ADP-ribosylation factors (ARFs) are ~20-kDa guanine nucleotide-binding proteins initially identified by their ability to enhance \( \text{in vitro} \) cholera toxin-catalyzed ADP-ribosylation and subsequently shown to participate in vesicular transport in the Golgi and other cellular compartments. By cDNA and genomic cloning, at least six mammalian ARFs were identified. Brefeldin A (BFA) disrupts Golgi membranes and inhibits binding of soluble high molecular weight proteins to Golgi fractions. We examined the effects of BFA on binding of ARF1, -3, and -5 to a Golgi fraction in the presence of an ATP-regenerating system and a fraction of soluble, high molecular weight, accessory proteins (SAP), presumably containing complexes identified by others as coatomers that are involved in vesicular transport. ARF binding in all instances was dependent on guanosine 5'-O-(3-thiotriphosphate) and increased by the ATP-regenerating system. Binding of ARF1 and -3, but not ARF5, was enhanced by SAP. BFA inhibited the SAP-dependent, but not the SAP-independent, binding of ARF1 and -3. It had no effect on the increment in binding produced by an ATP-regenerating system. B36, an inactive derivative of BFA, did not inhibit SAP-dependent binding of ARF1 and -3. Binding of ARF5, which was SAP-independent, was not affected by BFA. These observations are consistent with the conclusion that mammalian ARFs differ in their dependence on accessory proteins for interaction with Golgi and, perhaps, other cellular membranes and that BFA specifically inhibits SAP-dependent ARF binding.

ADP-ribosylation factors (ARFs)\(^1\) are ~20-kDa guanine nucleotide-binding proteins, initially identified by their ability to enhance \( \text{in vitro} \) cholera toxin-catalyzed ADP-ribosylation of \( \text{G}_\text{sa} \), the stimulatory guanine nucleotide-binding protein of the adenylcyclase system, and subsequently shown to serve as GTP-dependent, allosteric activators of the toxin A1 catalytic unit (reviewed in Ref. 1). In eukaryotic cells, ARFs are believed to participate in vesicular transport within the Golgi and other membrane compartments (2–7). As components of the Golgi transport system, ARFs act in concert with a high molecular weight soluble complex, termed coatamer, to promote vesicle transport involving Golgi, a process believed to involve also a heterotrimeric G protein (5, 8, 9).

At least six mammalian ARF members of a multigene family have been identified by cDNA and genomic cloning (10–14). Based on size, deduced amino acid sequence, phylogenetic analysis, and gene structure, they can be grouped into three classes (10–17): ARF1, -2, and -3 form Class I, ARF4 and -5 Class II, and ARF6 Class III (14). ARFs from all three classes activate cholera toxin-catalyzed ADP-ribosylation (18, 19). The stimulatory effects of certain phospholipids and detergents on this reaction are ARF-specific. Recombinant ARF2 (rARF2) was almost absolutely dependent on dimyristoylphosphatidylcholine plus cholate for activity, whereas rARF6 exhibited substantial activity in their absence (19).

ARFs are, at least in part, co-translationally modified by N-terminal myristoylation (20, 21). Activation of toxin by ARFs did not require myristoylation (18, 19), although it has been proposed that the N-terminal amino acid sequence may be necessary for this action (22). In addition to being activated by phospholipids, purified native and recombinant ARFs synthesized in \( \text{Escherichia coli} \) bound to phospholipids or Golgi membranes in the presence of nonhydrolyzable GTP derivatives (e.g. GTP\( \gamma \)S) but not GDP (23–27). In contrast to toxin activation, binding of ARF to Golgi membranes did require N-terminal myristoylation (25).

