Sorting and Intracellular Trafficking of a Glycosyolphosphatidylinositol-anchored Protein and Two Hybrid Transmembrane Proteins with the Same Ectodomain in Madin-Darby Canine Kidney Epithelial Cells*

(Received for publication, March 22, 1995, and in revised form, July 21, 1995)

Gladys Arreaza and Deborah A. Browing‡

From the Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, New York 11794-5215

We compared the trafficking of the glycosylphosphatidylinositol (GPI)-anchored placental alkaline phosphatase (PLAP) and two chimeric transmembrane proteins containing the PLAP ectodomain in stably transfected Madin-Darby canine kidney epithelial cells to determine whether different mechanisms might be used in apical sorting of GPI-anchored and transmembrane proteins. PLAP-G, which contained the transmembrane and cytoplasmic domains of the vesicular stomatitis virus glycoprotein, was delivered directly to the basolateral surface. PLAP-HA contained the transmembrane and cytoplasmic domains of influenza hemagglutinin. Both PLAP and PLAP-HA were delivered directly to the apical membrane. PLAP becomes insoluble in Triton X-100 during biosynthetic transport, as it associates with detergent-resistant membranes. Neither hybrid protein was detergent insoluble, though the small amount of PLAP that was missorted to the basolateral surface was insoluble. We examined the effects of three drugs known to interfere with membrane trafficking on sorting and delivery of PLAP and the hybrid proteins. Monensin had no effect on sorting or surface expression of any of the proteins. Nocodazole affected the sorting of both PLAP and PLAP-HA but not of PLAP-G. Brefeldin A appeared to disrupt the sorting of PLAP and PLAP-HA but not of PLAP-G. This conclusion was tempered by the observation that this drug affected the distribution of proteins at the cell surface. Thus, sorting and transport of GPI-anchored and apical transmembrane proteins are similar in a number of respects.

Polarized epithelial cells contain apical and basolateral plasma membrane domains that are separated from each other by tight junctions that maintain differences in the protein and lipid composition between the two surfaces (1–3). Compositional differences in the two membrane domains are generated by the sorting of proteins and lipids after intracellular synthesis. In the Madin-Darby canine kidney (MDCK) cell line, sorting occurs in the TGN before delivery to the cell surface (4, 5).

Efforts to understand the sorting process have focused on defining sorting signals in transported proteins. Signals in the cytoplasmic domains of several basolateral proteins are required for correct targeting (6–13). In some but not all cases, these regions overlap signals for internalization of the proteins in clathrin-coated pits.

Some proteins are anchored in membranes by glycosylphosphatidylinositol (GPI) instead of by conventional transmembrane peptides (reviewed in Refs. 14–16). GPI-anchored proteins are apically polarized in epithelial cells in culture and in tissues (17). The role of the membrane anchor in specifying this apical localization has been tested using hybrid proteins (18, 19). Hybrid proteins containing the ectodomains of normally basolateral proteins, or soluble proteins linked to GPI-anchors, are directed to the apical surface.

Two lines of investigation suggest that there may be differences between the intracellular trafficking of GPI-anchored proteins and apical transmembrane proteins. First, in two unusual cell lines, GPI-anchored proteins are not restricted to the apical membrane. One of these is a mutant MDCK cell line that is resistant to killing by concanavalin A (20, 21). GPI-anchored proteins are found in both domains of these cells. The other is the FRT thyroid cell line (22, 23). Most GPI-anchored proteins in FRT cells are present on the basolateral surface (24, 25). By contrast, many transmembrane proteins exhibit the same polarity in FRT cells as in other epithelial cells (26–28).

Metabolic studies provide another indication that the trafficking of GPI-anchored proteins and transmembrane proteins may differ. Two groups showed that cholesterol depletion specifically inhibits the cell-surface expression of GPI-anchored proteins. Growth in low density lipo protein-depleted serum lowered the expression of a hybrid GPI-anchored protein, gD1-DAF, in MDCK cells (64). Cholesterol depletion had a similar effect on the expression of the GPI-anchored protein CD14 on human monocytes (29).

