A Novel Type of Detergent-resistant Membranes May Contribute to an Early Protein Sorting Event in Epithelial Cells*§

Received for publication, May 31, 2005, and in revised form, October 13, 2005 Published, JBC Papers in Press, October 17, 2005 DOI 10.1074/jbc.M505924200

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One sorting mechanism of apical and basolateral proteins in epithelial cells is based on their solubility profiles with Triton X-100. Nevertheless, apical proteins themselves are also segregated beyond the trans-Golgi network by virtue of their association or nonassociation with cholesterol/sphingolipid-rich microdomains (Jacob, R., and Naim, H. Y. (2001) Curr. Biol. 11, 1444–1450). Therefore, extractability with Triton X-100 does not constitute an absolute criterion of protein sorting. Here, we investigate the solubility patterns of apical and basolateral proteins with other detergents and demonstrate that the mild detergent Tween 20 is adequate to discriminate between apical and basolateral proteins during early stages in their biosynthesis. Although the mannose-rich forms of the apical proteins sucrase-isomaltase, lactase-phlorizin hydrolase, aminopeptidase N, and dipeptidylpeptidase IV reveal similar solubility profiles comprising soluble and nonsoluble fractions, the basolateral proteins, vesicular stomatitis virus G protein, major histocompatibility complex class I, and CD46 are entirely soluble with this detergent. The insoluble Tween 20 membranes are enriched in phosphatidylinositol and phosphatidylglycerol compatible with their synthesis in the endoplasmic reticulum and the existence of a novel class of detergent-resistant membranes. The association of the mannose-rich biosynthetic forms of the apical proteins, sucrase-isomaltase, lactase-phlorizin hydrolase, aminopeptidase N, and dipeptidylpeptidase IV with the Tween 20-resistant membranes suggests an early polarized sorting mechanism prior to maturation in the Golgi apparatus.

The apical and basolateral domains in polarized epithelial cells exert specialized functions by virtue of their distinct protein and lipid compositions (1). This structural asymmetry is achieved and maintained by an active and continuous sorting process of newly synthesized components and by their regulated internalization. Current concepts have established that proteins destined for the apical or basolateral membranes are sorted in the trans-Golgi network (TGN) via selective specific signals. Initial progress in the characterization of sorting signals has been achieved with the identification of tyrosine-based cryptic short amino acid sequences, dileucine or leucine/isoleucine motifs in the cytoplasmic tails of basolaterally sorted proteins (2).

Signals for apical targeting are, unlike the basolateral signals, diverse in nature, structure, and location. These signals can be found in the extracellular domain, such as O- or N-glycans (3–8) and peptidic structures (9), membrane-anchoring domains (10), or even the cytosolic part of the apically sorted proteins (11–14). The diversity of the signals is comconitant with the existence of multiple transport mechanisms that deliver the sorted proteins in distinct carriers from their site of sorting to the apical membrane. These mechanisms could be discriminated on the basis of association of the proteins with detergent-insoluble lipid microdomains, also termed rafts, in the Golgi complex (15). Inclusion into rafts and subsequent apical sorting has been shown for some transmembrane proteins (10, 16) and for proteins anchored to the plasma membrane via the glycosylphosphatidylinositol anchor (17). However, several apical transmembrane proteins are entirely soluble in nonionic detergents, clearly indicating that they are not incorporated in lipid microdomains on their way to the apical (18–20) or axonal (21) surface. This applies also to many proteins that are secreted out of the apical domain to the external milieu (22, 23).

