Role of Lipid Modifications in Targeting Proteins to Detergent-resistant Membrane Rafts

MANY RAFT PROTEINS ARE ACYLATED, WHILE FEW ARE PRENYLATED*

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Sphingolipid and cholesterol-rich Triton X-100-insoluble membrane fragments (detergent-resistant membranes, DRMs) containing lipids in a state similar to the liquid-ordered phase can be isolated from mammalian cells, and probably exist as discrete domains or rafts in intact membranes. We postulated that proteins with a high affinity for such an ordered lipid environment might be targeted to rafts. Saturated acyl chains should prefer an extended conformation that would fit well in rafts. In contrast, prenyl groups, which are as hydrophobic as acyl chains but have a branched and bulky structure, should be excluded from rafts. Here, we showed that at least half of the proteins in Madin-Darby canine kidney cell DRMs (other than cytoskeletal contaminants) could be labeled with [3H]palmitate. Association of influenza hemagglutinin with DRMs required all three of its palmitoylated Cys residues. Prenylated proteins, detected by [3H]mevalonate labeling or by blotting for Rap1, Rab5, Gp, or Ras, were excluded from DRMs. Rab5 and H-Ras each contain more than one lipid group, showing that hydrophobicity alone does not target multiply lipid-modified proteins to DRMs. Partitioning of covalently linked saturated acyl chains into liquid-ordered phase domains is likely to be an important mechanism for targeting proteins to DRMs.

Increasing evidence suggests that cholesterol and sphingolipid-rich lipid microdomains or rafts exist in eukaryotic cell membranes and have important functions there (1–3). These rafts are likely to be important in the structure and function of caveolae, plasma membrane invaginations that are implicated in signal transduction (4, 5), endocytosis (6), transcytosis across endothelial cells (7, 8), and cholesterol trafficking (9–11). However, rafts are not restricted to caveolae (2, 3, 12) and recent evidence suggests that they act in signal transduction in cells that lack distinct caveolae, such as T lymphocytes (13–16) and basophils (17–19). Rafts have also been implicated in protein and lipid sorting in the secretory and endocytic pathways (1, 20–22).

Cholesterol and sphingolipid-rich detergent-resistant mem-

branes (DRMs)† can be isolated from mammalian cells (23). DRM lipids are in a state similar to the liquid-ordered (l0) phase (3, 24–26). The l1 phase, which requires cholesterol to form, is favored by lipids like sphingolipids, whose long saturated acyl chains give them a high degree of order and a high acyl-chain melting temperature (3). Acyl chain order explains the detergent-insolubility of DRMs (3). We hypothesize that DRMs are an in vitro correlate of rafts in intact membranes. It is important to note that detergent insolubility can underestimated the association of proteins and lipids with the l1 phase; some proteins and lipids that are in rafts can be solubilized (25). Nevertheless, DRM association provides a powerful tool for identifying molecules that are likely to have a high affinity for rafts.

DRMs isolated from cells contain a number of proteins (27–29) which are undoubtedly crucial for the function of the domains in vivo. For this reason, it is important to determine how proteins associate with DRMs. Three DRM targeting signals have been defined. First, glycosylphosphatidylinositol (GPI)-anchored proteins are targeted to DRMs through acyl chain interactions (23–25, 30). An N-terminal Met-Gly-Cys motif that is present in some 5′r family kinases and heterotrimeric G protein α subunits, in which Gly is myristoylated and Cys is palmitoylated, can also serve as a DRM targeting signal (31, 32). Third, dual palmitoylated Cys residues are required for raft association of the T cell adaptor protein LAT (15) and the neuronal protein GAP-43 (33).

The finding that DRM lipids are in an l1-like phase suggests a unifying mechanism for targeting of proteins to DRMs. Proteins with a high affinity for the ordered lipid environment of the l1 phase might spontaneously partition into the domains. In agreement with this model, all three of the DRM targeting signals listed above contain two closely spaced acyl chains. Myristate and palmitate, as well as most of the acyl chains on GPI-anchored proteins (34) are saturated, and thus should fit well into ordered lipid domains. This suggests that acylation, especially multiple acylation, may be a general DRM targeting signal.