Thus, GPI-anchored proteins and apical transmembrane proteins can show differential sorting and cell-surface transport in polarized epithelial cells. To compare the trafficking of GPI-anchored and transmembrane proteins in MDCK cells, we examined three similar proteins, PLAP, PLAP-G, and PLAP-HA (collectively called “PLAP proteins”). PLAP is an apical GPI-anchored protein. A hybrid protein, PLAP-G, contains the ectodomain of PLAP and the transmembrane and cytoplasmic domains of the vesicular stomatitis virus glycoprotein (VSV G) (30). We showed previously that PLAP-G is present on the basolateral surface of MDCK cells (18). Here, we describe a second hybrid protein, PLAP-HA, that contains the ectodomain of PLAP fused to the transmembrane and cytoplas-
The Asp residue of PLAP that is linked to the GPI anchor is indicated in C-terminal amino acid sequences of PLAP, PLAP-G, and PLAP-HA.

The same ectodomain in all three proteins (dark stipples) in PLAP-G, and the HA transmembrane domain (light stipples) in PLAP-HA. The Asp residue of PLAP that is linked to the GPI anchor is indicated (63). Residues beyond the site of GPI anchor attachment are not present in mature PLAP. The transmembrane domains of PLAP-G and PLAP-HA are underlined.

**EXPERIMENTAL PROCEDURES**

Materials, Cell Culture, and Plasmid Construction—All chemicals were from Sigma unless otherwise stated. MDCK strain II cells were grown as described (31). Stable transfection of these cells to generate independent lines expressing PLAP, PLAP-G, or PLAP-HA was as described (32). Plasmids pBC12/PLAP 513 encoding PLAP and pBC12/PLAP 489 encoding PLAP-G (30) were the gift of S. Udenfriend (Roche Institute, Nutley, NJ). The construction of plasmid pBC12/PLAP-HA, encoding PLAP-HA, was similar to that of pBC12/PLAP 489G (30). A 200-base pair fragment encoding the transmembrane and cytoplasmic domains of HA (H3 subtype) was excised from pSV2GH3A (33) by BamHI digestion. The ends were filled in, and the fragment was ligated into pBC12/PLAP 489 at the unique Hpal site, which was previously modified by insertion of a filled-in HindIII linker to obtain an in-frame fusion (Fig. 1).

**RESULTS**

Expression of PLAP, PLAP-HA, and PLAP-G in MDCK Cells—Although all three proteins were expressed at roughly similar levels, expression of PLAP-G was often lower than that of PLAP and PLAP-HA. Several independent stably transfected lines expressing each protein were used in the course of these studies. These varied in their expression level. In addition, expression of the transfected proteins decreased with time in culture. Thus, a precise comparison of the expression levels of the three proteins was difficult. None of the results reported here varied as a function of expression level. All the proteins were transported to a compartment in which their N-linked oligosaccharide chains became resistant to endoglycosidase H with half-times of about 20–30 min (data not shown). Most of each protein was delivered to the surface within 2 h of synthesis, as shown by the following experiment. The ratio of each [35S]methionine-labeled PLAP protein that could be recovered with immobilized streptavidin, to the amount of total labeled immunoprecipitated protein, was not exceeded about 50% and did not increase when more
Cells expressing PLAP (top panel) or PLAP-HA (bottom panel) were labeled for 5 min with $[^{35}S]$methionine and then incubated with unlabeled methionine for the indicated times (in min). After lysis, Triton X-100-soluble and -insoluble fractions were separated by centrifugation, and the pellets were solubilized in SDS. The PLAP proteins were immunoprecipitated from Triton-soluble (S) and Triton-insoluble (P) fractions, separated by SDS-PAGE, and analyzed by fluorography.