ARFs have been localized by immunoreactivity to Golgi membranes in tissue sections (28), although most ARF activity in homogenates appears to be soluble (20). Recent reports have suggested that ARFs may be involved in a variety of cellular processes in addition to Golgi transport, including endocytosis and nuclear membrane assembly (3, 4). Since several mammalian ARFs have been described, it seemed reasonable that they should have different functions and, likely, different intracellular localizations. Consistent with this hypothesis, ARF1, -3, and -5 clearly differed in their binding to a Golgi fraction (27). Since association of ARF with Golgi involves other soluble proteins (5, 9) and since Golgi membranes are disrupted by BFA (29, 30), the studies reported here were initiated to determine whether BFA and soluble accessory proteins (SAP) might affect differently the association of ARF1, -3, and -5 with Golgi fraction membranes.

**EXPERIMENTAL PROCEDURES**

**Materials**

B36, a BFA derivative, was a gift from Dr. Julie G. Donaldson, National Institute of Child Health and Human Development, NIH, Bethesda, MD. BFA was purchased from Epicentre Technologies.
**Sources of other materials are noted in earlier publications from this laboratory.**

**Methods**

**Preparation of Golgi Fraction, ARF Fraction, and SAP from Rat Brain Homogenates**—As described in detail previously (27, 31), fresh rat brain was homogenized (Teflon Dounce homogenizer) in 2 volumes of 0.25 mM sucrose, 1 mM MgCl₂, 1 mM dithiothreitol, 0.5 mM glycerophosphosulfate fluoride, soybean and lima bean trypsin inhibitors, leupeptin, and aprotinin, each 1 μg/ml, 10 mM Hepes buffer pH 7.5. The homogenate was diluted with another 2 volumes per weight of buffer A and centrifuged (850 × g, 10 min, Sorvall SS-54). The supernatant was further centrifuged (35 min, 175,000 g, SW 41, 37,000 rpm), and the pellet was dispersed in buffer A with 1.4 M instead of 0.25 M sucrose (volume equal to original tissue weight). A 4-ml sample of the suspension was transferred to the bottom of a centrifuge tube which contained layers (each 2 ml) of 0.25, 0.6, 0.85, and 1.15 M sucrose in buffer A without sucrose. After centrifugation (100 min, 175,000 g), the fraction at the interface of 0.85 and 1.15 M sucrose, which contained the highest specific activity of galactosyltransferase, a Golgi resident enzyme (32), was collected and stored in small portions at −70 °C.

To prepare partially purified rat brain ARFs, the supernatant after centrifugation at 175,000 g for 35 min was concentrated (Amicon Centriprep 10), and stored in small portions at −70 °C.

Based on ARF activity, mobility on SDS-PAGE, and immunoreactivity with rARF5 polyclonal antibodies, a native ARF 5 was partially purified from an enriched AcA 54 column fraction of rat brain by sequential chromatography on DEAE-Sephacel and CM-Sephrose. From the DEAE-Sephacel column, ARF5 was eluted with 107–116 mM NaCl and myristoylated rARF5 (mrARF5) with 90–110 mM NaCl. ARF5 active fractions, which were separated from most of ARF1 and -3 (based on reaction with sARF I1 and rARF5 antibodies), were titrated to pH 3. The supernatant fraction after centrifugation at 10,000 × g for 30 min was applied to a CM-Sepharose column. ARF5 did not bind and emerged in the void as described (28). The blot was then stained poorly with Coomassie Blue but well with silver stain. Based on silver staining ARF5 represented almost 10% of total protein.

**Columns**—Columns that contained proteins larger than ovalbumin (45 kDa) were pooled, concentrated (YM 10 membrane, Amicon) to half of its original volume. An 18-ml sample (~270 mg of protein) was applied to a column (2 × 95 cm, 279 ml) of Ultrogel Ac A 54 equilibrated and eluted with buffer B (0.25 M sucrose, 100 mM NaCl, 1 mM NaN₃, 5 mM MgCl₂, 1 mM EDTA, 2 mM dithiothreitol, 20 mM Tris, pH 8.0). Fractions (3 ml) were collected and assayed for ARF activity based on stimulation of CTA ADP-ribose transferase activity (33). Fractions containing ARF activity were pooled, concentrated (Amicon Centriprep 10), and stored in small portions at −70 °C.