Alternatively, however, it might be imagined that lipid modifications could target proteins to DRMs simply through hydrophobic interactions. Both models predict that many DRM proteins would be linked to lipids. The behavior of prenylated proteins should distinguish between the models, because pre-

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The abbreviations used are: DRMs, detergent-resistant membranes; MDCK, Madin-Darby canine kidney; GPI, glycosylphosphatidylinositol; PLAP, placental alkaline phosphatase; ECL, enhanced chemiluminescence; PAGE, polyacrylamide gel electrophoresis; HA, influenza hemagglutinin.
nyl groups are as hydrophobic as acyl chains, but have a bulky branched structure that should not fit well into the l1 phase. Because of the possibility that multiple hydrophobic modifications are required for DRM targeting, the behavior of dually or multiply lipid-modified proteins that are prenylated is especially informative in this regard. To test these models, we examined the lipid modifications of DRM proteins and the DRM association of prenylated proteins.

**EXPERIMENTAL PROCEDURES**

**Materials**—Mouse monoclonal anti-Rab5 antibodies were the gift of A. Wandinger-Ness or were from Transduction Laboratories (Lexington, KY). Mouse monoclonal anti-Rap1, anti-Ras, and anti-caveolin antibodies (Ig fraction) were from Transduction Laboratories. Rabbit polyclonal anti-Influenza hemagglutinin (HA) antibodies (35) were used. Rabbit anti-Gαi, antibody B960, against a C-terminal Gαi peptide, (36) was the gift of S. Mumby. Mouse monoclonal anti-transferrin receptor antibodies were a gift of I. Trowbridge. Rabbit polyclonal antibodies to p62+ (Yes) were generated by immunization of rabbits with a TrpE-Yes fusion protein as described (37). A purified Ig fraction was obtained using an immobilized Protein A column according to instructions from the supplier (Pierce, Rockville, IL). Rabbit polyclonal anti-transferrin was from Dako (Carpinteria, CA). Horseradish peroxidase-conjugated goat anti-mouse IgG (and M) was from Jackson Labs (West Grove, PA), and horseradish peroxidase goat anti-rabbit IgG was from Sigma. The enhanced chemiluminescence (ECL) reagent and Amplify (fluorography enhancement reagent) were from Amersham. Amphoteries were from Bio-Rad and prestained protein molecular weight standards were from Bio-Rad or Life Technologies (Gaithersberg, MD). EXPRE+35S+35S+35S protein labeling mixture (>1000 Ci/mmol; referred to as "[35S]methionine") was from NEN Life Science Products (Boston, MA). (Labeling media for MDCK strain II (40), COS-1 (33), and CV1 cells (35) were maintained as described previously. MDCK cells stably expressing PLAP (detected without butyrate induction) have been described (35). Briefly, oligonucleotide-directed mutagenesis was performed using a Bio-Rad GS-670 imaging densitometer as follows: 3% of protein in DRMs was performed using a Bio-Rad GS-670 imaging densitometer as follows: % of protein in DRMs = cpm in DRMs (gradient fractions 9–12)/ cpm in lysate (fractions 1–5) + cpm in DRMs. Although only the region of the blot showing the protein of interest is shown, no other bands were present. Two-dimensional gel electrophoresis, using non-equilibrium pH gradient electrophoresis in the first dimension and SDS-PAGE in the second dimension, was performed according to Jones (49, 50) after precipitation of proteins with acetone. In some cases, radiolabeled proteins on two-dimensional gels were transferred to nitrocellulose. After detection of Yes or caveolin by Western blotting, the ECL signal was quenched with 0.05% sodium azide. Blots were then air dried, sprayed with Amplify, and exposed to film to detect the [35S]methionine signal.