The existence of more than one mechanism of apical sorting that could be discriminated on the association or nonassociation of proteins with lipid rafts has been recently demonstrated for the intestinal proteins sucrase-isomaltase (SI) and lactase-phlorizin hydrolase (LPH) (24). Lipid rafts are focal assemblies of cholesterol and glycosphingolipids in plasma membranes and demonstrate decreased fluidity in comparison with the lipids in the surrounding portions of the membrane (15). A critical characteristic of these domains is their insolubility in detergents such as Triton X-100 at 4 °C and floating to lighter fractions on sucrose density gradients (25). Raft association occurs predominantly with apical proteins that are recruited to these structures through their transmembrane domains, e.g. in the case of the influenza virus proteins hemagglutinin (10) and neuraminidase (16), the glycosylphosphatidylinositol moiety (17), or putative sorting receptors that recognize sorting signals such as N- or O-linked glycans (3, 4, 26, 27). Other nonionic detergents, such as CHAPS, Lubrol, Brij, and Tween have been less widely used. The different physiochemical characteristics of detergents are reminiscent of different extraction profiles of membranes and their associated integral proteins. The variations in the extractability patterns and the altered degree of protein insolubility with a particular

ApN, aminopeptidase N; MHC, major histocompatibility complex; VSV, vesicular stomatitis virus; DRM, detergent-resistant membrane; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonic acid; MDCK, Madin-Darby canine kidney.
detergent are invariable in defining various types of membrane microdomains or subdomains and the associated biosynthetic and processed forms of proteins during various stages of their life cycle.

In this paper we employed several nonionic detergents to investigate the extractability of a number of apical and basolateral membrane proteins emphasizing the significance of protein-lipid associations in the sorting events in epithelial cells. We demonstrate the existence of a novel type of detergent-resistant membranes (DRMs) based on their solubility with Tween 20. These microdomains discriminate between apical and basolateral proteins at an early stage in the biosynthesis, prior to the Golgi apparatus, thus suggesting the presence of an early sorting mechanism.

MATERIALS AND METHODS

Immunochemical Reagents—SI was immunoprecipitated using a mixture of four different monoclonal antibodies (HBB 1/691, HBB 2/614, HBB 3/705, and HBB 2/219) (28). For immunoprecipitation of human LPH mouse monoclonal antibodies of hybridoma HBB 1/909 (28) and MLac 2, MLac 6, and MLac 8 (29) were used. The antibodies were generous gifts of Dr. Dallas Swallow (Medical Research Council, London, UK). Monoclonal anti-human MHC class I antibody was purchased from Serotec (Oxford, UK). Anti-CD46 antibody was obtained from Immunotech (Marseilles, France). Monoclonal anti-G protein of the vesicular stomatitis virus (VSV) and anti-Nfs1 were generous gifts of Dr. E. Rodriguez-Boulan (Weill Medical College of Cornell University, Ithaca, NY) and Dr. R. Lill (University of Marburg, Marburg, Germany) (30).

Tissue Culture, Transfections, and Biosynthetic Labeling—Colon carcinoma cells, Caco-2, were cultured in high glucose medium as described previously (31) and routinely used 5–6 days post-confluence, at which time SI becomes expressed. Caco-2 cells served also as a source for basolaterally located MHC class I. Another source for SI was a Madin-Darby canine kidney cell line that stably expresses SI (MDCK-SI) (4). ApN and DPPIV were analyzed in the colon intestinal cell line HT-29 (32) that expresses high levels of these two proteins. LPH was also isolated from the stably transfected epithelial MDCK cell line, MDCK-ML (33). The G protein of the vesicular stomatitis virus and CD46 (or measles virus receptor) membrane cofactor protein, two markers for a basolateral protein, were transiently expressed in COS-1 cells. The cDNA of the G protein used was kindly provided by Dr. M. G. Roth (Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX) and corresponded to the Indiana strain of the virus (34). The cDNA of CD46 was kindly provided by Dr. A. Maisner (University of Marburg) (30). The cells were transfected using DEAE-Dextran (Sigma) according to Naim et al. (35). The cells were biosynthetically labeled with [35S]methionine for different periods of time. The choice of the labeling periods depended on the transport kinetics of the individual proteins. For SI and LPH periods of 60 min and 4 h at 37 °C were chosen out. Because MHC class I and the G protein of VSV both are more rapidly transported (36, 37), a labeling period of 1 h was chosen. In pulse-chase analysis of SI, the cells were pulse-labeled for 30 min and chased for different periods of time. When used, 5 mM of deoxynojirimycin, a specific inhibitor of endoplasmic reticulum glucosidase I (38) was present in the culture medium during preincubation of the cells in methionine-deficient medium and during the labeling intervals as described before (39).