**Immunochemistry**—To investigate the interaction of ARFs with Golgi, a fraction enriched in ARFs from gel filtration of rat brain supernatant was used with or without an ARF-free fraction of SAP and/or an ATP-regenerating system. In the presence of GDPγS, ARF activity associated with Golgi membranes increased rapidly for 20 min and much more slowly thereafter (Fig. 1). The rate of binding was increased by addition of an ATP-regenerating system or SAP, or both (Fig. 1). The ATP-regenerating system increased binding of ARF1, -3, and -5 to Golgi membranes whether or not SAP was present, and SAP alone enhanced binding of ARF1 and -3 (data not shown), but not ARF5 (25).

**Effects of Brefeldin A on Binding of ARFs to Golgi**

**Immunodetection of ARF1 and -3 and mrARF5**—Two polyclonal antibodies, one raised against sARF II (ARF3) and the other against recombinant ARF5, were used (27). The former is relatively specific for class I ARFs (ARF1, -2, and -3), and the latter reacts well with class II, but not class I, ARFs. ARF1 and -3 differ in electrophoretic mobility. ARF5 differs from ARF4 (not detected in these experiments) as well as from ARF1 and -3 in electrophoretic mobility.

After precipitation with 7.5% trichloroacetic acid, proteins were separated by SDS-PAGE in 15% gels and transferred to nitrocellulose, which was incubated overnight with a mixture of the two antibodies (1:1000 dilution of rARF5 antibodies and IgG from anti-sARF II antisera, 1 μg/ml as described (35)). The blot was then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG followed by color development with 4-chloro-1-naphthol and H₂O₂. After incubation of Golgi and purified ARF in the presence of GDPβS, a small amount of immunoreactive ARF was associated with the membranes, although no ARF activity was detected. This was perhaps a result of the presence of some ARF that had been desaturated during the purification procedure.

**RESULTS AND DISCUSSION**

To investigate the interaction of ARFs with Golgi, a fraction enriched in ARFs from gel filtration of rat brain supernatant was used with or without an ARF-free fraction of SAP and/or an ATP-regenerating system. In the presence of GDPγS, ARF activity associated with Golgi membranes increased rapidly for 20 min and much more slowly thereafter (Fig. 1). The rate of binding was increased by addition of an ATP-regenerating system or SAP, or both (Fig. 1). The ATP-regenerating system increased binding of ARF1, -3, and -5 to Golgi membranes whether or not SAP was present, and SAP alone enhanced binding of ARF1 and -3 (data not shown), but not ARF5 (25).

**Fig. 1. Effect of an AR and SAP on association of ARFs with Golgi membranes in the presence of GDPγS**. Golgi membranes (12.5 μg) and rat brain ARF fraction (78 μg) were incubated (total volume 200 μl) with 0.2 mM GDPγS or GDPβS, with or without AR, SAP (240 μg), or both, at 37 °C for the indicated time before pelleting Golgi membranes. The pellet was dispersed in 150 μl of buffer A. Means are of duplicate 20-μl samples that were assayed for ARF activity. ○, GDPγS; △, GDPβS plus AR; ●, GDPβS plus SAP; ■, GDPγS, AR, and SAP; □, GDPβS and AR; ○, GDPβS, AR, and SAP. **TABLE 1.** Effects of Brefeldin A on Binding of ARFs to Golgi Membranes

| Time-min | ARF activity-nmol/h | ARF activity-nmol/h | ARF activity-nmol/h |
|----------|---------------------|---------------------|---------------------|
| 0        | 10                  | 10                  | 10                  |
| 20       | 20                  | 20                  | 20                  |
| 40       | 40                  | 40                  | 40                  |
| 60       | 60                  | 60                  | 60                  |

**REFERENCES**
BFA (25 or 100 µg/ml) did not inhibit basal or ATP-regenerating system-dependent binding of ARF activity to membranes (Fig. 2A). It did, however, inhibit the SAP-dependent binding of ARF activity regardless of whether an ATP-regenerating system was present (Fig. 2A). On immunoblots and silver staining, it appeared that BFA decreased the binding of ARF1 and -3 when SAP was present but had little effect on ARF5 (Fig. 2B and C).

