A human genetic disorder, Tangier disease, has been linked recently to mutations in ATP-binding cassette protein A1 (ABCA1). In addition to its function in apoprotein A-I-mediated lipid removal, ABCA1 was also shown to be a phosphatidylserine (PS) translocase that facilitates PS exofacial flipping. This PS translocation is crucial for the plasma membrane to produce protrusions enabling the engulfment of apoptotic cells. In this report, we show that ABCA1 also plays a role in endocytosis. Receptor-mediated endocytosis, probed by both transferrin and low density lipoprotein, is up-regulated by more than 50% in homozygous Tangier fibroblasts in comparison with controls. Fluid-phase uptake is increased similarly. We also demonstrate that bulk membrane flow, including lipid endocytosis and exocytosis, is accelerated greatly in Tangier cells. Moreover, endocytosis is similarly enhanced in normal fibroblasts when ABCA1 function is inhibited by glyburide, whereas glyburide has no effect on endocytosis in Tangier cells. In addition, we demonstrate a decreased annexin V binding in Tangier fibroblasts as compared with controls, supporting the notion that PS transmembrane distribution is indeed defective in the presence of ABCA1 mutations. Furthermore, adding a PS analog to the exofacial leaflet of the plasma membrane normalizes endocytosis in Tangier cells. Taken together, these data demonstrate that ABCA1 plays an important role in endocytosis. We speculate that this is related to the PS translocase function of ABCA1. A loss of functional ABCA1, as in the case of Tangier cells, enhances membrane inward bending and facilitates endocytosis.

ATP-binding cassette (ABC) protein A1 belongs to the ABC transporter superfamily, one of the largest and most highly conserved gene superfamilies (1). This family of transporters consists, minimally, of a highly conserved ABC-ATPase and a much less conserved multimembrane-spanning domain. By hydrolyzing ATP, ABC transporters are capable of transporting a wide variety of substrates including lipids across the membrane. The substrate specificity of ABC transporters is thought to be determined by the nature of the membrane-spanning domains. In humans, several clinical disorders are linked to defects in ABC transporters (2).

Tangier disease is a rare genetic disorder in humans characterized by extremely low plasma concentrations of high density lipoprotein cholesterol and apoprotein A-I (3). The absence of high density lipoprotein in Tangier patients was attributed to the impairment of apoprotein A-I-mediated cholesterol efflux (4). Recently, several mutations in ABCA1 have been linked genetically to the disease (5–8). This was confirmed further by studies using a knockout mouse model (9, 10). These animals also demonstrate a nearly complete absence of high density lipoprotein. The mechanisms by which ABCA1 facilitates apoprotein A-I-mediated lipid efflux, however, remain largely unknown. ABCA1 may serve as a receptor on the cell surface for apo-AI as indicated by chemical crosslinking studies (11, 12). Apo-AI and ABCA1, however, were shown to have rather distinct diffusional coefficients on the plasma membrane, indicating that direct interaction between apo-AI and ABCA1 is limited (13). ABCA1 is known to specifically transport phosphatidylserine (PS) from the internal leaflet of the plasma membrane to the exofacial leaflet (PS translocase) (14). This could influence the lipid microenvironment on the cell surface and may sequentially facilitate apoprotein A-I binding (13). In addition, the plasma membrane, together with endosomal membranes, is the main reservoir of cellular free cholesterol (15). Endosomal membrane trafficking may be linked directly to apo-AI-mediated cholesterol and phospholipid efflux (16).

We have hypothesized that impairment of PS translocase caused by functional mutations in ABCA1 or inhibition of ABCA1 would result in the alteration in endosomal membrane trafficking. In the present report, we provide evidence demonstrating that a loss of functional ABCA1 results in an increase in endocytosis. Both receptor-mediated endocytosis and fluid-phase uptake are enhanced in Tangier fibroblasts. Membrane recycling in the endosomal system is also accelerated. Importantly, this enhanced endocytosis can be duplicated in normal fibroblasts by pharmacologically inhibiting ABCA1 function. Furthermore, endocytosis in Tangier cells can be attenuated by lyso-PS, a phospholipid analog specifically inserted into the exofacial leaflet of the plasma membrane.