Results

Association of Various Biosynthetic Forms of SI with Lipid Rafts—SI as well as several other apically sorted proteins, most notably glyco-
Novel Detergent-resistant Membranes and Polarized Sorting

Extractability of SI in different detergents. Caco-2 or MDCK cells were biosynthetically labeled with [35S]methionine for 4 h (A) or 30 min followed by a chase for the indicated time intervals (B). The cells were lysed with Triton X-100 (TX-100), CHAPS, Brij 96, or Tween 20, and the cell extracts were centrifuged at 100,000 x g for 1 h at 4 °C. Supernatants (S) and pellets (P) were immunoprecipitated with the monodonal antibody anti-SI and analyzed by SDS-PAGE on 6% gels and with phosphorimaging.

For this purpose, we studied the solubilization profiles of SI with several nonionic detergents, Triton X-100, CHAPS, Brij 96, and Tween 20, in biosynthetically labeled Caco-2 cells or MDCK cells stably expressing SI (4). As shown in Fig. 1A the complex glycosylated mature form of SI (SIc) was found to be associated with the Triton X-100-insoluble fraction, confirming previous data on the association of this form with cholesterol/sphingolipid-rich microdomains in the TGN (48, 49). An essentially similar pattern of extractability was obtained with CHAPS suggestive of similar solubilization properties of Triton X-100 and CHAPS and implying that the insoluble microdomains revealed upon solubilization with either one of these detergents are similar in their composition. The Brij 96 solubilization profile differed from those with the two previous detergents in that the mannose-rich ER-located form of SI (SIh) was more efficiently solubilized than SIc. A substantially different separation pattern was obtained with Tween 20. Tween 20 is a detergent with relatively lower polar headgroups than Triton X-100, Brij 96, and CHAPS, and its hydrophilic-hydrophobic balance value (50) of 16.7 is also higher than these detergents (for example, the hydrophilic-hydrophobic balance values of Triton X-100 and Brij 96 are 13.5 and 12.4, respectively). Its solubilization properties would therefore be expected to differ from the previous ones. As shown in TABLE ONE, quantitative differences are revealed in the protein/detergent ratios following lysis with different detergents. TABLE TWO depicts the protein content in the gradient fractions of Tween 20- and Triton X-100-extracted cells. It was higher in the upper fractions and lower in the final three fractions of the Triton X-100 gradient as compared with their counterparts in the Tween 20 gradient. In addition to these quantitative differences, qualitative differences were observed between the Tween 20 and Triton X-100 pellets. In fact, the Tween 20-insoluble pellet contained the ER-located mannose-rich glycosylated SIh as well as the complex glycosylated mature SIc and differed in this respect quantitatively from the insoluble pellets obtained with Triton X-100, Brij 96, and CHAPS, which contained predominantly the mature SI species (Fig. 1A). The solubilization profiles of SI with all four detergents in Caco-2 cells were confirmed in another polarized cell line, MDCK stably expressing SI (Fig. 1A, lower panels). An important finding in these studies therefore is that the Tween 20-insoluble extracts/DRMs contain almost half the total amounts of the early biosynthetic form of SI, the mannose-rich SIh species, and therefore substantially more than found in the pellets of other detergents. The discrimination by Tween 20 of this early biosynthetic form suggests the presence of early insoluble membrane domains containing mannose-rich SIh. We therefore set out to investigate the kinetics of this putative association.