To determine whether PLAP-HA or PLAP-G associated with detergent-resistant membranes, transfected cells were subjected to a pulse-chase protocol as described in Fig. 2. After lysis on ice, Triton-soluble and insoluble fractions were separated by centrifugation, and the pellets were solubilized in SDS (43). The hybrid proteins were recovered from both fractions by immunoprecipitation and analyzed by SDS-PAGE and fluorography. This experiment was previously performed on cells expressing PLAP (34). PLAP shifted from the Triton-soluble to the Triton-insoluble fraction with a half-time of about 30 min. After 3 h of chase, approximately 95% of the protein was insoluble in Triton (Fig. 1 in Ref. 34). By contrast, both PLAP-G and PLAP-HA were recovered exclusively from the Triton-soluble fraction at all times of chase, showing that neither protein associated with the detergent-resistant membranes (Fig. 3).

Basolateral PLAP is Insoluble in Triton X-100—A small amount of PLAP is missorted to the basolateral surface. To determine whether this protein was insoluble in Triton X-100, a pulse-chase experiment was performed on cells expressing PLAP as described in Fig. 2, using a 5-min pulse and a 3-h chase. After domain-specific biotinylation and lysis, the detergent-soluble and -insoluble fractions were separated. $[^{35}S]$Methionine-labeled, biotinylated PLAP was recovered and analyzed as described in Fig. 2. A fluorograph is shown in Fig. 4. The small amount of PLAP that was present on the basolateral surface was largely insoluble in Triton X-100.

Monensin, Nocodazole, Brefeldin A, and Transport—Three drugs have been reported to affect sorting or biosynthetic transport of transmembrane proteins. These drugs, monensin, nocodazole, and BFA, are thus useful tools for characterizing the trafficking of a GPI-anchored protein. Examining two closely related chimeric proteins also provides a unique opportunity to compare the effects of the drugs on apical and basolateral transport.

Monensin Does Not Block Surface Delivery of PLAP, PLAP-HA, or PLAP-G—The monovalent cation ionophore monensin blocks transport along the secretory pathway in the cis or medial Golgi in most mammalian cells (44). In MDCK cells, however, the effect is different. Early studies using virally

---

2 K. Melkonian and D. Brown, unpublished data.
grown cells expressing PLAP were labeled with [35S]methionine for 20 min and then transferred to media containing unlabeled methionine for 2 h. Filters were biotinylated on the apical (A) or basolateral (B) surface, and cells were lysed in buffer containing Triton X-100. Triton X-100-soluble and -insoluble fractions were separated by centrifugation, and the pellets were solubilized from Triton-soluble (S) and Triton-insoluble (P) fractions, separated by SDS-PAGE, and analyzed by fluorography.

infected cells showed that apical transport of influenza HA was unaffected, while basolateral transport of VSV G was blocked (45–47). We studied the effect of monensin on transport and sorting of the PLAP proteins. Cells treated with or without monensin were subjected to the pulse-chase procedure described in Fig. 2 and subjected to domain-specific biotinylation. Biotinylated, [35S]methionine-labeled PLAP proteins were recovered and detected by fluorography (Fig. 5). In contrast to the earlier results cited above, we found that monensin had little effect on the sorting or cell-surface transport of any of these proteins. However, the electrophoretic mobility of all three proteins was altered (Fig. 5). The proteins migrated faster after monensin treatment, consistent with the previously described effects of this drug on glycosylation (46).

Nocodazole Affects the Sorting of PLAP and PLAP-HA but Not of PLAP-G—Treatment of epithelial cells with microtubule-disrupting drugs specifically affects polarized delivery to the apical membrane (48–52). To ensure that nocodazole disrupted microtubules, MDCK cells grown on coverslips were treated with (Fig. 6A) or without (Fig. 6B) nocodazole and examined by immunofluorescence using anti-tubulin antibodies. Only a diffuse cytoplasmic staining was observed after drug treatment. We then determined the effect of nocodazole on expression of the PLAP proteins using a pulse-chase protocol. Results are shown in Fig. 7. Only 50% of PLAP and 60% of PLAP-HA was targeted to the apical membrane after microtubule disruption, while 94% of PLAP-G was expressed on the basolateral surface. Thus, in accord with previous findings, sorting of a basolateral protein was not altered by the drug. Recent results suggest that this may result from incomplete microtubule disruption (see "Discussion"). Despite this possibility, our results clearly demonstrate that polarized delivery of a GPI-anchored protein, like that of apical transmembrane proteins, requires intact microtubules.