EXPERIMENTAL PROCEDURES

Cell Culture—Tangier fibroblasts (TD1 and TD2) and two normal control fibroblasts (N1 and N2) were obtained from Dr. J. Oram (Washington University, Seattle, WA). These cells were immortalized by transfection with human papillomaviruses E6 and E7 (17). Another independent Tangier primary fibroblast cell line (TD3) and two normal...
control primary fibroblast cell lines (N3 and N4), characterized by one of the authors (J. G.) were also studied. The described mutations were as follows: TD1, Arg-527 to tryptophan (homozygous), and TD2, Gin-537 to arginine (a compound heterozygote in which the second allele failed to produce detectable mRNA) (18). TD3 was a compound heterozygote. All fibroblasts cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 units/ml). Cells were used before 20 passages. 2–3 days before experiments, cells were seeded onto 35-mm coverslip-bottom dishes coated with poly-lysine (MatTek Corp., Ashland, MA).

Materials and Reagents—LDL was obtained from the sera of healthy individuals by density centrifugation. Dil-LDL was prepared as described (19). Human transferrin (Sigma) was column-purified and Cy3-labeled following manufacturer instructions (Amersham Pharmacia Biotech). All the fluorescent reagents were checked by competition experiments with unlabeled materials. Fluorescein dextran (70 kDa), BODIPY-C5-SM, FM 1-43, and Alexa Fluor 488 conjugated annexin V were purchased from Molecular Probes (Eugene, OR). Glyburide was from Sigma, and 1-oleyl-2-hydroxy-sn-glycero-3-phospho-L-serine (trisodium) was from Avanti Polarlipids, Inc. (Alabaster, AL). All live cell experiments were carried out in medium 1 (lyso-phosphatidylserine or lyso-PS) was from Avanti Polarlipids, Inc.

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FIG. 1. ABCA1 inhibitor, glyburide, increases Tf endocytosis in normal fibroblasts but not in Tangier cells. Both control (a) and Tangier (b) fibroblasts were preincubated with or without glyburide (100 μM) for 30 min at 37°C and then incubated with Cy3-Tf (+glyburide) for 10 min. Tf uptake in glyburide-treated cells was normalized to that of nontreated correspondent cells and presented as relative Tf uptake: (FI/cell) + g(FI/cell) – g. There is a significant increase in Tf endocytosis after glyburide treatment in control (a) (*, p < 0.001) but not in Tangier fibroblasts (b).

RESULTS

ABCA1 Inhibitor, Glyburide, Increases Endocytosis in Human Fibroblasts—Because ABCA1 has been shown to influence lipid distribution in the plasma membrane, we first asked if the inhibition of ABCA1 would affect endocytosis. Normal human fibroblasts were preincubated with glyburide for 30 min and then incubated with Tf in the presence of glyburide for an additional 10 min at 37°C. Tf uptake was measured and compared with correspondent untreated cells. As shown in Fig. 1a, glyburide treatment resulted in an increase in Tf endocytosis in two independent fibroblast cell lines ranging from 30 to 50%. This strongly suggested that ABCA1 is indeed involved in membrane trafficking. We then tested glyburide on three Tangier fibroblast cell lines. The inhibition of ABCA1 by glyburide, as expected, had no detectable effect on all the Tangier fibroblasts tested (Fig. 1b). Because Tangier fibroblasts express only nonfunctional ABCA1, we then used these Tangier cells to