Kinetics of Association of Early Biosynthetic Forms of SI with Tween 20-insoluble Lipid Microdomains—The association of SI with Tween 20-insoluble membranes was further analyzed in a kinetic study. As a control, the other two detergents, Triton X-100, and Brij 96 were utilized (CHAPS revealed basically similar results to Triton X-100). Caco-2 cells were pulse for 30 min with [35S]methionine and chased for different time intervals. After 3 h of chase, cell lysis with Triton X-100 and Brij 96 results in the appearance of complex glycosylated mature SIs in the detergent-insoluble pellet (Fig. 1B). The insoluble proportion of SIh increased further at 4 h of chase compatible with its association with the insoluble microdomains following complex glycosylation. The distribution of the SI precursor and mature forms after lysis with Tween 20 revealed a different pattern. Approximately half the amount of the mannose-rich SIh polypeptides was retained in the insoluble pellet after 30 min of pulse (Fig. 1B). Given that the half-life for the conversion of the SIh to complex glycosylated SIh is about 70 min (39), the insolubility of almost 50% of SIh at the 30-min pulse time point is indicative of an early association, perhaps immediately after synthesis of these SIh...
forms, with Tween 20 DRMs. This percentage increases during the transport of SI to the cell surface, and ultimately only a minor proportion of SI molecules remain in the soluble pool. Later into the chase, complex glycosylated SI was predominantly precipitated from the insoluble material. These data indicate therefore that in the beginning the mannose-rich form associates with Tween 20 DRMs, and this type of domains harbors also the complex glycosylated SI, which appears later into the chase in the Golgi. This phase of insolubility with Tween 20 encompasses also the TGN stage, in which complex glycosylated SI is additionally also Triton X-100-insoluble (3).

To corroborate the finding of an early association of mannose-rich SIh with Tween 20 DRMs, two different approaches were used. In the first one, biosynthetic labeling of the cells was performed for 1 h at 15 °C, at which temperature proteins are blocked in the ER and also the ER/Golgi intermediate compartment (51). Fig. 2A shows that the main form generated at 15 °C is SIh, as compared with SI, and SIh, revealed at 37 °C (the left two lanes). At 15 °C and upon solubilization with Tween 20, a significant proportion of mannose-rich SIh was retained in the Tween 20-insoluble pellet. By contrast, this SIh form was entirely soluble with Triton X-100, CHAPS, and Brij 96, because the corresponding insoluble pellets were devoid of SIh. The second approach employed deoxynojirimycin in biosynthetic labeling experiments to inhibit ER-located glucosidase I and glucose trimming of the core oligosaccharides in the ER and affect subsequent protein transport out of the ER. This treatment reveals exclusively the core glycosylated SI protein with an apparent molecular weight similar to that of mannose-rich SIh (39). As shown in Fig. 2B, a large proportion of this early biosynthetic form was recovered in the insoluble pellet, indicating that the association with Tween 20 DRMs occurs at an early stage in the life cycle of SI.

The Tween 20 DRMs Are Enriched in Phosphatidylinositol and Phosphatidylglycerol—In view of the distinct solubilization behavior of SI with Tween 20, we utilized high pressure liquid chromatography to determine the lipid composition of the Tween 20-insoluble pellet/DRMs obtained from extracts of Caco-2 cells (Fig. 3 and TABLE THREE). Further, these lipid components were compared with those in Triton X-100 DRMs in the same cell line. These analyses revealed major relative differences in the lipid classes by taking into consideration the different ratios of lipid versus protein in the insoluble pellets. The phospholipid fraction of the Tween 20 pellets contains less sphingomyelin as compared with the Triton X-100 pellets but significantly more phosphatidylinositol and phosphatidylglycerol, whereas no difference could be observed for phosphatidylethanolamine, phosphatidylserine, and phosphatidyicholine (Fig. 3A). Because sphingomyelin-synthesis is located in the cis-compartment of the Golgi (52, 53), a low content of this lipid strongly suggests that Tween 20 DRMs are formed in an earlier step in the secretory pathway and supports thus the protein data shown above. On the other hand, phosphatidylinositol and phosphatidylglycerol are synthesized in the cytoplasmic leaflet of the ER (54, 55); thus, their increase in the Tween 20 pellets could be related to their subcellular synthesis in the ER. Remarkable is the reduced protein content in Tween 20 DRMs (Fig. 3B), which indicates that these DRMs are not as highly packaged as the Triton X-100-insoluble ones (56). The cholesterol content was difficult to determine in the Tween 20 pellets because of its low abundance and the interfering high background of the detergent. The biochemical data have clearly demonstrated the association of the mannose-rich ER form of SI as well as its complex glycosylated Golgi form with Tween 20 DRMs. To determine whether these two forms are associated with DRMs that are derived from the ER and Golgi, we performed a sucrose gradient centrifugation to isolate the ER and Golgi membranes. Western blot analysis with anti-BiP, anti-GM130, and anti-Nfs1 antibodies located the ER in fractions 1–3, the Golgi in fractions 7/8, and the mitochondria in fractions 2/3 (Fig. 3C). The ER and Golgi fractions were collected, Tween 20 DRMs were isolated, and the lipid components were analyzed as shown above. As shown in Fig. 3B and TABLE THREE, the similar lipid composition of these insoluble pellets was obtained in both organelles with marked concentrations of phosphatidylinositol and phosphatidylglycerol.