**RESULTS**

**Acylated Proteins in DRMs**—To determine how many DRM proteins from MDCK cells were palmitoylated, cells were incubated with [3H]palmitic acid before preparation of DRMs. For comparison, DRMs were also prepared from cells incubated with [35S]methionine to metabolically label all proteins. DRM proteins were separated by SDS-PAGE and visualized by fluorography (Fig. 1). As previously reported (29), about 20–25 major proteins were observed (lane 1). Many of the proteins were labeled with palmitate (lane 2). The same pattern of [3H]palmitate-labeled proteins was observed in DRM prepared from cells without sodium carbonate at pH 7.5 (data not shown). In initial experiments, we also labeled cells with [3H]myristate using a similar protocol, in an attempt to visualize N-myristoylated proteins in DRMs. Many of the proteins that were labeled with [3H]palmitate also incorporated [3H]myristate (not shown). However, S-acylated proteins can be labeled biochemically with [3H]myristate (51), and we were unable to

**Preparation of Total Cell Membranes and DRMs**—For total cell membranes, cells were scraped from dishes, washed in phosphate-buffered saline (150 mM NaCl, 20 mM sodium phosphate, pH 7.4) and then in hypotonic buffer (10 mM Hepes, pH 7.4, 0.5 mM EDTA), resuspended in 1 ml of hypotonic buffer, and broken by passage through a 25-gauge needle 40 times. Nuclei and debris were removed by centrifugation at 3000 x g for 10 min at 4 °C, and light membranes were collected directly in gel loading buffer for analysis by SDS-PAGE. For DRMs, cells in 1 confluent 10-cm dish were lysed in 1 ml of TNE buffer (25 mM Tris-Cl, pH 7.5, 150 mM NaCl, 5 mM EDTA) containing 1% Triton X-100 (TNE/Triton X-100), and DRMs were isolated by flotation on sucrose gradients as described (40). Unless otherwise indicated, the lysate buffer and all sucrose gradient solutions were adjusted to pH 11 with NaOH. In most cases, the floating membrane band was harvested and diluted to about 12 ml with TNE. Membranes were harvested by centrifugation for 1 h at 120,000 x g. Alternatively, where indicated 1-ml fractions were collected from the bottom of the sucrose gradient with an isco (Lincoln, NB) Model 185 density gradient fractionator. All buffers were ice-cold and contained the following protease inhibitors: 0.5 µg/ml leupeptin, 0.7 µg/ml pepstatin, and 0.2 mM phenylmethylsulfonyl fluoride.
to show that [3H]myristate labeling of DRM proteins was specific. For this reason, these studies were not pursued further.

It was important to verify that the [3H]palmitate label was present as thioester-linked fatty acid. Thus, proteins labeled with either [3H]palmitate or [35S]methionine were separated on the same gel and treated with or without hydroxylamine, which cleaves thioester bonds (52), as shown in Fig. 2. As expected, the [35S]methionine label was resistant to hydroxylamine (compare lanes 1 and 2) while the [3H]palmitate label was removed (compare lanes 3 and 4).

**Not All Acylated Proteins Are in DRMs**—We next determined how many of the palmitoylated proteins in the cell were present in DRMs. Cells were incubated with [3H]palmitate and lysed. DRMs were then prepared after saving a small fraction of the whole cell lysate. Both fractions were analyzed by SDS-PAGE and fluorography (Fig. 3). Very different patterns of labeled proteins were seen in whole cell lysate and DRMs. Thus, only a subset of palmitoylated proteins associated efficiently with DRMs.

**Two-dimensional Gel Analysis of DRM Proteins**—We extended our analysis of DRM proteins using two-dimensional gels, separating [35S]methionine-labeled proteins in the first dimension by non-equilibrium pH gradient electrophoresis, and in the second by size by conventional SDS-PAGE. We first compared total cell proteins (Fig. 4A) with DRM proteins prepared from cells with or without carbonate during detergent extraction (Fig. 4, B and C). For orientation, MDCK cells stably expressing the GPI-anchored protein PLAP were used in this experiment. PLAP was prominent among the DRM proteins (Fig. 4, B and C), and could be detected in the whole cell lysate and DRMs. Thus, only a subset of palmitoylated proteins associated efficiently with DRMs.