FIG. 1A

Enhanced Endocytosis in Normal Fibroblasts

**A**

**B**

**C**

**D**

**E**

**F**

**G**

**H**

**I**

**J**

**K**

**L**

**M**

**N**

**O**

**P**

**Q**

**R**

**S**

**T**

**U**

**V**

**W**

**X**

**Y**

**Z**

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Fig. 2. Transferrin endocytosis is up-regulated in Tangier fibroblast. Both control and Tangier fibroblasts were incubated with Cy3-Tf for 10 min at 37 °C, and the cells were then fixed for fluorescence microscopy. Intracellular Tf distributions in two Tangier (TD1 and TD2) and two control (N1 and N2) cell lines are shown in a. Tf uptake is shown in b. The uptake of Tf by three Tangier (TD1, TD2, and TD3) and three control (N1, N2, and N3) fibroblasts was quantified by fluorescence microscopy following an established method (20, 21). Each data point represents an average of F1/cell from 5–8 fields of cells. Error bars represent the standard deviations among the fields.

study in detail the effect of impaired ABCA1 function on endocytosis.

Receptor-mediated Endocytosis Is Increased in Tangier Fibroblasts—We then compared receptor-mediated endocytosis in control and Tangier fibroblasts. Tf and LDL are known to bind to surface receptors and to be internalized through clathrin-coated pits (22). Fibroblasts were incubated with Cy3-Tf for 10 min at 37 °C before microscopy observation. A typical Tf intracellular distribution in control fibroblasts is shown in Fig. 2a (left). Tf is seen in punctate endosome structures throughout the cells. There were also bright perinuclear clusters in most of the cells, presumably the recycling endosomes. Tf uptake in two Tangier fibroblasts (TD1 and TD2) evidently is greater compared with the two normal (N1 and N2) cell lines. This was verified by quantitative measurements in three Tangier fibroblasts and three normal controls as shown in Fig. 2b. There is an ~50% increase in Tf endocytosis during a 10-min incubation in Tangier cells in comparison with controls. Increased endocytosis of Tf is not caused by an altered surface receptor expression, because Tf surface binding was similar in all six cell lines (Fig. 2b, light gray bar).

We next measured LDL uptake in these cells. Both control and Tangier fibroblasts were incubated with Dil-LDL for 30 min at 37 °C and then fixed for microscopy measurements. Typical Dil-LDL distribution is shown in Fig. 3a. To correct for possible differences in surface receptor expression, LDL surface binding was first measured by incubating cells with Dil-LDL on ice for 30 min (Fig. 3b, black bars). This was used to normalize final Dil-LDL uptake in each cell line (Fig. 3b). Similar to Tf, Dil-LDL uptake in all three Tangier cell lines is more than double that of control cells after a 30-min incubation. Together with Tf uptake results, we conclude that the receptor-mediated endocytosis is up-regulated in Tangier cells including both immortalized and primary Tangier fibroblasts.

Fluid-phase Uptake Is also Increased in Tangier Fibroblasts—In addition to receptor-mediated endocytosis, extracellular nutrients can also be taken up by cells through other mechanisms such as pinocytosis (23). A fluid-phase marker, fluorescein-dextran, was used to measure overall endocytosis. The cells were incubated with dextran for 30 min at 37 °C, and the amount of uptake was quantified. The two Tangier cell lines accumulated more than double the amount of dextran during a 30-min period as compared with normal control cells (Fig. 4).

Both Membrane Lipid Endocytosis and Recycling Is Accelerated in Tangier Fibroblasts—Another method to examine endocytosis is to monitor membrane lipid flow. A short chain sphingomyelin fluorescence analog (BODIPY-C5-SM) was used to assay membrane endocytosis (24). Cells were incubated on ice with liposomes containing BODIPY-C5-SM to allow the sphingomyelin analog to insert into the cell surface. The cells then were rinsed with dye-free medium and chased at 37 °C for 10 or 30 min to allow endocytosis to occur. By this time, a population of endosomes containing BODIPY-C5-SM became visible (Fig. 5a). The majority of the labeling was still on the cell surface, and the overall degree of labeling was similar in Tangier and control cells. The cells were then cooled down on ice once again, and the remaining surface BODIPY-C5-SM was removed by back exchange with fatty acid-free BSA. The only BODIPY-C5-SM remaining with cells at this point was within the endosomes. Intracellular BODIPY-C5-SM was then quantified as shown in Fig. 5b. BODIPY-C5-SM endocytosis was more than doubled in Tangier fibroblasts in comparison with normal controls at both 10 and 30 min. This is in agreement with results described earlier.

