Recombinant Expression of the MAL Proteolipid, a Component of Glycolipid-enriched Membrane Microdomains, Induces the Formation of Vesicular Structures in Insect Cells*

(Received for publication, March 24, 1997, and in revised form, May 7, 1997)

Rosa Puertollano‡§, Shengwen Li¶, Michael P. Lisanti†‡‡, and Miguel A. Alonso‡ ‡‡

From the §Centro de Biología Molecular “Severo Ochoa,” Universidad Autónoma de Madrid, Consejo Superior de Investigaciones Científicas, Cantoblanco, 28049 Madrid, Spain and ¶The Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142-1479

The MAL proteolipid has been identified as a component of glycolipid-enriched membrane microdomains resistant to detergent solubilization in epithelial Madin-Darby canine cells, as well as in T lymphocytes and in myelin-forming cells. To study the function of the MAL proteolipid we have ectopically expressed a tagged form of MAL in both mammalian and insect cellular backgrounds. Immunofluorescence analysis in transiently transfected COS-7 cells showed the presence of MAL in large vesicular structures, and biochemical analysis identified MAL in the fraction of membranes resistant to Triton X-100 solubilization. Electron microscopic analysis showed that the expression of MAL in SF21 cells morphologically resulted in the intracellular accumulation of large vesicles with a diameter from 200 to greater than 700 nm that were absent in uninfected or control infected cultures. Thus, ectopic expression of MAL in this heterologous expression system was sufficient to drive the formation of vesicles with a size similar to that of the vesicles detected in mammalian cells. These vesicles were clearly different from the caveolae-like vesicles induced by caveolin expression, as evidenced by co-infection experiments using a recombinant caveolin baculovirus. Taken together, these results suggest that the MAL proteolipid might play a role as a component of the machinery of vesiculation of glycolipid-enriched membranes.

Protein recruitment plays a role in a number of cellular processes including adhesion, signal transduction, and protein transport. The confinement of certain proteins into specialized membrane microdomains resistant to nonionic detergent (i.e. Nonidet P-40, Triton X-100) solubilization is emerging as one of the mechanisms used by the cell to recruit specific proteins (1–3). The high content in both glycolipids and cholesterol makes these microdomains insoluble in detergent (4). In epithelial Madin-Darby canine kidney (MDCK) cells, insoluble membranes have been found at the trans-Golgi network (TGN) and the plasma membrane (3, 5). It has been proposed that the membrane microdomains at the TGN are involved in the transport of glycosylphosphatidylinositol-anchored proteins, a limited number of transmembrane proteins, and glycolipids to the apical surface (1). According to this model, the self-association of glycolipids and cholesterol would form the biophysical basis for formation of the insoluble microdomains. However, to be operative as a route of transport, the glycolipid-enriched microdomains require protein sorting machinery that would minimally consist of a set of proteins to achieve the processes of vesicle formation, cargo recruiting, targeting, and fusion to the apical surface (1). The MAL cDNA was initially identified during a search for genes selectively expressed during T cell development (6). The MAL gene is present in human chromosome 2 (7) and is organized into four exons, each of which encodes a hydrophobic segment and an adjacent short hydrophilic sequence (8). The MAL protein displays unusual lipid-like properties that render MAL soluble in the organic solvents commonly used to extract cell lipids (9). This feature allowed the assignment of MAL to the proteolipid group, which includes other proteins displaying similar lipid-like characteristics (10). More recently, despite the restricted pattern of MAL gene transcription, MAL expression has also been detected in epithelial MDCK cells (11) and during the maturation of myelin-forming cells (12). Thus, MAL gene expression is both tissue- and differentiation-specific and appears to be modulated by elements distal to its 5′-proximal promoter region (13). In all of the cell types in which is expressed, MAL has been identified as a component of detergent-insoluble membrane microdomains (11, 12, 14). This fact, the identification of MAL in TGN-related vesicles in epithelial cells (14), and its predominance in apical transport vesicles in MDCK cells (11) have led to the proposal that MAL is a component of the protein sorting or vesiculation machinery of the glycolipid-mediated pathway of transport (11, 14).

