the phenotype of ARF1 deactivation (Figs. 1 and 2). These findings lend further support to our suggestion that the phenotype of ARF1 deactivation upon overexpression of GAP is a result of increased GAP catalytic activity that prevents the generation of active, GTP-bound ARF1 (17).

A second set of mutations resulted in proteins that were progressively truncated at the carboxyl-terminal end. These mutants, which contained intact GAP domains, were expected to possess full catalytic GAP activity (15, 16). However, whereas a protein with a relatively small truncation (possessing the first 359 of 415 residues) was nearly as effective as wild-type protein in causing the phenotype of ARF1 deactivation, mutants that had further truncations and possessed 335 amino acids or less were rather ineffective (Figs. 3 and 4). These findings were first revealed morphologically by the ability of various GAP mutants to disassemble the Golgi complex. Subsequently the results were substantiated by a biochemical assay that measures specifically Golgi redistribution to the ER (17). Thus, whereas both wild-type GAP and GAP$_{1-355}$ induced Golgi-specific glycosylation of the ER glycoprotein Tac–E19, the shorter mutants (GAP$_{1-257}$, GAP$_{1-277}$, and GAP$_{1-335}$) all had a similar and significantly reduced effect (see Fig. 4). This set of studies suggested that GAP activity in vivo requires not only the catalytic domain but also a second functional domain that extends toward the carboxyl-terminal part.

In further support for this interpretation, we ruled out two alternate possibilities that might account for the difference in activity in vivo between full-length and truncated GAPs. First, previous findings that bacterially expressed GAP$_{1-257}$ is as active as tissue-purified protein (15, 16) were confirmed in this study with full-length and truncated proteins expressed and purified from COS cells. Thus, differences in catalytic activity cannot account for the differential in vivo activity of GAP$_{1-257}$ and wild-type GAP. Additionally, we found that neither wild-type GAP nor GAP$_{1-257}$ self-oligomerizes when overexpressed, ruling out differences in oligomerization as a reason for the differential in vivo effects of wild-type and truncated GAP.

Thus, what could be the basis for the inactivity of GAP$_{1-257}$ in vivo? One clue is that GAP dynamically distributes between cytosol and the Golgi membrane (16). Our previous studies with artificial phospholipid micelles have suggested that the productive interaction between ARF and GAP requires that both proteins are bound to a micelle (25). Thus the activity of GAP requires its recruitment to a membrane site where it can...
encounter its membrane-bound substrate, GTP-bound ARF1. Since efficient binding to lipid micelles was observed with GAP_{1-257} (25), it is unlikely that the carboxyl-terminal part is involved in binding to lipids. Rather, this noncatalytic domain could be involved in targeting of GAP to a specific membrane compartment by mediating the interaction of GAP with a membrane “receptor” protein. The findings that GAP_{359} is highly active, whereas GAP_{335} is rather inactive, suggest that the putative targeting domain terminates before amino acid 359, although it is not known at present how far this domain extends toward the amino-terminal part of the protein. In support of a role of the noncatalytic domain in targeting, a catalytically inactive, full-length GAP mutant (C22A) showed a Golgi-like localization similar to endogenous GAP, whereas the shorter carboxyl-terminally truncated proteins (GAP_{257} and GAP_{227}) appear to have lost this capacity.

Based on the results of this and previous studies, we propose that GAP function in vivo requires a bimodal interaction with membranes mediated by direct binding to lipids through the amino-terminal part as well as interaction of a noncatalytic part with a membrane protein. Such a mechanism can explain the partial activity observed in mutants that lack the noncatalytic domain, as the mass-action effect of overexpression could result in sufficient binding of such mutants to the Golgi complex through the lipid binding domain.

Interestingly, ARF GAP belongs to an extensive family of zinc finger proteins that possess a domain with high similarity to GAP catalytic domain, whereas other parts of these proteins do not show significant similarity to GAP (26). An active ARF GAP, previously referred to as GAP2 (27), was recently identified as a member of this zinc finger family. Perhaps the distinct noncatalytic domains of these two GAPs bear information for targeting to different membrane compartments.

In this context, we have shown previously that the transmembrane KDEL receptor acts to recruit ARF GAP to membranes (17, 28). Moreover, in studies to be published elsewhere, a good correlation was observed between the in vivo activity of carboxyl-terminal truncated mutants and their ability to interact with the KDEL receptor. These findings suggest that the KDEL receptor interacts with a noncatalytic part of GAP (either directly or through additional mediator proteins) and that this interaction plays an important role in the targeting of GAP. Future studies of the interaction between GAP and membrane receptors will likely reveal important mechanisms that regulate the GTPase cycle of ARF1.