Effect of BFA on Sorting of PLAP Proteins and on Cell Polarity—Low concentrations of the fungal metabolite BFA do not affect secretion in MDCK cells but have been reported to alter membrane trafficking in these cells (see "Discussion"). To examine the effect of BFA on sorting of the PLAP proteins, cells expressing them were pulse labeled with [35S]methionine for 5 min and then incubated for 3 h with unlabeled methionine with or without BFA. BFA had a dramatic effect on the localization of all three proteins (Fig. 8A and Table I). As the distribution of all three proteins was affected, we were concerned about the specificity of the BFA effect. We performed a control experiment to determine whether BFA altered the distribution of proteins that were already on the plasma membrane when drug treatment began. Cells were incubated with [35S]methionine and then chased for 2–3 h to allow newly synthesized plasma membrane proteins to reach the cell surface. Cells were then treated for 3 h with or without 3.5 μM BFA. Monolayers were subjected to domain-specific biotinylation, and the [35S]methionine-labeled, biotinylated proteins were recovered and detected as described in Fig. 2. Results are shown in Fig. 8B and Table I. The distribution of all three proteins was affected by this treatment. This effect did not appear to result from disruption of the tight junctions, as we detected little effect of BFA on the leakage of fluorescein isothiocyanate-dextran (M, 3860) across the monolayer in 3 h (data not shown). BFA thus affects a step in sorting, trafficking, or distribution of surface proteins that is not related to biosynthetic sorting in the TGN. A likely explanation of this behavior is the recently described effect of BFA on transcytosis (see "Discussion").

We wondered whether a shorter exposure to BFA might minimize this effect and allow us to detect any changes in intracellular sorting. Proteins were pulse labeled and then incubated with or without BFA for 1 h before domain-specific biotinylation. We included an internal control to measure the effect of the drug on cell-surface proteins. Cells on parallel
surface. Biotinylated PLAP proteins were recovered and analyzed as in panel A of nocodazole was calculated as in Fig. 5.

of each protein localized to the apical surface with (+) by scanning densitometry. The average values are shown. The percent distribution of cell-surface proteins allowed us to determine the effect of a 1-h exposure to BFA on

filters were pulse labeled, incubated for 2.5 h without BFA to filters on GPI-anchored and Hybrid Proteins

FIG. 7. Effect of nocodazole on polarized expression of PLAP proteins. A, filter-grown cells expressing the indicated protein were incubated with (+) or without (−) nocodazole. Cells were labeled with [35S]methionine for 20 min and then incubated with unlabeled methionine for 3 h before biotinylation from the apical (A) or basolateral (B) surface. Biotinylated PLAP proteins were recovered and analyzed as in Fig. 2 and detected by fluorography. B, bands on fluorographs from three experiments similar to the one shown in panel A were quantitated by scanning densitometry. The average values are shown. The percent of each protein localized to the apical surface with (+) or without (−) nocodazole was calculated as in Fig. 5. P-HA, PLAP-HA; P-G, PLAP-G.

PLAP
+ −
ΔA B ΔA B
PLAP-HA
+ −
ΔA B ΔA B
PLAP-G
+ −
ΔA B ΔA B

FIG. 8. Effect of BFA on polarized expression of PLAP proteins. Filter-grown cells expressing the indicated PLAP protein were labeled with [35S]methionine for 5 min. A, cells were then incubated with unlabeled methionine with (+) or without (−) BFA for 3 h before biotinylation from the apical (A) or basolateral (B) surface. B, after labeling, cells were incubated with unlabeled methionine for 3 h. Cells were then treated with (+) or without (−) BFA for 2 h (PLAP and PLAP-HA) or 3 h (PLAP and PLAP-HA). C, after labeling, cells were incubated with (+) or without (−) BFA for 1 h or for 2.5 h without BFA and then for 1 h with BFA (+). A–C, domain-specific biotinylation was performed from the apical (A) or basolateral (B) side, and biotinylated PLAP proteins were recovered and processed as in Fig. 2 and detected by fluorography.