Here, we have approached the study of MAL function using overexpression of MAL in both COS-7 cells and SF21 insect cells. Transient expression of MAL in COS-7 cells, as in other cell lines (11, 14), produces the accumulation of MAL in large vesicles. In this work, we have adopted the baculovirus expression system to study the possible vesiculation induced by MAL

*Present address: Dept. of Molecular Pharmacology, The Albert Einstein College of Medicine, 1300 Morris Park Ave., The Bronx, NY 10461.
**Recipient of a predoctoral fellowship from the Comunidad de Madrid.
†Recipient of NCI, National Institutes of Health, Postdoctoral Fellowship CA-71326.
‡‡Recipient of a postdoctoral fellowship from the Comunidad de Madrid.
‡‡‡Recipient of a postdoctoral fellowship from the Elsa U. Pardee Foundation (to M. P. L.), a grant from the W. M. Keck Foundation to the Whitehead Fellows Program (to M. P. L.), and a grant from the W. M. Keck Foundation to the Whitehead Institute Program (to M. P. L.), and the American Institute of Cancer Research (to M. P. L.). This work was supported in part by grants from the Comisión Interministerial de Ciencia y Tecnología PB93-0175 and PM96-0004 (to M. A. A.), a grant from Comunidad de Madrid (to M. A. A.), National Institutes of Health FIRST Award GM-50443 (to M. A. A.), a grant from the Whitehead Institute Program (to M. A. A.), National Institutes of Health FIRST Award GM-50443 (to M. A. A.), a grant from the W. M. Keck Foundation to the Whitehead Fellows Program (to M. P. L.), and an institutional grant to Centro de Biología Molecular “Severo Ochoa” from Fundación Ramón Areces. 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 1754 solely to indicate this fact.

The abbreviations used are: MDCK, Madin-Darby canine kidney; mAb, monoclonal antibody; Mes, 4-morpholineethanesulfonic acid; PBS, phosphate-buffered saline; TGN, trans-Golgi network.
expression. Similar to the case in COS-7 cells, the expression of the MAL proteolipid in Sf21 insect cells induced the de novo formation of numerous intracellular vesicles ranging in size from 200 to more than 700 nm that almost completely filled the cytoplasm of the infected cells. These vesicles were clearly different from the caveolae-like vesicles induced by caveolin expression (15), as evidenced by co-infection experiments. The interaction of MAL with glycolipid-enriched membranes and its ability to generate extensive vesiculation suggest that MAL might be involved in vesiculation of the glycolipid-enriched microdomains.

**EXPERIMENTAL PROCEDURES**

**Materials**—The mouse hybridoma producing mAb 9E10 (IgG1) against the human c-Myc epitope EQKLISEED (16) was purchased from the American Type Culture Collection. Peroxidase-conjugated antibodies were from Pierce. Texas Red-conjugated antibodies were from Southern Biotech. Octyl glucoside and Triton X-100 were from Sigma. The baculovirus expression kit for recombinant baculovirus production was from CLONTECH.

**Cell Culture Conditions**—COS-7 cells were grown at 37 °C in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% (v/v) fetal bovine serum (Life Technologies), penicillin (50 units/ml), and streptomycin (50 μg/ml) in an atmosphere of 5% CO2, 95% air. Insect Spodoptera frugiperda (Sf21) cells were provided by Drs. Takashi Okamoto and Ikuo Nishimoto (Massachusetts General Hospital/Harvard Medical School). Sf21 cells were grown at 27 °C in TC100 medium (Life Technologies) containing 10% fetal bovine serum (Life Technologies), penicillin (50 units/ml), and streptomycin (50 μg/ml).