Acknowledgments—We thank Dr. H. P. Hauri for providing us with antibodies against giantin and Sagie Zuck for help with editing the manuscript.

REFERENCES
1. Donaldson, J. G., and Klausner, R. D. (1994) Curr. Opin. Cell Biol. 6, 527–532
2. Rothman, J. E., and Wieland, F. T. (1996) Science 272, 227–233
3. Schekman, R., and Orci, L. (1996) Science 271, 1526–1532
4. Donaldson, J. G., Cassel, D., Kahn, R., and Klausner, R. D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6408–6412
5. Palmer, D. J., Helms, J. B., Becker, C. J., Orci, L., and Rothman, J. E. (1993) J. Biol. Chem. 268, 12083–12089
6. Donaldson, J. G., Finazzi, D., and Klausner, R. D. (1992) Nature 360, 350–352
7. Helms, J. B., and Rothman, J. E. (1992) Nature 360, 352–354
8. Peyroche, A., Paris, S., and Jackson, C. L. (1996) Nature 384, 479–481
9. Chardin, P., Paris, S., Antonny, B., Robinewu, S., Beraud-Dufour, S., Jackson, C., and Chabre, M. (1996) Nature 384, 481–484
10. Morinaga, N., Tsai, S. C., Moss, J., and Vaughan, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 12656–12660
11. Meacci, E., Tsai, S. C., Adamik, R., Moss, J., and Vaughan, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1745–1748
12. Donaldson, J. G., Kahn, R. A., Lippincott-Schwartz, J., and Klausner, R. D. (1991) Science 254, 1197–1199
13. Tanigawa, G., Orci, L., Amherdt, M., Ravazola, M., Helms, J. B., and Rothman, J. E. (1993) J. Cell Biol. 123, 1365–1371
14. Teal, S. B., Hsu, V. W., Peters, P. J., Klausner, R. D., and Donaldson, J. D. (1994) J. Biol. Chem. 269, 3135–3138
15. Makler, V., Cukierman, E., Rotman, M., Admon, A., and Cassel, D. (1995) J. Biol. Chem. 270, 5232–5237
16. Cukierman, E., Huber, I., Rotman, M., and Cassel, D. (1995) Science 270, 1999–2002
17. Aoe, T., Cukierman, E., Lee, A., Cassel, D., Peters, P. J., and Hsu, V. W. (1997) EMBO J. 16, 7305–7316
18. Klausner, R. D., Donaldson, J. D., and Lippincott-Schwartz, J. (1992) J. Cell Biol. 116, 1071–1080
19. Peters, P. J., Hsu, V. W., Ooi, C. E., Finazzi, D., Teal, S. B., Oorschot, V., Donaldson, J. D., and Klausner, R. D. (1995) J. Cell Biol. 129, 1003–1017
20. Dascher, C., and Balch, W. E. (1994) J. Biol. Chem. 269, 1437–1448
21. Weiss, O., Holden, J., Rulka, C., and Kahn, R. A. (1989) J. Biol. Chem. 264, 21066–21072
22. Franco, M., Chardin, P., Chabre, M., and Paris, S. (1995) J. Biol. Chem. 270, 1337–1341
23. Chu, G., Hayakawa, H., and Berg, P. (1987) Nucleic Acids Res. 15, 1311–1326
24. Linstedt, A. D., and Hauri, H. P. (1993) Mol. Biol. Cell 4, 679–693
25. Antonny, B., Huber, I., Paris, S., Chabre, M., and Cassel, D. (1997) J. Biol. Chem. 272, 30848–30851
26. Hammond-Odie, L. P., Jackson, T. R., Profit, A. A., Blader, I. J., Turck, C. W., Prestwich, G. D., and Theibert, A. B. (1996) J. Biol. Chem. 271, 18859–18866
27. Randazzo, P. (1997) Biochem. J. 324, 413–419
28. Aoe, T., Lee, A. J., van Donselaar, E., Peters, P. J., and Hsu, V. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1624–1629

2 M. T. Brown, J. Andrade, H. Radhakrishna, J. G. Donaldson, J. A. Cooper, and P. A. Randazzo, submitted for publication. 3 T. Aoe, I. Huber, C. Vasudevan, G. Romero, D. Cassel, and V. W. Hsu, submitted for publication.