Ten proteins that were especially abundant in the whole cell lysate (Fig. 4A, arrows) were detected in DRMs prepared without carbonate (Fig. 4B, asterisks). These are likely to be cytoskeletal or other structural proteins. Consistent with this possibility, fibrous material could sometimes be observed in DRM preparations by electron microscopy (not shown). Most of these proteins were present in lower abundance in DRMs prepared with carbonate (Fig. 4C, asterisks). Actin, the most abundant cellular protein, was among these proteins. Actin could also be detected in DRMs on one-dimensional gels by Western blotting (not shown). Although these abundant proteins may associate specifically with DRMs, it is likely instead that they adhere nonspecifically during DRM preparation.

Finding these 10 abundant proteins in DRMs raised the possibility that most of the spots on our two-dimensional gels were contaminants. Proteins that associated specifically with DRMs, and were enriched there, might be present at such low
levels that they could not be seen. To test this idea, as described next, we identified several proteins that are known to associate specifically with DRMs. Our ability to detect these proteins suggested that many of the other spots also corresponded to bona fide DRM proteins.

We first examined [35S]methionine-labeled GPI-anchored proteins released from DRMs by phosphatidylinositol-specific phospholipase treatment (Fig. 5B). (No proteins were observed when phosphatidylinositol-specific phospholipase was omitted from the reaction (not shown).) These were analyzed in parallel with total [35S]methionine-labeled DRM proteins (Fig. 5A). (For orientation, major cell proteins identified in Fig. 4 are labeled with asterisks.) As found by others (53), GPI-anchored proteins of 50 and 80 kDa could be detected in the whole DRM pattern by alignment of the films. These are labeled GPI in Fig. 5, A and C. We next identified two known DRM proteins on two-dimensional gels by Western blotting. DRM proteins from [35S]methionine-labeled MDCK cells were separated on two-dimensional gels and transferred to nitrocellulose. The positions of the Src family non-receptor tyrosine kinase Yes and of caveolin, a marker for caveolae (4, 54), were determined by Western blotting (not shown). Blots were then exposed to film for detection of all [35S]methionine-labeled DRM proteins. Alignment of the films allowed identification of Yes and caveolin in the [35S]methionine-labeled pattern. Spots corresponding to Yes and caveolin (labeled Cav) are labeled in Fig. 5, A and C. (The caveolin spot is faint because much of the caveolin was in a high molecular weight oligomeric form (55). Oligomeric caveolin was detected by Western blotting, but could not be aligned unambiguously with an [35S]methionine-labeled spot.) Thus, although these proteins are not the most abundant DRM proteins, they are easily detectable, increasing our confidence that many of the unidentified proteins are also specific.

We next separated DRM proteins from MDCK cells labeled with [3H]palmitate (Fig. 5D, contrast enhanced by image processing) or [35S]methionine (Fig. 5C) on parallel two-dimensional gels. The [3H]palmitate-labeled proteins were numbered, both on the [3H]palmitate-labeled gel (Fig. 5D) and the corresponding [35S]methionine-labeled gel (Fig. 5C). The positions of GPI-anchored proteins, Yes, and caveolin were indicated (Fig. 5C). Finally, unidentified [35S]methionine-labeled proteins not labeled with [3H]palmitate were marked with letters (Fig. 5C). As expected, except for faint labeling of a protein of about 30 kDa (Fig. 5C, marked 10,*), the major cell proteins in DRMs were not labeled with [3H]palmitate. Neither could we detect [3H]palmitate labeling of the two GPI-anchored proteins. Although palmitate is present in GPI anchors, the anchors are preassembled and added to proteins en bloc (56). Thus, a 2-h labeling period was probably not sufficient to allow detectable labeling of these proteins. However, in agreement with data from the one-dimensional gels, a large fraction of the other proteins were labeled with [3H]palmitate. Twenty-three palmitoylated proteins (including caveolin) and only 12 non-palmitoylated proteins (other than cytoskeletal or GPI-anchored proteins) are labeled in Fig. 5C, the former with numbers corresponding to [3H]palmitate-labeled proteins in Fig. 5D, and the latter with capital letters. The fact that we did not detect [3H]palmitate labeling of caveolin, although it is triply palmitoylated (57), suggests that other less-abundant DRM proteins might also be palmitoylated, but not detectable in Fig. 5D.