proteins had reached the plasma membrane, about 70–80% of each protein was found on the apical surface (Fig. 8C, +*). This was similar to the value for the “no-BFA” control (Fig. 8C, −). This result shows that 1 h of BFA treatment did not affect the polarity of proteins already present on the apical surface. We conclude that BFA affected the intracellular sorting of both proteins.

The results were different for PLAP-G. In the absence of BFA, 97% of the protein was delivered to the basolateral surface (Fig. 8C, −). When BFA was added at the beginning of the chase, only 70% of PLAP-G was correctly localized to the basolateral surface (Fig. 8C, +). However, when BFA was added after PLAP-G had reached the plasma membrane, a similar value of 78% of the protein was detected on the basolateral membrane (Fig. 8C, +*). It appeared that the main effect of BFA on PLAP-G was to alter the distribution of the protein after it reached the cell surface. BFA seemed to have little effect on the intracellular sorting of PLAP-G.

Discussion

Signals in the cytoplasmic domains of several proteins can specify basolateral targeting (6–11). Casanova et al. (6) showed that a transmembrane form of PLAP with a very short cytoplasmic domain was expressed apically but could be redirected basolaterally by the addition of a sequence containing a basolateral sorting signal. The cytoplasmic domain of VSV G contains a basolateral sorting signal (53). Thus, a sorting signal in the cytoplasmic domain of PLAP-G is likely to be responsible for its basolateral localization.

If proper targeting of basolateral proteins requires positive signals, does transport of apical proteins occur by default, without the need for specific signals? If so, then apical proteins should be correctly sorted or missorted coordinately. The finding that GPI-anchored proteins are not apically polarized in a concanavalin A-resistant MDCK cell line (21) and in FRT cells (24, 25) suggested that apical transmembrane and GPI-anchored proteins may be sorted by different mechanisms. This prompted us to characterize the trafficking of PLAP and PLAP-HA in detail.

Solubility of the PLAP Proteins in Triton X-100—PLAP-HA and PLAP-G were fully soluble in Triton X-100, while both apical and basolateral pools of PLAP were insoluble. Simons and van Meer (2) suggested that apical proteins associate with glycolipid patches or rafts in the TGN and that this association is important in apical sorting. Based on this model, we suggested that association of PLAP with glycolipid-rich detergent-resistant membrane domains in the TGN might play a role in

TABLE I
Percent of each protein on the correct membrane

| Protein | PLAP | PLAP-HA | PLAP-G |
|---------|------|---------|--------|
| − BFA* | 85 ± 13 | 88 ± 9 | 86 ± 4 |
| + BFA* | 28 ± 15 | 42 ± 7 | 58 ± 5 |
| − BFA BFA* | 83 ± 5 | 86 ± 8 | 94 ± 3 |
| + BFA (3 h)$ | 67 ± 7 | 56 ± 6 | 73 ± 2 |
| − BFA* | 78 ± 6 | 69 ± 10 | 97 ± 1 |
| + BFA* | 39 ± 8 | 15 ± 10 | 70 ± 5 |
| + BFA (1 h) | 79 ± 6 | 76 ± 7 | 78 ± 3 |

* During 3-h chase.
$ During 3-h chase.
* After 1-h chase.
* After 2.5-h chase.
While this work was in progress, Lafont et al. (59) observed that nocodazole polarized expression of PLAP and PLAP-HA but not PLAP-G.

proteins in the TGN. We found that nocodazole affected the polarized delivery of apical but not basolateral transmembrane proteins. Treatment with microtubule-disrupting drugs disrupts the polarity of apical and basolateral transport vesicles. The small fraction of PLAP that is missorted basolaterally could easily associate with glycolipid-rich microdomains after delivery to that surface.