Different Extractabilities of Early Forms of Apical and Basolateral Membrane Proteins with Tween 20 Is Suggestive of a Sorting Event Prior to the Golgi—By virtue of the biochemical and analytical identification of the Tween 20 DRMs with a different lipid composition as compared with Triton X-100 rafts, we asked whether the early association of mannose-rich SIh with this type of insoluble membranes can be also observed with other membrane proteins and has subsequent implications on their trafficking or sorting mechanisms in epithelial or polarized cells. For this we investigated the extractability profiles of other

### Table Two

| Protein concentrations in eight fractions of 5–35% sucrose gradients were assessed according to Bradford (40) |
|---------------------------------------------------------------|
| Fraction | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|-----------------|---|---|---|---|---|---|---|---|
| **Triton X-100** | 1.4 | 1.2 | 2.0 | 2.1 | 1.8 | 0.9 | 0.8 | 0.8 |
| **Tween 20** | 0.5 | 0.4 | 0.7 | 0.8 | 1.0 | 1.3 | 1.4 | 1.5 |

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**Figure 2.** Detergent extractability of ER-resident forms of SI. A, Caco-2 cells were biosynthetically labeled at 15 °C with [35S]methionine for 1 h and lysed with Triton X-100 (TX-100), CHAPS, Brij 96, or Tween 20. The cell extracts were centrifuged at 100,000 × g for 1 h at 4 °C. Supernatants (S) and pellets (P) were immunoprecipitated with monoclonal antibody anti-SI and analyzed by SDS-PAGE. Mannose-rich and complex glycosylated forms of SI were immunoprecipitated from cell extracts with sodium deoxycholate as a control. B, after biosynthetic labeling with [35S]methionine for 4 h in the presence or absence of deoxynojirimycin (dNM), Caco-2 cells were lysed with Tween 20 or sodium deoxycholate as a control. The extracts were processed as described for A, and the immunoprecipitates were analyzed by SDS-PAGE and phosphorimaging.
proteins, apical and also basolateral, with Tween 20. The common feature of these proteins is that they differ from SI with respect to their association with cholesterol/sphingolipid-rich microdomains. One of these is LPH, a highly polarized apical protein that is entirely extractable with Triton X-100 and transported to the membrane in different vesicular carriers as those of SI (24, 49). Two other apical marker proteins are aminopeptidase N (ApN) and DPPIV, whereas the other basolaterally sorted proteins are the G protein of the VSV (57), MHC class I (58), and the membrane cofactor protein of the complement system (CD46) (30).

The extraction properties of these proteins with Tween 20 were examined to determine whether this detergent discriminates between apical and basolateral proteins on one hand and also between apical proteins themselves, as Triton X-100 does, on the other.

ApN and MHC class I were examined in biosynthetically labeled Caco-2 cells, DPPIV in HT29 cells, CD46 and the VSV G protein in transfected COS-1 cells, and LPH in stably transfected MDCK cells (Fig. 4A). All of the basolateral proteins were exclusively found in the soluble fractions of cellular extracts that were solubilized with Triton X-100 or Tween 20 (Fig. 4B). The apical proteins ApN and LPH, on the other hand, were entirely soluble in Triton X-100, but not

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**TABLE THREE**

The phospholipid patterns of Triton X-100 and Tween 20 pellets are presented as μg/μg protein.