**DNA Constructions and Transfections**—The human MAL cDNA was tagged at its COOH terminus with sequences encoding the c-Myc epitope and a polyhistidine (His7) tag by the polymerase chain reaction product was subcloned into the pBacPAK9 transfer plasmid. A mixture of 2 μg of recombinant plasmid pBacPAK-MAL and 1 μg of purified engineered baculovector viral vector DNA BacPa64 linearized by Bsu36I digestion were co-transfected into Sf21 cells using Lipofectin-based standard protocols (Life Technologies, Inc.). Four days later, culture supernatants were removed and centrifuged at low speed. Clarified supernatants containing wild-type and recombinant baculoviruses were plaque-assayed on Sf21 cell monolayers. Occlusion-negative Clarified supernatants containing wild-type and recombinant baculovirus stock for subsequent experiments.

**Characterization of MAL Expression in Sf21 Cells Using the Baculovirus System**—The expression of MAL protein in the baculovirus system was evaluated by SDS-polyacrylamide gel electrophoresis and Western blot analysis. Samples were separated by 15% acrylamide gels under reducing conditions and transferred to Immobilon-P membranes (Millipore). After blocking with 5% (w/v) nonfat dry milk, 0.05% (v/v) Tween-20 in phosphate-buffered saline (PBS), blots were incubated with mAb 9E10 culture supernatant at a ratio of 1:2 for 1 h, washed several times, and incubated with goat anti-mouse IgG antibodies coupled to horseradish peroxidase diluted at 1:5,000 in PBS/Tween 20. Blots were developed using an enhanced chemiluminescence Western blotting kit (ECL, Amersham).

**Immunofluorescence Microscopy**—COS-7 cells grown on coverslips were washed with PBS, fixed in 4% (w/v) paraformaldehyde in PBS for 15 min, rinsed, and treated with 10% glycerine in PBS for 10 min to quench the aldehyde groups. The cells were permeabilized with 0.2% (v/v) Triton X-100 for 10 min, rinsed, and incubated with 3% (w/v) bovine serum albumin for 20 min. Coverslips were then incubated with 9E10 mAb culture supernatant for 1 h, rinsed several times, and incubated for 1 h with goat anti-mouse IgG antibodies conjugated to Texas Red used at 1:1,000. After washing, the coverslips were mounted on slides. The cells were photographed with a Zeiss Axioskop photomicroscope using Kodak T-Max 400 film.

**Transmission Electron Microscopy**—Samples were fixed with glutaraldehyde, postfixed with osmium tetroxide, and stained with uranyl acetate and lead citrate, as detailed by Sargiacomo et al. (17) and Lisanti et al. (18).

**Detergent Extraction Procedures**—Triton X-100-insoluble complexes were separated by centrifugation to equilibrium in sucrose density gradients essentially as described by Brown and Rose (5) and Sargiacomo et al. (17). Cells grown to confluence in 100-mm dishes were rinsed with PBS and lysed with 1.8 ml of 25 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100 at 4 °C. The lysate was scraped from the dishes with a rubber policeman, homogenized by passing the sample through a 22-gauge needle, brought to 40% sucrose (w/v) in a final volume of 4 ml, and finally placed at the bottom of an 8-ml 5–30% linear sucrose gradient. Gradients were centrifuged for 15–18 h at 39,000 rpm at 4 °C in an SW41 rotor (Beckman Instruments). Fractions of 1 ml were collected from the bottom of the tube, and aliquots were subjected to immunoblot analysis.

**Velocity Gradient Centrifugation**—For the estimation of the oligomerization state of recombinant MAL, samples were loaded at the top of a 5–40% linear sucrose gradient (4.3 ml) prepared in 25 mM Mes, pH 6.5, 150 mM NaCl buffer, and 60 mM octyl glucoside. After centrifugation at 50,000 rpm for 10 h in an SW60 rotor (Beckman Instruments), the gradient was fractionated from the top. Aliquots of the different fractions were subsequently subjected to immunoblot analysis using 9E10 mAb. Molecular mass standards (Sigma) were as follows: carbonic anhydrase (29 kDa), bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), α-amylase (200 kDa), and apoferritin (443 kDa).