Monensin—Treatment of virally infected MDCK cells with monensin inhibited the surface expression of the basolateral VSV G protein but did not affect the polarity of influenza HA (45, 47). These results suggested that monensin affected transport of apical and basolateral proteins differently. We found that monensin did not alter the expression or polarity of PLAP, PLAP-HA, or PLAP-G, and we conclude that the drug has no general effect on apical or basolateral transport in MDCK cells.

In previous work, we used monensin in an attempt to block transport of PLAP out of the Golgi apparatus in MDCK cells (34). We found that the protein was insoluble in Triton X-100 after monensin treatment and concluded that it was insoluble while in the Golgi. Our current findings suggest that transport was not actually blocked in the earlier experiment. However, a separate result in the earlier paper also supported the same conclusion (Ref. 34, Fig. 2). As monensin was not used in this experiment, the conclusion is still valid.

Nocodazole—Most previous studies have indicated that treatment with microtubule-disrupting drugs disrupts the polarized delivery of apical but not basolateral transmembrane proteins (48–52). It is likely that microtubule disruption affects targeting of apical transport vesicles rather than sorting of proteins in the TGN. We found that nocodazole affected the polarized expression of PLAP and PLAP-HA but not PLAP-G. While this work was in progress, Lafont et al. (55) reported that nocodazole affected transport of both apical and basolateral proteins in permeabilized MDCK cells. They suggested that the drug does not fully disrupt microtubules in intact cells. This may explain why delivery of PLAP-G, and other basolateral proteins studied earlier, was not affected by nocodazole. Our most significant result, however, was that both PLAP and PLAP-HA were affected to the same extent. We conclude that microtubules are important in the polarized delivery of both GPI-anchored and apical transmembrane proteins.

Brefeldin A—Low concentrations (1–3 μM) of BFA have little effect on the efficiency of secretion in MDCK cells (56–58). However, apically directed proteins are missorted in the presence of the drug (57, 59–61).

Several groups have also studied the effect of 3.5 μM BFA on basolateral proteins. Three groups found no effect on basolateral sorting (58, 59, 61). In contrast, targeting of the low density lipoprotein receptor was reported to be affected by BFA (60).

We found that treatment with BFA for 3 h affected the polarity of proteins on the cell surface, as measured by domain-specific biotinylation. The explanation for this effect is unknown. An attractive possibility is that BFA may stimulate transcytosis of the PLAP proteins. The drug is known to increase the rate of basolateral to apical transcytosis of nonspecific markers (62), although specific transcytosis of the polymeric immunoglobulin receptor is blocked (56). This stimulation of transcytosis probably reflects an effect of BFA on normal sorting in an early endosomal compartment. An alternate explanation of the effect of BFA that we observed, that the integrity of tight junctions is disrupted, is unlikely (56, 62).

Regardless of the mechanism, however, these data show that apparent effects of BFA on sorting in the TGN may be complicated by changes in other membrane transport processes.

Treatment of cells with BFA for 1 h had little effect on the distribution of proteins that were already present on the apical surface when drug treatment began. However, apical proteins were missorted if exposed to BFA while they were in intracellular compartments. Thus, we agree with others that BFA affects the sorting of apical proteins. Qualitatively, BFA had the same effect on sorting of a GPI-anchored protein (PLAP) as an apical transmembrane protein (PLAP-HA).

About 20–30% of a [35S]methionine-labeled basolateral protein (PLAP-G) was present on the apical surface after 1 h of incubation in BFA, whether the drug was applied during biosynthetic sorting or after the protein had reached the plasma membrane. Thus, there appears to be little additional effect of BFA on the intracellular sorting of PLAP-G beyond the effect on the protein already present at the cell surface. This phenomenon may have been responsible for the apparent effect of BFA on biosynthetic sorting of the low density lipoprotein receptor (60) and may explain the discrepancy between the fact that TGN sorting of this protein appeared to be affected by BFA, while that of other basolateral proteins did not (58, 59, 61).