Finding that so many DRM proteins were palmitoylated was...
pared from MDCK cells in two 10-cm dishes labeled with [35S]methionine but not by [3H]palmitate are identified by corresponding [35S]methionine-labeled spots (C) are numbered. Proteins labeled by [35S]methionine but not by [3H]palmitate are identified by capital letters in C. Yes, actin, GPI-anchored proteins, and caveolin (Cav) are indicated. Exposure times: A, 7 days; B, 14 days; C, 4 days; D, 6 months.

consistent with the idea that palmitoylation is a DRM targeting signal. Thus, we next examined several known palmitoylated proteins for DRM association, in order to test the role of palmitoylation in DRM targeting directly. Vesicular stomatitis virus glycoprotein does not associate with DRMs (23, 33). Similarly, we found that endogenous MDCK cell transferrin receptor (another palmitoylated transmembrane protein (58)) was not in DRMs (data not shown). In contrast, influenza HA, which is triply palmitoylated (35), associates with DRMs in several cell types (45, 59, 60). In agreement with this result, we found that 50% of HA expressed in MDCK cells associated with DRMs (not shown).

**DRM Association of Influenza Hemagglutinin Requires Palmitoylated Cys Residues**—To test the role of the palmitoylated Cys residues in DRM targeting, HA proteins mutated in one, two, or all three Cys residues were expressed in CV1 cells. After detergent extraction and separation of soluble and insoluble fractions, HA was recovered from both fractions by immunoprecipitation as described (60), and the percent insoluble was determined. Using this procedure, 29% of wild-type HA was insoluble. The difference between this and the 50% of HA found in DRMs in transiently transfected MDCK cells may reflect cell type or other procedural differences. It is also possible that the exogenously expressed HA was incompletely palmitoylated.) Mutation of any Cys, or any combination of Cys, essentially abolished detergent insolubility (Fig. 6). Thus, all three palmitoylated Cys residues are essential for targeting HA to DRMs.

**Prenylated Proteins in DRMs**—We examined DRM proteins for possible prenylation by incubating MDCK cells with [3H]mevalonate, a precursor of prenyl groups. Cells were then lysed as usual, except that sodium carbonate was omitted and lysis was performed at pH 7.5.

To detect all cellular prenylated proteins, proteins in 10% of the lysate were precipitated with trichloroacetic acid and analyzed by SDS-PAGE (Fig. 7A, WCL). DRMs were isolated from the remaining 90% of the lysate (Fig. 7A, DRM). Although [3H]mevalonate-labeled proteins were easily seen in whole cell lysates, they were virtually undetectable in DRMs.

Most mammalian cells take up mevalonate poorly, making it difficult to detect prenylated proteins by [3H]mevalonate labeling. For this reason, we repeated the experiment shown in Fig. 7A using met-18b-2 cells (42), which express a mutant mevalonate transporter that allows faster uptake of mevalonate (61). Results are shown in Fig. 7B. As expected, [3H]mevalonate labeling was more efficient than in MDCK cells. As was seen for MDCK cell DRMs, very few met-18b-2 cell DRM proteins were labeled.

In contrast to our findings, three prenylated proteins, Rab5 (62), Rap1 (27, 62), and the βy component of heterotrimeric G proteins (63), have been detected by others in DRMs. For this reason, we next examined MDCK cell DRMs for the presence of these proteins. Proteins in total cell membranes or in DRMs prepared from at least 10 times as many cells were separated by SDS-PAGE and transferred to nitrocellulose. Although both Rab5 and Rap1 were easily detected in total cell membranes (Fig. 8, WM), they were barely detected in DRMs (Fig. 8, DRM). Approximately 1% of the total cellular Rap1 was present in DRMs.