Membrane recycling was measured with a fluorescence lipophilic dye, FM 1-43. This dye partitions between membrane and aqueous phase and is fluorescent only in the membrane (a 105 increase in quantum yield) (25). Cells were incubated with FM 1-43 for 15 min at 37 °C to label endosomal compartments. The cells then were rinsed several times with medium to wash off the dye on the cell surface. The only dye left associated with cells at this time was in the endosomal compartments. The cells were then chased in a dye-free medium. During the chase, lipids in the endosomal compartments move back to the cell surface (membrane recycling), and FM 1-43 loses its fluorescence upon reaching the surface by rapidly dissociating from membrane. The rate of cell-associated fluorescence decay, therefore, is an indicator of membrane exocytosis (26). When this experiment was performed with control cells, membrane lipids recycle back to the cell surface with t1⁄2 ~ 9 min (Fig. 6), similar to the rate observed by others (26). In Tangier cells, however, the fluorescence decay is faster with t1⁄2 ~ 5 min. This indicates that the membrane flow from endosomal compart-
ments back to the cell surface is accelerated in Tangier fibroblasts. Together with our observations with BODIPY-C₅-SM described above, we conclude that the rate of membrane recycling in Tangier cells is about twice that of normal control fibroblasts.

Fig. 3. LDL uptake is increased in Tangier fibroblasts. Both control and Tangier fibroblasts were incubated with DiI-LDL for 30 min at 37 °C. Fluorescence microscopic images of DiI-LDL uptake are shown in a. Surface receptor expression was measured by incubating cells on ice with DiI-LDL for 30 min. The quantitative measurements of DiI-LDL uptake are indicated in b. The amount of DiI-LDL uptake (FI/cell) is presented as a light gray bar, and surface binding is presented as a black bar.

Exogenous Lyso-PS Attenuates Endocytosis in Tangier Fibroblasts But Not in Normal Cells—ABCA1 has shown to be a PS translocase that transports PS from the internal leaflet to the exofacial leaflet of the membrane. The loss of functional ABCA1, such as in Tangier fibroblasts, could lead to an alteration of PS distribution across the bilayers of the plasma membrane. PS distribution between bilayers is thought to be maintained minimally by two flippases (27): an aminophospholipid flippase flops PS inward, and ABCA1 flips outward. At steady state, most PS is on the internal leaflet of the plasma membrane with a small fraction in the exofacial leaflet. With impaired ABCA1 function and a normal aminophospholipid flippase, however, Tangier fibroblasts may have less PS in the exofacial leaflet. This might be expected to have an impact on membrane bending, which consequently could influence endocytosis. To verify that Tangier cells indeed have an altered PS distribution, Alexa Fluor 488 conjugated annexin V was used to quantitate the amount of PS in the outer leaflets. Annexin V is known to have a high affinity for PS and is widely used to detect...
Both normal and Tangier fibroblasts were incubated with fluorescein-exa 488 annexin V surface binding (1 h at 0 °C) to detect apoptotic cells where the membrane asymmetry is lost (50). As shown in Fig. 8, Tf endocytosis was decreased by 30% in the two Tangier cell lines treated with lyso-PS, whereas in Fig. 9, LDL uptake was increased significantly in Tangier fibroblasts. We therefore used Tangier cells to probe the role of this transporter in endocytosis. It is not entirely clear at present how ABCA1 might regulate endocytosis. Signaling processes such as activation of phospholipases D and C were shown to be partially impaired in Tangier cells (33). This could have an impact on membrane trafficking. Alternatively, as suggested by the “rescue effect” of lyso-PS in Tangier fibroblasts, ABCA1 is necessary for the maintenance of cross-leaflet PS distribution of the plasma membrane. Lipids in the plasma membrane are distributed asymmetrically (36), with phosphatidylcholine and SM mainly in the exofacial leaflet and phosphatidylethanolamine and PS in the internal leaflet of the membrane. This asymmetry is maintained actively by flippases and translocases that utilize ATP (37). Such a system provides cells with a stable yet highly responsive and dynamic membrane. Within a lipid bilayer, for example, any unidirectional lipid flipping or flopping between two leaflets would cause an alteration of relative surface area of the leaflets and therefore a change in membrane curvature (27). This would facilitate either inward (invagination) or outward membrane bending (evagination) (38). Membrane bending then initiates vesiculation leading to either endocytosis or blebbing.