The effects of BFA, ATP-regenerating system, and SAP on binding of purified native or recombinant ARFs were also evaluated. With the native ARF1 (Fig. 3) or ARF3 (Fig. 4) gene products purified from bovine brain cytosol, as noted with the crude ARF fraction, GTPγS, but not GDPβS, enhanced binding of ARF activity to Golgi following apparently a slight delay (Figs. 3 and 4). In the presence of GTPγS, either an ATP-regenerating system or SAP enhanced association of ARF1 (Fig. 3) or ARF3 (Fig. 4) with Golgi and the former perhaps diminished the delay time.

In the presence of GTPγS and an ATP-regenerating system, the effect of increasing SAP on binding of ARF1 (Fig. 5) or ARF3 (Fig. 6) to membranes was biphasic. As the enhanced ARF binding observed with smaller amounts of SAP was not reproduced by the addition of BSA, it was presumably not a nonspecific “stabilizing” effect. Without the regenerating system...
Effects of Brefeldin A on Binding of ARFs to Golgi

FIG. 5. Effect of SAP on ARF1 binding to Golgi membranes in the presence of GTP\(_\gamma\)S with or without AR. Golgi membranes (12.5 µg), ARF1 (3.3 µg), 0.2 mM GTP\(_\gamma\)S, BSA (40 µg), and the indicated amount of SAP or BSA, with or without AR and other additions as described under "Methods" (total volume 200 µl) were incubated at 37 °C for 40 min. GTP\(_\gamma\)S (○) or GTP\(_\gamma\)S plus AR (▲) with indicated amount of SAP, and GTP\(_\gamma\)S (□) or GTP\(_\gamma\)S plus AR (■) with indicated amount of BSA are plotted.

FIG. 6. Effect of SAP on ARF3 binding to Golgi membranes in the presence of GTP\(_\gamma\)S with or without AR. Golgi membranes (6.25 µg), ARF3 (2 µg), BSA (20 µg), 0.2 mM GTP\(_\gamma\)S, and SAP or BSA (total volume 100 µl) with other components as described under "Methods" were incubated at 37 °C for 40 min as described in Fig. 5. GTP\(_\gamma\)S (○) or GTP\(_\gamma\)S plus AR (▲) with indicated amount of SAP, and GTP\(_\gamma\)S (□) or GTP\(_\gamma\)S plus AR (■) with indicated amount of BSA are plotted.

FIG. 7. Effect of SAP on mrARF5 binding to Golgi membranes in the presence of GTP\(_\gamma\)S with or without AR. Binding of mrARF5 (3.6 µg) was carried out as described in the legend for Fig. 6 except that only 5 µg of BSA was present. GTP\(_\gamma\)S (○) or GTP\(_\gamma\)S plus AR (▲) with indicated amount of SAP, and GTP\(_\gamma\)S (□) or GTP\(_\gamma\)S plus AR (■) with indicated amount of BSA are plotted.

TABLE I
Effect of brefeldin A on association of ARF1, ARF3, or mrARF5 with Golgi membranes in the presence of AR and SAP

| Addition | None | AR | SAP | AR/SAP |
|----------|------|----|-----|--------|
| ARF1     | 3.2  | 6.1 | 9.1 | 9.8    |
| BFA      | 3.2  | 6.6 | 4.2 | 7.5    |
| ARF3     | 3.7  | 7.9 | 8.9 | 13.7   |
| BFA      | 4.2  | 7.9 | 4.5 | 9.5    |
| mrARF5   | 11.5 | 24.3| 12.2| 15.6   |
| BFA      | 13.7 | 23.4| 12.5| 16.4   |

The effects of BFA on binding of purified native or recombinant ARF proteins were assessed in the presence of SAP and an ATP-regenerating system (Table I, Fig. 8). BFA (4 µg) inhibited the SAP-stimulated binding of ARF1 and ARF3 to Golgi membranes but did not inhibit mrARF5 binding under any conditions (Table I). As shown in Fig. 8, inhibitory effects of BFA on binding of ARF1 and ARF3 were concentration-dependent. B36, an inactive derivative of BFA (30), did not inhibit the binding of ARF1 or ARF3 to Golgi in the presence of SAP (Table I).