The prenylated Gβ subunit is responsible for membrane targeting of the Gβγ complex. 30% of this complex was found in DRMs isolated from a neuroblastoma cell line grown with serum (63). In contrast, very little Gβγ was found in chicken gizzard DRMs (27). We subjected lysates of MDCK cells stably expressing PLAP to sucrose gradient ultracentrifugation, fractionated the gradients, and examined the distribution of Gβγ and PLAP between the Triton-soluble lysate fractions and floating DRMs (Fig. 9A, panels 1 and 2). Although 85% of PLAP was recovered in the DRM fractions, Gβγ was barely detectable.
of the total was in DRMs (Fig. 9). From the remaining 99% of the lysate showed that less than 1% of the protein is DRM targeted. This suggests that although palmitoylation can increase the affinity of proteins for DRMs, this effect is not always strong enough to mediate stable association between proteins and DRMs.

Palmitoylation can also contribute to the overall affinity of the protein for DRMs. Other proteins might be targeted to DRMs indirectly, by binding to more tightly associated proteins or to other lipid modifications. However, not all palmitoylated proteins are DRM targeted. This suggests that acylation is a commonly used signal for DRM targeting. Other proteins might be targeted to DRMs indirectly, by binding to more tightly associated proteins or to other lipid modifications.

Emerging functions for rafts in the structure and function of caveolae, in signal transduction, and in sorting in the secretory and endocytic pathways (1, 2, 5) highlight the importance of determining how lipids and proteins associate with them. If rafts are l phase microdomains, as we propose, then acyl chain order should be a key determinant of their formation. As expected, lipids with saturated acyl chains, whose extended structure fits well into an ordered environment, are enriched in DRMs isolated from cells (23) and model membranes (24, 25). Proteins, too, might be targeted to DRMs by modification with saturated acyl chains. In agreement with his idea, the best defined DRM-targeting signals (GPI anchorage (30, 39), tandem myristoylation and palmitoylation (31, 32), and dual palmitoylation (15, 33) all consist of saturated acyl chains. The role of palmitoylation in targeting proteins to DRMs was tested further in this paper. An important finding of this work is that a high fraction of the proteins in DRMs is acylated. This suggests that acylation is a commonly used signal for DRM targeting.

Several findings suggest that cholesterol and sphingolipid-rich l phase microdomains or rafts can exist in cell membranes and can be isolated as DRMs (1, 3). First, GPI-anchored proteins and gangliosides, which are enriched in DRMs, can exhibit a clustered distribution in the plasma membrane (64–69). Furthermore, the physical properties of DRMs isolated from cells are very similar to those of the l phase (24). In a complementary approach, we demonstrated the plausibility of phase separation in biological membranes by showing that l phase microdomains form spontaneously at 37 °C in liposomes containing physiologically reasonable levels of sphingolipids and cholesterol (26).

**DISCUSSION**

Several findings suggest that cholesterol and sphingolipid-rich l phase microdomains or rafts can exist in cell membranes.
chains should enhance DRM association. By contrast, a membrane-spanning peptide might not pack easily into such an environment. In agreement with this idea, to our knowledge all proteins examined to date that lack membrane-spanning domains but are modified with dual saturated acyl chains are targeted to DRMs. This includes the myristoylated and palmitoylated protein endothelial cell nitric oxide synthase, although it associates with DRMs less efficiently than other such proteins (70).

The “rules” for how palmitoylation can target transmembrane proteins to DRMs are less straightforward. We showed here that dual palmitoylation is not sufficient for targeting of vesicular stomatitis virus glycoprotein G or the transferrin receptor to DRMs, and that three palmitate groups are required to target HA to DRMs. As HA is trimeric, each molecule is modified with a total of nine palmitate chains. This high concentration of saturated acyl chains may be required to overcome packing difficulties and allow efficient targeting to DRMs.