Although some invaginations on the cell surface are assisted by protein coatings such as clathrin-coated pits or caveolae, a large number of vesiculation events occur without apparent coating. This is evident by the fact that fluid-phase uptake, a measure of overall vesiculation events, is rather insensitive to the inhibition of clathrin-coated pit endocytosis (39). This implies that vesiculation events could occur simply by the modifications of membrane leaflets, possibly through a dynamic adjustment of relative surface areas in a lipid bilayer. Hydrolysis of sphingomyelin (an exofacial lipid leaflet on the plasma membrane), for example, results in extensive vesiculations in the absence of visible coatings and is independent of ATP (40). This has been attributed to a decrease of surface area in the exofacial leaflet of the membrane, which in turn forces inward membrane bending (40). Similarly, enrichment of the internal leaflet with exogenous PS is known to produce an increase in endocytosis (41) that was dose-dependent and relying on PS being translocated into the internal leaflet of the plasma membrane by an aminophospholipid flippase (42). A lyso-PS, similar to the one employed in our study, had an inhibitory effect on endocytosis by remaining on the exofacial leaflet of the membrane (28). Interestingly, both receptor-mediated and fluid-phase endocytosis was affected in this case, similar to our observations. When lyso-PS was added in a concentration that did not have any significant impact on the endocytosis of cells that have functional ABCA1, we found that endocytosis in Tangier cells was attenuated significantly. We cannot rule out the possibility that Tangier cells may have other metabolic alterations.
defects causing enhanced endocytosis. The facts that Tangier cells have less PS on exofacial leaflet of the membrane and lyso-PS can differentially affect Tangier and normal cells, however, point to the importance of PS in Tangier phenotypes including endocytosis.

If indeed ABCA1 functions as PS flippase, it would be responsible for supplying extra PS to the exofacial leaflet of the membrane while depleting PS from internal leaflet. Both these movements favor an outward membrane bending (43). This in fact is consistent with the observation that ABCA1 or its homologue is required for the engulfment of apoptotic cells (31, 44), a process characterized by membrane protrusion initiated by an outward membrane bending (45). A loss of functional ABCA1 would lead to relatively more PS in the inner leaflet of the membrane. This relative excess inner leaflet area not only suppresses membrane protrusion (engulfment), but also favors membrane inward bending, which in turn facilitates endocytosis. Our interpretation is also in line with the recent finding that another ABC transporter, Drs2p, influences the formation of clathrin-coated vesicles from the trans-Golgi network in yeast (46). This ABC transporter was shown to be an amino-phospholipid flippase (47) that translocates PS or phosphatidylethanolamine from luminal leaflet to cytoplasmic leaflet on the trans-Golgi network. This may imply that although the budding of clathrin-coated vesicles depends on protein coats, changes in membrane bending energy caused by defects in lipid

Fig. 5. Membrane lipid endocytosis in increased in Tangier fibroblasts. Both normal and Tangier fibroblasts (TD1) were labeled on ice with liposomes containing BODIPY-C₅-SM for 30 min, rinsed with ice-cold medium free of lipid analog, and then warmed to 37 °C for a 10- or 30-min chase. The live cells were then imaged with fluorescence microscopy. BODIPY-C₅-SM is seen on the plasma membrane and in the punctate dots, the endosomal compartments (a). The cells then were cooled on ice and washed with 5% BSA to remove surface-remaining BODIPY-C₅-SM. This resulted in BODIPY-C₅-SM only in the intracellular compartments, representing the amount of membrane lipid endocytosed during the chase at 37 °C. The amount of cell-associated BODIPY-C₅-SM was then measured, and the results are shown in b.
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flippase (Drs2P) or translocase (ABCA1) could add an additional driving force to inhibit or promote initial invagination.