Prior studies had demonstrated an inhibitory effect of BFA...
Effects of Brefeldin A on Binding of ARFs to Golgi

Fig. 8. Inhibition by brefeldin A of association of ARF1 and ARF3 with Golgi membranes in the presence of SAP with or without an AR. Golgi membranes (12.5 μg), ARF1 or ARF3 (4 μg), SAP (164 μg), ARF1 and ARF3, were incubated at 37 °C for 10 min. 10 μl of 4 mM GTPγS was added, and the samples were incubated at 37 °C for 40 min. All samples contained 1% ethanol. Pellets were suspended in 150 μl of buffer A. A, duplicate 10-μl samples were assayed for ARF activity. 1, ARF1; 2, ARF3; 3, ARF1, AR; 4, ARF3, AR. (It is noted that one or another of the values for ARF1 in the absence of BFA appeared to be in error in this experiment. In all other experiments, ARF binding was increased by the ATP-regenerating system.) B, 88-μl samples were used for SDS-PAGE and immunodetection of ARF1 and -3. Densitometric readings of ARF1 and ARF3 (sample without BFA in each group set as 1.0) from left to right, ARF1, without AR: 0.48 (samples contained GDP;S, not GTPγS); 1.0, 1.17, 1.06, 0.67; ARF1 with AR: 1.0, 0.82, 0.49, 0.73; ARF3, without AR: 1.0, 0.67, 0.59, 0.46; ARF3 plus AR: 1.0, 0.8, 0.4, 1.7; ARF3, without AR: 0.18 (GDPγS). C, 13-μl samples were subjected to electrophoresis and silver staining. Densitometric readings of ARF1 and ARF3 (sample without BFA in each group set as 1.0) from left to right, ARF1 without AR: 1.0, 0.57, 0.51, 0.44; ARF1 plus AR: 1.0, 0.84, 0.71, 0.54; ARF3 without AR: 1.0, 1.0, 0.75, 1.0; ARF3 plus AR: 1.0, 1.05, 0.85, 0.65.

Table II

| Drug added | ARF activity associated with Golgi |
|------------|-----------------------------------|
|            | ARF1 | ARF3 |
| No×e       | 10.0 | 8.5  |
| BFA, 2 μg  | 5.7  | 6.2  |
| BFA, 5 μg  | 5.3  | 4.7  |
| B36, 2 μg  | 9.8  | 8.9  |
| B36, 5 μg  | 10.5 | 9.8  |

Effects of brefeldin A and B36 on ARF1 or ARF3 binding to Golgi in the presence of SAP

Golgi (6.25 μg), SAP (107 μg), ARF1 (2.5 μg) or ARF3 (2 μg), and other additions with either BFA or B36, a derivative of BFA (2 or 5 μg in 5 μl of 10% ethanol), were incubated at 21 °C for 10 min (95 μl total volume) as in Fig. 8. After addition of 5 μl of 4 mM GTPγS, incubation was continued at 37 °C for 40 min. Pellets were assayed for ARF activity.

and ARF3 was increased, whereas the binding of ARF5 was either unaffected, or when an ATP-regenerating system was present, was inhibited. Consistent with this observation, BFA inhibited only the binding of ARF1 and -3, specifically the SAP-dependent increment in binding ARF1 and -3, and not ARF5. Initially, we had some concern that the lack of an effect of SAP and BFA on mARF5 binding might reflect a defect in the recombinant proteins not detected by the cholera toxin activation assay. The observation that ARF5 partially purified from rat brain behaved similarly (data not shown) supports the conclusion that the mARF5, for intrinsic rather than artifactual reasons, behaved differently from ARF1 and ARF3 in response to BFA and SAP.