We have shown that the sorting and transport pathways of GPI-anchored proteins and a closely-related transmembrane apical protein have several key features in common. Further definition of these transport mechanisms and determination of whether GPI-anchored and transmembrane proteins inhabit the same transport vesicles remain challenges for the future.

Acknowledgments—We thank J. Rose, in whose laboratory PLAP-HA was constructed, S. Udenfriend and J. Volwerk for plasmids, B. Theurkauf for anti-tubulin antibodies, and N. Dean, B. Haltiwanger, and J. Crosby for critically reading the manuscript.

REFERENCES

1. Simons, K., and Fuller, S. D. (1985) Annu. Rev. Cell Biol. 1, 243–288.
2. Simons, K., and van Meer, G. (1988) Biochemistry 27, 6197–6202.
3. Rodriguez-Boulan, E., and Nelson, W. J. (1989) Science 245, 718–725.
4. van Meer, G., Stelzer, E. H. K., Wijnaendts- van-Resandt, R. W., and Simons, K. (1989) J. Cell Biol. 105, 285–320.
5. Mostov, K., Apodaca, G., Arendt, B., and Okamoto, C. (1992) J. Cell Biol. 116, 577–583.
6. Casanania, J. E., Apodaca, G., and Mostov, K. E. (1991) Cell 66, 65–75.
7. Hunziker, W., Harter, C., Matter, K., and Meliman, I. (1991) Cell 66, 907–920.
8. Le Bivic, A., Sambuy, Y., Patzak, A., Patil, N., Chao, M., and Rodriguez-Boulan, E. (1991) J. Cell Biol. 115, 607–618.
9. Brauer, C. B., and Roth, M. G. (1981) J. Cell Biol. 94, 413–421.
10. Yokode, M., Patthak, R. K., Hammer, R. E., Brown, M. S., Goldstein, J. L., and Anderson, R. G. W. (1992) J. Cell Biol. 117, 39–46.
11. Matter, K., Hunziker, W., and Meliman, I. (1992) Cell 71, 741–753.
12. Hunziker, W., and Fumey, C. (1994) EMBO J. 13, 2963–2969.
13. Matter, K., Yamamoto, E. M., and Meliman, I. (1994) J. Cell Biol. 126, 991–1004.
14. Ferguson, M. A. J., and Williams, A. F. (1988) Annu. Rev. Biochem. 57, 285–320.
15. Low, M. (1989) Biochim. Biophys. Acta 988, 427–454.
16. McCray, M. J., and Ferguson, M. A. J. (1993) Biochem. J. 294, 305–324.
17. Lisanti, M. P., Sargiacomo, M., Graeve, L. L., Saltiel, A. R., and Rodriguez-Boulan, E. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9557–9561.
18. Brown, D. A., Crise, B., and Rose, J. K. (1989) Science 245, 1499–1501.
19. Lisanti, M. P., Caras, I. W., Davitz, M. A., and Rodriguez-Boulan, E. (1989) J. Cell Biol. 109, 2145–2156.
20. Miess, H. K., Green, R. F., and Rodriguez-Boulan, E. J. (1982) Mol. Cell. Biol. 2, 1287–1294.
21. Lisanti, M. P., Le Bivic, A., Saltiel, A. R., and Rodriguez-Boulan, E. (1990) J. Membr. Biol. 113, 155–167.
22. Amberg-Imbottamo, F. S., and Coon, H. G. (1979) Int. Rev. Cytol. 50, (suppl.) 163–172.
23. Nitsch, L., Tramontano, D., Amberg-Imbottama, F. S., Quarto, N., and Nitsch, L. (1992) J. Cell Biol. 115, 99–105.
24. Zurzolo, C., Le Bivic, A., Quarini, A., Nitsch, L., and Rodriguez-Boulan, E. (1992) EMBO J. 11, 2337–2344.