**RESULTS**

**Expression of Tagged MAL Protein in COS-7 and Sf21 Cells**—The MAL proteolipid has been found as a component of detergent-insoluble membranes in MDCK cells (11), oligodendrocytes (12), and T lymphocytes (14). Transient expression of the MAL protein in mammalian cells produces the accumulation of the MAL proteolipid in large vesicular structures containing membranes resistant to detergent solubilization (11, 14). We adopted a baculovirus system to express the MAL protein tagged at its COOH terminus with a c-Myc epitope and a polyhistidine tag in Sf21 insect cells. This expression system was used with the double aim of (i) studying the possible vesiculation induced by MAL expression and (ii) purifying MAL protein for in vitro experiments. Our current model for MAL structure (8) as well as a schematic of the tagged protein are shown in Fig. 1.

Although previous studies have established that tagging with either c-Myc or polyhistidines does not generally interfere with the normal properties of proteins, we first analyzed the incorporation of the tagged MAL protein into detergent-insoluble membranes by centrifugation to equilibrium and determined its presence in vesicular structures by immunofluorescence analysis. Transiently transfected COS-7 cells were extracted in 1% Triton X-100 at 4 °C, and the extracts were subjected to centrifugation to equilibrium on sucrose density

**FIG. 1.** Schematic representation of the tagged MAL protein used for COS-7 and Sf21 cell overexpression. The top panel shows our current model for the MAL protein (8). The bottom panel shows the localization of the c-Myc and polyhistidine tags introduced into the MAL molecule.
gradients to separate lipid-rich resistant membranes from the Triton X-100-soluble material (5). After gradient fractionation, aliquots from the different fractions were subjected to immunoblot analysis using 9E10 mAb. Fig. 2 shows that MAL was confined to the low density “floating” fractions (fractions 5–12), in agreement with the reported presence of MAL in detergent-insoluble membranes (11, 12, 14). To analyze the doubly tagged MAL protein, COS-7 cells were observed by immunofluorescence at 24 h post-transfection. Fig. 3 shows that MAL overexpression caused the accumulation of the tagged protein along the exocytic pathway and in large vesicles at the cells’ periphery.

To express the doubly tagged MAL protein in insect SF21 cells, a recombinant baculovirus was generated by standard techniques (19). A band of the corresponding size of the tagged protein was specifically detected in the infected samples when cell extracts from uninfected and infected cells were analyzed by immunoblotting with 9E10 mAb (Fig. 4), demonstrating that the protein was efficiently expressed in SF21 cells using the baculovirus system.

Expression of the MAL Proteolipid in SF21 Cells Induces de Novo Formation of Large Intracellular Vesicles—Baculovirus-driven expression of caveolin has been successfully used to generate caveolae-like structures in the cytoplasm of SF21 insect cells (15). We have used this system to address whether expression of MAL can induce vesicles as well. Electron microscopic analysis of uninfected cells (Fig. 5A) or cells infected with a baculovirus expressing control proteins (not shown) did not show any remarkable difference in their cytoplasmic vesicle content. However, MAL expression induced a massive de novo production of large vesicles in the cytoplasm of the infected cultures (Fig. 5, B–E). More than 100 vesicles were counted, and their sizes were determined. Fig. 5F shows that the range of vesicle size was from 200 to more than 700 nm in diameter. Quantitative analysis indicated that the major group (36% of the total number of vesicles examined) includes vesicles with a diameter of ~300–400 nm, whereas 80% of the vesicles have a diameter of ~200–600 nm.