The SAP fraction was used in our experiments as a potential source of coaters (and perhaps other proteins) that might influence ARF binding and/or vesicle formation, recognizing that its heterogeneity could result in multiple effects. It seems quite possible, for example, that the inhibition of ARF binding that occurred with increasing amounts of SAP in the presence of the ATP-regenerating system is a result of inhibition or destruction of the source of ATP. In addition, it is unclear just what ARF binding means in functional terms in this system. We believe that some, possibly large, fraction of ARF binding, although GTP-dependent, is "nonspecific," perhaps analogous to the GTP-dependent binding of ARF to phospholipids (23, 26). The BFA inhibition of binding of ARF1 and -3 would appear to define one relatively specific component, i.e. that which is dependent on something(s) in the SAP fraction. These observations, which are very clear-cut and reproducible, seem to be consistent with the view that ARF binding is an early step in Golgi vesicle formation, and it is this step that is inhibited by BFA (8, 9, 36).

While this paper was being completed, there were two more reports of effects of BFA that are believed to be due to its interference with ARF binding (37, 38). In these studies, ARF binding, evaluated for the most part as an exchange of protein-bound guanyl nucleotide produced by incubation of myristoylated recombinant ARF1 with Golgi, was essentially completely inhibited by BFA. This BFA inhibition corresponds to a specific functional step, the result of interaction of an ARF molecule with a specific nucleotide exchange protein, which had been earlier postulated as a mechanism for triggering vesicle formation when an ARF molecule with bound GDP interacts with a specific membrane protein that activates it, i.e. catalyzes the replacement of GDP with GTP. This is a very plausible, in fact appealing, mechanism, but it leaves us with the question of why BFA-inhibited binding in our studies...
Effects of Brefeldin A on Binding of ARFs to Golgi

was dependent on SAP. We think it quite possible that the Golgi preparations used by other workers, which are obtained from different tissue sources, contain the ARF-binding protein(s) that is missing from our rat brain Golgi fractions but is supplied by SAP.

BFA is believed to affect several types of membranes, in addition to the Golgi (29). Thus, it might be expected that its effects on ARF binding would include ARFs that interact with membranes other than Golgi. Current studies are directed toward identifying the membranes involved in the ARF-specific interactions. In any case, the observation that ARF5 binding was unaffected by BFA or SAP is consistent with the view that it does not bind to the same membranes as ARF1 or ARF3.

Acknowledgments—We thank Dr. Julie Donaldson for kindly providing the brefeldin A derivative B36 and Carol Kosh for expert secretarial assistance.