The most striking phenotype of Tangier disease is the total absence of lipid efflux from these cells. Recently, a retroendocytosis model has been proposed as a possible mechanism for lipid efflux (16). Lipid acceptors, either apo-AI or high density lipoprotein, may constantly traffic through endosomes and then recycle back to the cell surface. Possibly within endosomal organelles, the acceptors acquire lipids. Tangier fibroblasts have an enhanced endocytosis yet totally lack lipid efflux. This apparent paradox may be resolved by the fact that the binding of apo-AI is correlated positively with the expression of a functional ABCA1 (12, 13). Without functional ABCA1, Tangier cells would not be able to bind to apo-AI efficiently or to shed lipid. Apo-AI cell association and binding in these fibroblasts is extremely low, and in these studies we failed to detect any significant binding or association (data not shown).

Fig. 6. Relative Tf uptake in control cells but not in control cells. Control fibroblasts (N1, N2, N3, and N4) and Tangier (TD1 and TD3) fibroblasts were preincubated with or without lyso-PS (10 μM) for 20 min at 37 °C and then incubated with Cy3-Tf (+ lyso-PS) for an additional 10 min. Tf uptake in lyso-PS-treated cells was normalized to nontreated correspondent cells as described in the Fig. 1 legend. *, p < 0.001.

An outstanding question regarding ABCA1 is how a PS translocase activity possibly facilitates lipid efflux. Using cell lines with or without ABCA1 expression, Fielding et al. (32) recently demonstrated that to acquire cholesterol apo-AI must first be lipidad with phospholipids, especially phosphatidylcholine. Lipidated apo-AI can then efficiently acquire cholesterol independent of ABCA1 expression. This is in line with recent evidence that ABCA1-mediated cholesterol efflux does not depend on “rafts,” a membrane microdomain rich in cholesterol and sphingomyelin but depleted in phosphatidylcholine (48). It seems unlikely that PS can interact with cholesterol directly. In contrast, the relationship between PS and phosphatidylcholine may be important and remains to be explored.

In summary, we report that ABCA1 plays an important role in endosomal membrane trafficking. These data support recent studies indicating that ABCA1 functions as a PS translocase (14) and are in line with reports that ABCA1-green fluorescent protein is mainly localized to the plasma membrane (14, 149). Further work is required to understand whether ABCA1 also functions in other vesicular transport processes.

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REFERENCES
1. Holland, I. B., and Blight, M. A. (1999) J. Mol. Biol. 293, 381–399
2. Schmitz, G., Kaminski, W. E., and Orso, E. (2000) Curr. Opin. Lipidol. 11, 493–501
3. Assman, G., Schmitz, G., and Brewer, H. B. (1989) in The Metabolic Basis of Inherited Diseases (S crippen, C. R., Beaudet, L. A., Sly, W. S., and Valle, D., eds) pp. 1267–1282, McGraw-Hill Inc., New York
4. Schmitz, G., Assmann, G., Robenek, H., and Brennhausen, B. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 6305–6309
5. Brooks-Wilson, A., Marciel, M., Clee, S. M., Zhang, L. H., Roop, K., van Dam, M., Yu, L., Brewer, C. – Collins, J. A., Molhuizen, H. O., Loubser, O., Ouellette, B. F., Fichter, K., Ashbourne-Excroix, K. J., Sensen, C. W., Scherer, S., Mott, S., Denis, M., Martindale, D., Frohlich, J., Morgan, K., Koup, B., Pimstone, S., Kastelein, J. J., Hayden, M. R. (1999) Nat. Genet. 22, 336–345
6. Marciel, M., Brooks-Wilson, A., Clee, S. M., Roop, K., Zhang, L. H., Yu, L., Collins, J. A., van Dam, M., Molhuizen, H. O., Loubser, O., Ouellette, B. F., Sensen, C. W., Fichter, K., Mott, S., Denis, M., Boucher, B., Pimstone, S., Genest, J., Kastelein, J. J., and Hayden, M. R. (1999) Lancet 354, 1341–1346
7. Orso, E., Brocardo, C., Kaminski, W. E., Botcher, A., Liebisch, G., Drobnik, W., Gotz, A., Chambenoit, O., Diederich, W., Langmann, T., Spruss, T., Luciani, M. F., Rothe, G., Lackner, K. J., Chimin, G., and Schmitz, G. (2000) Nat. Genet. 24, 192–196
Enhanced Endocytosis in Tangier Fibroblasts