In summary, GPI-anchored proteins, Src family kinases, Caveolin, which binds cholesterol tightly (76) and can induce signals, as at least one protein, caveolin, associates with DRMs (15).

In agreement with this idea, to our knowledge all proteins examined to date that lack membrane-spanning domains but are modified with dual saturated acyl chains are targeted to DRMs through hydrophobic interactions that do not depend on the structure of the lipid. Prenyl groups are hydrophobic, but would not be expected to fit in an lα domain. Thus, the finding that prenylated proteins are excluded from DRMs (71, 72). (CD44 is palmitoylated (73), and the role of this modification appears to be common mechanism of increasing the affinity of proteins for DRMs, and may be the primary targeting mechanism for proteins without membrane spans. As the role of rafts in cellular function is becoming increasingly clear (1, 2, 15, 16), it is becoming increasingly important to understand in molecular detail how proteins and lipids are organized into these domains.

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References

1. Simons, K., and Ikonen, E. (1997) Nature 387, 569–572
2. Brown, D. A., and Landon, E. (1998) Annu. Rev. Cell Dev. Biol. 14, 111–136
3. Brown, D. A., and London, E. (1998) J. Membr. Biol. 164, 103–114
4. Okamoto, T., Schlegel, A., Scherer, P. E., and Lisanti, M. P. (1998) J. Biol. Chem. 273, 5419–5422
5. Anderson, R. G. W. (1998) Annu. Rev. Biochem. 67, 199–225
6. Porton, R. G., Jogerst, B., and Simons, K. (1994) J. Cell Biol. 127, 1199–1216
7. Sznitwer, J. E., Oh, P., Pinney, E., and Allard, J. (1994) J. Cell Biol. 127, 1137–1152
8. Middleton, J., Neil, S., Wintle, J., Clarke-Lewis, I., Moore, H., Lam, C., Anzud, M., and Rot, A. (1997) Cell 91, 385–395
9. Smart, E. R., Ying, Y., Donzelli, W. C., and Anderson, R. G. W. (1996) J. Biol. Chem. 271, 29427–29434
10. Babitt, J., Trigatti, B., Rigotti, A., Smart, E. J., Anderson, R. G. W., Xu, S., and Krieger, M. (1997) J. Biol. Chem. 272, 13242–13249
11. Bist, A., Fielding, P. E., and Fielding, C. J. (1997) J. Biol. Chem. 272, 4723–4732
12. Brown, D. (1993) Curr. Opin. Immunol. 5, 349–354
13. Stulig, T. M., Berger, M., Sigmund, T., Stockinger, H., Horejsi, V., and Waldhaeusl, W. (1997) J. Biol. Chem. 272, 19242–19247
14. Zeng, W., Tribe, R. P., and Samelson, L. E. (1998) Immunity 9, 239–246
15. Xavier, R., Brennan, T., Li, Q., McCormack, C., and Seed, B. (1998) Immunity 9, 723–732
16. Fields, K. A., Holowka, D., and Baird, B. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9201–9205
17. Draherová, L., Amou, M., and Draher, P. (1996) Immunology 87, 141–148
18. Field, K. A., Holowka, D., and Baird, B. (1997) J. Biol. Chem. 272, 4726–4729
19. Lisanti, M. P., Tang, Z., and Sargiacomo, M. (1993) J. Cell Biol. 127, 3966–3971
20. Mayer, S., Sabharanjak, S., and Maxfield, F. R. (1998) EMBO J. 17, 4626–4638
21. Brown, D. A., and Rose, J. K. (1992) Cell 68, 538–544
22. Schroeder, R., London, E., and Brown, D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12130–12134
23. Schroeder, R. J., Ahmed, S. N., Zhu, Y., London, E., and Brown, D. A. (1998) J. Biol. Chem. 273, 1150–1157
24. Ahmed, S. N., Brown, D., and London, E. (1997) Biochemistry 36, 10944–10953
and Shiigi, S. M., eds) pp. 398–439, Freeman, San Francisco, CA