REFERENCES

1. Serventi, I. M., Moss, J., and Vaughan, M. (1992) Curr. Top Microbiol. Immunol. 175, 43-67
2. Balch, W. E., Kahn, R. A., and Schwesinger, R. (1992) J. Biol. Chem. 267, 13003-13006
3. Boman, A. L., Taylor, T. C., Melançon, P., and Wilson, K. L. (1992) Nature 354, 512-514
4. Lenhard, J. M., Kahn, R. A., and Stahl, P. D. (1992) J. Biol. Chem. 267, 13047-13052
5. Rothman, J. E., and Orci, L. (1992) Nature 355, 409-415
6. Taylor, T. C., Kahn, R. A., and Melançon, P. (1992) Cell 70, 69-79
7. Zeusen, S., Feick, P., Zimmermann, P., Hasse, W., Kahn, R. A., and Schultz, L. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6619-6623
8. Donaldson, J. G., Kahn, R. A., Lippincott-Schwarz, J., and Klausner, R. D. (1991) Science 254, 1197-1199
9. Serafini, T., Orci, L., Amherdt, M., Brunner, M., Kahn, R. A., and Rothman, J. E. (1991) Cell 67, 239-253
10. Price, S. R., Nightingale, M., Tasi, S.-C., Williamson, K. C., Adamik, R., Chen, H.-C., Moss, J., and Vaughan, M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5488-5491
11. Sewell, J. L., and Kahn, R. A. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4620-4624
12. Bobak, D. A., Nightingale, M. S., Murtagh, J. J., Price, S. R., Moss, J., and Vaughan, M. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6101-6105
13. Monaco, L., Murtagh, J. J., Newman, K. B., Tasi, S.-C., Moss, J., and Vaughan, M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2206-2210
14. Tsuchiya, M., Price, S. R., Tasi, S.-C., Moss, J., and Vaughan, M. (1991) J. Biol. Chem. 266, 2737-2772
15. Tasi, S.-C., Haun, R. S., Tsuchiya, M., Moss, J., and Vaughan, M. (1991) J. Biol. Chem. 266, 23063-23069
16. Lee, C.-M., Haun, R. S., Tasi, S.-C., Moss, J., and Vaughan, M. (1992) J. Biol. Chem. 267, 9025-9034
17. Serventi, I. M., Cavanaugh, E., Moss, J., and Vaughan, M. (1993) J. Biol. Chem. 268, 4893-4897
18. Weiss, O., Holden, J., Rulks, C., and Kahn, R. A. (1989) J. Biol. Chem. 264, 21066-21072
19. Price, S. R., Welch, C. F., Haun, R. S., Stanley, S. J., Moss, J., and Vaughan, M. (1992) J. Biol. Chem. 267, 9025-9034
20. Kahn, R. A., Goddard, C., and Newmark, H. (1992) J. Biol. Chem. 283, 8282-8287
21. Kunz, B. C., Mucynski, R. A., Tasi, S.-C., Adamik, R., Chang, P. P., Moss, J., and Vaughan, M. (1990) J. Cell Biol. 147, 167
22. Kahn, R. A., Randazzo, P., Serafini, T., Weiss, O., Rulks, C., Clark, J., Amherdt, M., Roller, P., Orci, L., and Rothman, J. E. (1992) J. Biol. Chem. 267, 13039-13046
23. Kahn, R. A. (1991) J. Biol. Chem. 266, 15595-15597
24. Regazzi, R., Ulrich, S., Kahn, R. A., and Wollheim, C. B. (1991) Biochem. J. 275, 639-644
25. Haun, R. S., Tasi, S.-C., Adamik, R., Moss, J., and Vaughan, M. (1993) J. Biol. Chem. 268, 7064-7068
26. Walker, M. W., Bobak, D. A., Tasi, S.-C., Moss, J., and Vaughan, M. (1992) J. Biol. Chem. 267, 3230-3232
27. Tasi, S.-C., Adamik, R., Haun, R. S., Moss, J., and Vaughan, M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9272-9276
28. Stearns, T., Willingham, M. C., Botstein, D., and Kahn, R. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 1238-1242
29. Donaldson, J. G., Lippincott-Schwarz, J., Bloom, G. S., Kreis, T. E., and Klausner, R. D. (1990) J. Cell Biol. 111, 2288-2296
30. Klausner, R. D., Donaldson, J. G., and Lippincott-Schwarz, J. (1991) J. Cell Biol. 116, 1071-1080
31. Cariappa, R., and Kilberg, M. S. (1990) J. Biol. Chem. 265, 1470-1475
32. Fleischer, B. (1974) Methods Enzymol. 31, 181-191
33. Tasi, S.-C., Noda, M., Adamik, R., Chang, P. P., Chen, H.-C., Moss, J., and Vaughan, M. (1988) J. Biol. Chem. 263, 7686-7672
34. Murayama, T., Tasi, S.-C., Adamik, R., Moss, J., and Vaughan, M. (1993) Biochemistry 32, 561-566
35. Towbin, H., Staehlin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350-4354
36. Donaldson, J. G., Cassel, D., Kahn, R. A., and Klausner, R. D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6408-6412
37. Donaldson, J. G., Finazzi, D., and Klausner, R. D. (1992) Nature 360, 350-352
38. Helms, J. B., and Rothman, J. E. (1992) Nature 360, 352-354