| Phospholipid            | Triton X-100 pellets | Tween 20 pellets |
|-------------------------|----------------------|------------------|
| Phosphatidylcholine      | 97.2 ± 37.2          | 302.3 ± 124      |
| Phosphatidylethanolamine | 55.7 ± 33.2          | 149.2 ± 78.1     |
| Phosphatidylglycerol     | 12.5 ± 5.9           | 64.6 ± 37.4      |
| Phosphatidylinositol     | 13.7 ± 11.2          | 86.7 ± 22.4      |
| Phosphatidylserine       | 11.6 ± 7.8           | 31.7 ± 16.2      |
| Sphingomyelin            | 20.9 ± 11.7          | 21.9 ± 12.3      |

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**FIGURE 3. Lipid composition of Triton X-100 and Tween 20 DRMs.**

A, distribution of phospholipid classes in Triton X-100- and Tween 20-insoluble total pellets or Tween 20 pellets derived from ER- or Golgi-containing fractions. The relative amounts (%) of phospholipids are shown. B, protein content of Tween 20 and Triton X-100 (TX-100) pellets. The amounts of protein per phospholipid are presented. In A and B at least six different preparations were analyzed. The means ± S.D. are presented. Significant differences (p < 0.05) are indicated with asterisks. C, isolation of ER and Golgi membranes from Caco-2 cells by sucrose gradient centrifugation. Isolated fractions were separated by SDS-PAGE, blotted, and analyzed with antibodies directed against BiP, GM130, and Nfs1.
with Tween 20. Here, and in a fashion similar to the SI, the mannose-rich as well as complex glycosylated forms of ApN and LPH were retained in the Tween 20-insoluble pellet, whereas the soluble fraction contained almost exclusively the mannose-rich glycosylated pool (Fig. 4A). This accumulation of mannose-rich polypeptides in DRMs could also be observed for apical DPPIV after Tween 20 lysis.

However, in contrast to ApN and LPH, complex glycosylated DPPIVc remained in the Triton X-100 DRMs, which indicates its association with these membranes after passage through the Golgi apparatus. In this respect DPPIV and SI have a similar distribution pattern after lysis with Triton X-100 and Tween 20. As a conclusion, a direct comparison of the Tween 20 extraction properties of the apical proteins SI, ApN, LPH, and DPPIV on one hand and the basolateral proteins CD46, MHC class I, and the G protein of VSV on the other unravels a subtle difference. Basolateral proteins remain soluble, whereas apical proteins can be detected in Tween 20 DRMs already at an early stage of their biosynthetic cycle, most likely in the ER.

The extraction properties of the apical and basolateral proteins with Triton X-100 and Tween 20 were further evaluated in sucrose gradients in which DRMs were retained in the floating fractions on the top of the gradient (Fig. 5). After cell lysis with Triton X-100, the complex glycosylated SIc polypeptides were immunoprecipitated from light floating as well as denser fractions of the gradient (Fig. 5A), whereas mannose-rich Sih was mainly revealed in denser fractions. Tween 20 on the other hand generates floating micelles that contain both SIc and Sih molecules. The same distribution pattern could be observed for DPPIV. Furthermore, the other two apical polypeptides, ApN and LPH, that are absent from floating fractions generated by Triton X-100 lysis could be detected in micelles that float after lysis with Tween 20 in fractions 5–8. By contrast, the basolateral proteins, CD46, MHC class I, and the G protein of VSV, were found exclusively in the soluble gradient fractions (Fig. 5B).

Taken together, the flotation and sedimentation experiments support the notion that a difference in the profile of association of basolateral and apical proteins with DRMs exists already prior to terminal matura-
tion in the Golgi, strongly suggesting that a polarized sorting of these proteins occurs early in their biosynthetic life cycle.