50. Jones, P. P. (1984) Methods Enzymol. 108, 452–466

51. Gu, X., Trigatti, B., Xu, S., Acton, S., Babitt, J., and Krieger, M. (1998) J. Biol. Chem. 273, 26338–26348

52. Pepperberg, D. R., Morrison, D. F., and O’Brien, P. J. (1995) Methods Enzymol. 250, 348–361

53. Fiedler, K., Kobayashi, T., Kurzchalia, T. V., and Simons, K. (1993) Biochemistry 32, 6365–6373

54. Rothberg, K. G., Heuser, J. E., Donzell, W. C., Ying, Y.-S., Glenney, J. R., and Anderson, R. G. W. (1992) Cell 69, 673–682

55. Monier, S., Parton, R. G., Vogel, F., Behlke, J., Henlake, A., and Kurzchalia, T. V. (1995) Mol. Biol. Cell 6, 911–927

56. Doering, T. L., Masterson, W. J., Hart, G. W., and Englund, P. T. (1995) J. Biol. Chem. 265, 611–614

57. Dietzen, D. J., Hastings, W. R., and Lublin, D. M. (1995) J. Biol. Chem. 270, 6838–6842

58. Jing, S. Q., and Trowbridge, I. S. (1987) EMBO J. 6, 327–331

59. Skibbens, J. E., Roth, M. G., and Matlin, K. S. (1989) J. Cell Biol. 108, 821–832

60. Scheiffele, P., Roth, M. G., and Simons, K. (1997) EMBO J. 16, 5501–5508

61. Kim, C. M., Goldstein, J. L., and Brown, M. S. (1992) J. Biol. Chem. 267, 23113–23121

62. Lisanti, M. P., Scherer, P. E., Vidugiriene, J., Tang, Z., Hermanowski-Vosatka, A., Tu, Y.-H., Cook, R. F., and Sargiacomo, M. (1994) J. Cell Biol. 126, 111–120

63. Rehm, A., and Ploegh, H. L. (1997) J. Cell Biol. 137, 305–317

64. Spiegel, S., Kassis, S., Wilchek, M., and Fishman, P. H. (1984) J. Cell Biol. 99, 1575–1591

65. Rothberg, K. G., Ying, Y.-S., Kamen, B. A., and Anderson, R. G. W. (1990) J. Cell Biol. 111, 2931–2938

66. Parton, R. G. (1994) J. Histochem. Cytochem. 42, 155–166

67. Harder, T., Scheiffiele, P., Verkade, P., and Simons, K. (1993) J. Cell Biol. 141, 929–942

68. Varma, R., and Mayor, S. (1998) Nature 394, 798–802

69. Friedrichson, T., and Kurzchalia, T. V. (1996) Nature 394, 802–805

70. Garcia-Cardeza, G., Oh, P., Liu, J., Schnitzer, J. E., and Sessa, W. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6448–6453

71. Neame, S. J., Uff, C. R., Sheikh, H., Wheatley, S. C., and Isacke, C. M. (1995) J. Cell Sci. 108, 3127–3133

72. Perschl, A., Lesley, J., English, N., Hyman, R., and Trowbridge, I. S. (1995) J. Cell Sci. 108, 1033–1041

73. Bourgignon, L. W., Kalomiris, E. L., and Lokeshwar, V. B. (1991) J. Biol. Chem. 266, 11761–11765

74. Zhang, W., Sloan-Lancaster, J., Kitchen, J., Trible, R. P., and Samelson, L. E. (1998) Cell 92, 83–92

75. Farnsworth, C. C., Seabra, M., Ericsson, L. H., Golb, M. H., and Glomset, J. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11963–11967

76. Murata, M., Peränen, J., Schreiner, R., Wieland, F., Kurzchalia, T. V., and Simons, K. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10339–10343

77. Fra, A. M., Williamson, K., Simons, K., and Parton, R. G. (1996) Proc. Natl. Acad. Sci. U. S. A. 92, 8655–8659

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