Co-expression of MAL and Caveolin in SF21 Cells Produces Different Populations of Vesicles—MAL and caveolin are two protein candidates to be elements of the vesicular machinery for protein sorting, operating in the glycolipid-mediated route of transport (11, 20). In addition, caveolin appears to function in the organization of caveolar architecture (15, 21–23). Whereas caveolin expression in SF21 cells induced the formation of vesicular structures with a diameter of 50–100 nm resembling intracellular caveolae (15), we have shown here that MAL expression induces much larger vesicles. To investigate whether there is any effect on the morphology of their respective vesicles under condition of simultaneous expression, we examined the cytoplasm of cells co-infected with recombinant viruses expressing MAL, caveolin-1α, and caveolin-1β. Fig. 6 shows the simultaneous presence in these cells of two different populations of vesicles: large ones with a size corresponding to those induced by MAL and small ones corresponding to the intracellular caveolae-like vesicles induced by caveolin (15). This suggests that the vesiculation induced by MAL and caveolin are two separate processes.

Oligomerization of Recombinant MAL in SF21 Cells—Caveolin is known to form large homo-oligomers in both mammalian (24, 25) and insect cells (15). To address whether MAL is also able to form homo-oligomers, we assessed the oligomeric state of recombinant MAL in insect cells by employing an established velocity gradient system (24). Fig. 7 indicates that, in contrast to caveolin, MAL does not form large oligomers, although dimers, trimers, and even tetramers were detectable. Similar results were obtained in stably transfected epithelial cells (not shown). This indicates that the generation of vesicles induced by MAL expression does not require the formation of large MAL homo-oligomers.

**DISCUSSION**

The identification of the components of the protein machinery involved in vesicle budding, transport, and fusion is a major...
focus in modern cell biology (26, 27). Especially challenging is the study of the mechanisms of vectorial transport in epithelial cells in which apical and basolateral plasma membrane proteins are sorted at the TGN by inclusion into separate vesicular carriers (28, 29). It has been proposed that one of the routes to vectorial transport to the apical surface. The induction of two different types of vesicles in insect cells (15). MAL, which is mainly located in apical vesicles has the potential to observe a great magnification of the effects produced by the protein; these effects are sometimes difficult to detect with lower levels of protein expression. A second advantage of this system is that it allows the analysis of the effects of ectopic protein expression in a nonmammalian protein background. To study the possible formation of vesicles induced by MAL expression, we developed an experimental system in which the human MAL proteolipid has been overproduced in Sf21 cells by using a baculovirus-based vector. Similar to the results of transient expression experiments in mammalian cells, MAL overexpression in the infected insect cells resulted in a large number of intracellular vesicles ranging in size from 200 to more than 700 nm in diameter. These vesicles were absent in infections with control baculoviruses as evidenced by electron microscopy analysis.

Caveolin is preferentially located in mammalian cells in specialized invaginations of the plasma membrane called caveolae (21) and consistently induces the formation of caveolae-like vesicles in insect cells (15). MAL, which is mainly located in TGN-derived vesicular structures (14), produces large vesicles in insect cells clearly different from those induced by caveolin as shown in co-infection experiments. It is plausible that both caveolin and MAL may belong to the vesicular transport machinery specific for the glycolipid-enriched microdomains but acting in the generation of different classes of vesicular carriers. Thus, it is possible that more that one glycolipid-mediated route of transport can take place in the cell. For instance, caveolin-induced vesicles appear related to specific transport to caveolae, whereas MAL-induced vesicles might be involved in transport to the apical surface. The induction of two different types of vesicles by the simultaneous ectopic expression of MAL and caveolin in insect cells is in agreement with our recent results showing segregation of MAL and caveolin into distinct

FIG. 5. Transmission electron microscopy of Sf21 cells expressing recombinant MAL. A, a mock-infected Sf21 cell. Bar, 200 nm. B, an Sf21 cell infected with the recombinant MAL baculovirus. Bar, 500 nm. Note that this cell has accumulated hundreds of large vesicles that are absent in mock-infected cells. C, D, and E, higher magnification views of an Sf21 cell expressing MAL. Bars represent 500 nm (in C) or 200 nm (in D and E). F, quantitation of the size distribution of MAL-induced vesicles in Sf21 cells. The diameters of over 100 vesicular profiles were measured and grouped into intervals of 100 nm, and their frequency was tabulated.