8. Remaley, A. T., Rust, S., Rosier, M., Knapper, C., Naudin, L., Broccardo, C., Peterson, K. M., Koch, C., Arnould, I., Prades, C., Duverger, N., Funke, H., Assman, G., Dinger, M., Dean, M., Chimini, G., Santamarina-Fojo, S., Fredrickson, D. S., Denefle, P., and Brewer, H. B. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 12685–12690

9. McNeish, J., Astello, R. J., Guyot, D., Turi, T., Gabel, C., Aldinger, C., Hoppe, K. L., Rosch, M. L., Royer, L. J., de Wet, J., Broccardo, C., Chimini, G., and Fancone, O. L. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 4245–4250

10. Rust, S., Rosier, M., Funke, H., Real, J., Amoura, Z., Piette, J. C., Deleuze, J. F., Brewer, H. B., Duverger, N., Denefle, P., and Assmann, G. (1999) *Nat. Genet.* 22, 352–355

11. Oram, J. F., Lawn, R. M., Garvin, M. R., and Wade, D. P. (2000) *J. Biol. Chem.* 275, 34508–34511

12. Wang, N., Silver, D. L., Costet, P., and Tall, A. R. (2000) *J. Biol. Chem.* 275, 33053–33058

13. Chambenoit, O., Hamon, Y., Marguet, D., Rigneault, H., Rosseneu, M., and Chambenoit, O., Hamon, Y., Broccardo, C., Luciani, M. F., Toti, F., Chaslin, S., Freyssinet, J. M., Devauxa, P. F., McNeish, J., Marguet, D., and Chimini, G. (2001) *Nat. Cell Biol.* 2, 399–406

14. Mukherjee, S., Zha, X., Tabas, I., and Maxfield, F. R. (1998) *Biophys. J.* 75, 1915–1925

15. Takahashi, Y., and Smith, J. D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 11358–11363

16. Oram, J. F., Mendez, A. J., Lymp, J., Kavanagh, T. J., and Halbert, C. L. (1999) *J. Lipid Res.* 40, 1769–1781

17. Lawn, R. M., Wade, D. P., Garvin, M. R., Wang, X., Schwartz, K., Porter, J. G., Seilhamer, J. J., Vaughan, A. M., and Oram, J. F. (1999) *J. Clin. Invest.* 104, R25–R31

18. Fitter, R. K., Innerarity, T. L., Weinstein, J. N., and Mahley, R. W. (1981) *Arteriosclerosis* 1, 177–185

19. Dunn, K. W., and Maxfield, F. R. (1999) in *Noninvasive Techniques in Cell Biology* (Foskette, J. K., and Grinstein, S. eds) pp. 153–176, Wiley-Liss, Inc., New York

20. Zha, X., Tabas, I., Leopold, P. L., Jones, N. L., and Maxfield, F. R. (1997) *Arterioscler. Thromb. Vasc. Biol.* 17, 1421–1431