FIG. 6. Transmission electron microscopy analysis of Sf21 cells co-expressing MAL and caveolin-1α and β. A, an Sf21 cell co-infected with recombinant baculoviruses expressing MAL, caveolin-1α, or caveolin-1β. Bar, 500 nm. B, a higher magnification of a cell co-expressing MAL and both caveolin-1 isoforms. Bar, 200 nm. Note the simultaneous existence of small vesicles corresponding to caveolae-like structures documented previously (15) and of large vesicles with sizes corresponding to the vesicles induced by MAL expression.

FIG. 7. MAL forms small homo-oligomers in insect cells. Sf21 cells were infected with recombinant MAL baculovirus, and after 72 h of infection cells were lysed in the presence of 60 mM octyl glucoside to solubilize glycolipid-enriched membranes (5), and the lysate was loaded at the top of a 5–40% sucrose gradient containing octyl glucoside and subjected to velocity centrifugation for 10 h. After fractionation from the top of the gradient, aliquots were analyzed by immunoblotting with mAb 9E10. The migration of molecular mass standards was as indicated.
lipid microenvironments in MDCK cells (33).

Caveolin expression in insect cells induces intracellular caveolae-like vesicles but not surface caveolae (15). One interpretation of these findings is that these vesicles probably represent transient intermediates on the way toward fusion with the plasma membrane to form plasma membrane-attached caveolae. Thus, other factors absent in Sf21 cells may exist to connect these putative precursors to the plasma membrane. Similarly, MAL-induced vesicles in Sf21 cells were also intracellular, and we did not detect any fusion event between the vesicles and the plasma membrane. This suggests that the machinery for fusion of MAL-induced vesicles with the plasma membrane is not also operative in Sf21 cells. However, the heterogeneous size of the vesicles induced by MAL expression suggests that homotypic fusion events might occur, generating larger vesicles.

It has been shown that caveolin is found in mammalian cells as large homo-oligomers of up to ~400 kDa (24, 25). These oligomerization properties were preserved in insect cells expressing recombinant caveolin (15). On the contrary, the analysis of the oligomeric state showed that MAL does not form large oligomers but that it can be found as dimers, trimers, and even tetramers in both mammalian and insect cells. The interaction of MAL with itself and with selected endogenous lipid components might provide the basis for membrane vesiculation.

In summary, based on the specific presence of MAL in transport vesicles containing detergent-insoluble membranes (11, 14), MAL has been proposed to play a role as a component of the transport machinery of the glycolipid-mediated pathway. The results presented in this work showing the induction of extensive vesicle formation by MAL expression in insect cells are consistent with a role for MAL in vesiculation of glycolipid-enriched microdomains.

Acknowledgments—We thank members of Dr. Lisanti’s and Dr. Alonso’s laboratories for encouragement and helping discussions. We also thank Ya-Huei Tu (Whitehead Institute) for electron microscopy.

REFERENCES
1. Simons, K., and Wandinger-Ness, A. (1990) Cell 62, 207–210
2. Lisanti, M. P., Scherer, P., Tang, Z.-L., and Sargiacomo, M. (1994) Trends Cell Biol. 4, 231–235
3. Parton, R. G., and Simons, K. (1995) Science 269, 1388–1399
4. Hanada, K., Nishijima, M., Akamatsu, Y., and Pagano, R. E. (1995) J. Biol. Chem. 270, 6265–6268
5. Brown, D. A., and Rose, J. H. (1992) Cell 70, 533–544
6. Alonso, M. A., and Weissman, S. M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1997–2001
7. Alonso, M. A. Barton, D. E., and Francke, U. (1988) Immunogenetics 27, 91–95
8. RancanÒ, C., Rubino, T., and Alonso, M. A. (1994) Genomics 21, 447–450
9. RancanÒ, C., Rubino, T., Correas, I., and Alonso, M. A. (1994) J. Biol. Chem. 269, 14159–14164
10. Schlesinger, M. J. (1981) Annu. Rev. Biochem. 50, 193–206
11. Zacchetti, D., Pera, J., Murata, M., Fiedler, K., and Simons, K. (1995) FEBS Lett. 377, 465–469
12. Kim, T., Fiedler, K., Madison, D. L., Krueger, W. H., and Pfeiffer, S. E. (1995) J. Neurosci. Res. 42, 413–422
13. Tugores, A., Rubino, T., RancanÒ, C., and Alonso, M. A. (1997) DNA Cell Biol. 16, 245–255
14. Millan, J., Puertollano, R., Fan, L., RancanÒ, C., and Alonso, M. A. (1997) Biochem. J. 321, 247–252
15. Li, S., Song, K. S., Koh, S. S., Kikuchi, A., and Lisanti, M. P. (1996) J. Biol. Chem. 271, 28847–28854
16. Evan, G. I., Lewis, G. K., Ramsay, G., and Bishop, J. M. (1985) Mol. Cell. Biol. 5, 3610–3616
17. Sargiacomo, M., Sudd, M., Tang, Z., and Lisanti, M. P. (1993) J. Cell Biol. 122, 789–807
18. Lisanti, M. P., Scherer, P. E., Vidugiriene, J., Tang, Z.-L., Hermanskovic, V., Tu, Y. H., Cook, R. F., and Sargiacomo, M. (1994) J. Cell Biol. 126, 111–126
19. King, L. A., and Possee, R. D. (1992) The Baculovirus Expression System: A Laboratory Guide, Chapman and Hall, New York
20. Kurzchalia, T. V., Dupree, P., Parton, R. G., Kellner, R., Virta, H., Lehner, M., and Simons, K. (1995) J. Cell Biol. 121, 1003–1014
21. Rothberg, K. G., Heuser, J. E., Donzell, W. C., Ying, Y.-S., Glenney, J. R., and Anderson, R. G. W. (1992) Cell 68, 673–682
22. Dupree, P., Parton, R. G., Raposo, G., Kurzchalia, T. V., and Simons, K. (1993) EMBO J. 12, 1597–1605
23. Fra, A. M., Williamson, E., Simons, K., and Parton, R. G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8655–8659
24. Sargiacomo, M., Scherer, P. E., Tang, Z.-L., Kubler, E., Song, K. S., Sanders, M. C., and Lisanti, M. P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9407–9411
25. Monier, S., Parton, R. G., Vogel, F., Behlke, J., Henske, A., and Kurzchalia, T. V. (1995) Mol. Biol. Cell 6, 911–927
26. Rothman, J. E. (1994) Nature 372, 55–63
27. Rothman, J. E., and Wieland, F. T. (1996) Science 272, 227–229
28. Rodriguez-Boulan, E., and Powell, S. K. (1992) Annu. Rev. Cell Biol. 8, 395–427
29. Matter, K., and Mellman, I. (1994) Curr. Opin. Cell Biol. 6, 545–554
30. Wandianger-Ness, A., Bennett, M. K., Antony, C., and Simons, K. (1990) J. Cell Biol. 111, 987–1000
31. Fiedler, K., Parton, R. G., Kellner, R., Etzold, T., and Simons, K. (1994) EMBO J. 13, 1729–1740
32. Fiedler, K., Lafont, F., Parton, R. G., and Simons, K. (1995) J. Cell Biol. 128, 1043–1053
33. Millan, J., Puertollano, R., Fan, L., and Alonso, M. A. (1997) Biochem. Biophys. Res. Commun. 233, 707–712