21. Brown, M. S., and Goldstein, J. L. (1983) *Annu. Rev. Biochem.* 52, 233–261

22. Ellis, S., and Mellor, H. (2000) *Trends Cell Biol.* 10, 85–88

23. Koval, M., and Pagano, R. E. (1991) *Biochim. Biophys. Acta* 1082, 113–125

24. Setz, W. J., Man, F., and Smith, C. B. (1996) *Curr. Opin. Neurobiol.* 6, 365–371

25. Hao, M., and Maxfield, F. R. (2000) *J. Biol. Chem.* 275, 15279–15286

26. Devaux, P. F. (2000) *Biochimie (Paris)* 82, 497–509

27. Farge, E., Ojcius, D. M., Subtil, A., and Dautry-Varsat, A. (1999) *Am. J. Physiol.* 276, C725–C733

28. Haidar, B., Mott, S., Boucher, B., Lee, C. Y., Marcil, M., and Genest, J. (2001) *J. Lipid Res.* 42, 249–257

29. Wang, N., Silver, D. L., Thiele, C., and Tall, A. R. (2001) *J. Biol. Chem.* 276, 23742–23747

30. Böst, F., Hamon, Y., Bajetto, A., Gola, M., Verrier, B., and Chimini, G. (1997) *J. Biol. Chem.* 272, 2695–2699

31. Fielding, P. E., Nagao, K., Hakamata, H., Chimini, G., and Fielding, C. J. (2000) *Biochemistry* 39, 14113–14120

32. Walter, M., Reinecke, H., Gerdes, U., Nofer, J. R., Hobbel, G., Seedorf, U., and Assmann, G. (1996) *J. Clin. Invest.* 98, 2315–2323

33. Shen, Y., Xu, L., and Foster, D. A. (2001) *Mol. Cell. Biol.* 21, 595–602

34. Neckers, L. M., Vidal, C., McGlennen, R., and Colamonici, O. (1996) *Exp. Cell Res.* 166, 151–160

35. Zachowski, A. (1993) *Biochem. J.* 294, 1–14

36. Pomorski, T., Hrafnssdottir, S., Devauxa, P. F., and Meer, G. (2001) *Semin. Cell Dev. Biol.* 12, 129–148

37. Sackmann, E. (1994) *FEBS Lett.* 346, 3–16

38. Damler, H., Baha, T., van der Bieke, A. M., and Schmid, S. L. (1995) *J. Cell Biol.* 131, 69–80

39. Zha, X., Pierini, L. M., Leopold, P. L., Skiba, P. J., Tabas, I., and Maxfield, F. R. (1998) *J. Cell Biol.* 140, 39–47

40. Rauch, C., and Farge, E. (2000) *Biophys. J.* 78, 3036–3047

41. Farge, E. (1995) *Biophys. J.* 69, 2501–2506

42. Sheetz, M. P., and Singer, S. J. (1974) *Proc. Natl. Acad. Sci. U. S. A.* 71, 4457–4461

43. Marquet, D., Luciani, M. F., Moynault, A., Williamson, P., and Chimini, G. (1999) *Nat. Cell Biol.* 1, 454–456

44. Chimini, G., and Chavrier, P. (2000) *Nat. Cell Biol.* 2, E191–E196

45. Chen, C. Y., Ingram, M. F., Rosal, P. H., and Graham, T. R. (1999) *J. Cell Biol.* 147, 1225–1236

46. Tang, X., Halleck, M. S., Schlegel, R. A., and Williams, P. (1996) *Science* 272, 1495–1497

47. Mendez, A. J., Lin, G., Wade, D. P., Lawn, R. M., and Oram, J. F. (2001) *J. Biol. Chem.* 276, 3158–3166

48. Neufeld, E. B., Remaley, A. T., Demosky, S. J., Stonik, J. A., Cooney, A. M., Comly, M., Dwyer, N. K., Zhang, M., Blanchette-Mackie, J., Santamarina-Fojo, S., and Brewer, H. B. (2001) *J. Biol. Chem.* 276, 27584–27590

49. van Engeland, M., Nieland, L. J., Roach, M. L., Royer, L. J., de Wet, J., Chimini, G., Santamarina-Fojo, S., and Brewer, H. B. (1998) *Cytometry* 31, 1–9