**DISCUSSION**

Current concepts of protein sorting in epithelial cells propose that apical and basolateral proteins are segregated in the TGN and are transported separately in distinct vesicular carriers (59). One of the main sorting criteria is the association or nonassociation of these proteins with detergent-insoluble lipid microdomains enriched in cholesterol and sphingolipids. An essential role of the lipid composition of transport carriers in the sorting event of transported cargo has also been demonstrated for other types of polarized cells like hepatocytes (60) and neuronal cells (61). On the other hand, epithelial cells and hepatocytes transport many proteins with high fidelity to the apical membrane by a mechanism that is independent of Triton X-100-insoluble lipid microdomains or rafts (3, 19, 62). Strikingly, an additional sorting step of apical proteins takes place beyond the TGN. In fact, we have clearly demonstrated the existence of multiple sorting pathways for apically sorted proteins based on their association or nonassociation with lipid rafts in MDCK cells. Here, LPH and SI are transported in distinct vesicular carriers from the TGN to the cell surface, and these vesicles differ significantly in their protein contents (24, 63–65). In correlation with these observations, hepatocytes also transport apical cargo by two pathways. In these cells an indirect apical pathway transports the classical Triton X-100-insoluble microdomains, and a direct route is taken by Triton X-100-soluble but Lubrol WX-insoluble membrane components (62). Interestingly, observations made in epithelial cells by live cell imaging also suggest the presence of indirect and direct deliveries of apical proteins in epithelial cells (66).

As a result the discrimination between apical and basolateral proteins through their association or nonassociation with the classical Triton X-100-insoluble, cholesterol/sphingolipid-rich microdomains does not constitute an absolute sorting criterion. We asked therefore whether extractability patterns of apical and basolateral proteins with other detergents are more exclusive and sensitive than with Triton X-100. As our paper clearly demonstrates, Tween 20 is one such detergent that discriminates between apical and basolateral proteins. All of the apical proteins examined reveal a similar solubility profile comprising soluble and nonsoluble fractions. By contrast, basolateral proteins are entirely soluble with this detergent. The Tween 20 DRMs are enriched in phosphatidylinositol and phosphatidylglycerol concomitant with the synthesis of these lipids in the ER (54). These lipids are synthesized by CDP-diacylglycerol synthase via CDP-diacylglycerol, whereas phosphatidylethanolamine, phosphatidylserine, and phosphatidylethanolamine are synthesized via a different pathway with diacylglycerol as an intermediate (67). The composition of the Tween 20-insoluble membranes suggests the existence of a novel class of DRMs or rafts that is assembled in an early compartment prior to the cis-Golgi, most likely in the ER. These data are in line with previous observations that suggest an involvement of lipid microdomains early in the secretory pathway (68).

Of particular interest are the early biosynthetic forms of the apical proteins that associate with these insoluble Tween 20 DRMs. In all of the proteins analyzed, a predominant mannose-rich biosynthetic form is revealed compatible with an early association of these proteins with the Tween 20 DRMs prior to the Golgi apparatus. By contrast, neither of the basolateral proteins investigated associates with this type of DRMs. Therefore, the variable solubilization profiles of early forms of apical and basolateral proteins with Tween 20 and the presence of a new type of DRMs are strongly suggestive of a novel polarized sorting mechanism for apical and basolateral proteins occurring early in their biosynthesis at a level prior to the Golgi. The apical proteins, on the other hand, are further segregated at a later stage in the TGN, depending on whether or not they are associated with Triton X-100 lipid rafts (24). In this case, the transport of proteins associated with Triton X-100 lipid rafts occurs along actin tracks in the cellular periphery, whereas the Triton X-100-soluble apical proteins are transferred in an actin-independent fashion to the apical membrane (63).

**REFERENCES**

1. Rodriguez-Boulan, E., and Powell, S. K. (1992) Annu. Rev. Cell Biol. 8, 395–427
2. Matter, K. and Mellmann, I. (1994) Curr. Opin. Cell Biol. 6, 545–554
3. Alfalah, M., Jacob, R., Preuss, U., Zimmer, K. P., Naim, H., and Naim, H. Y. (1999) Curr. Biol. 9, 593–596
4. Jacob, R., Alfalah, M., Grunberg, J., Obendorf, M., and Naim, H. Y. (2000) J. Biol. Chem. 275, 6566–6572
5. Kaether, C., Skehel, P., and Dotti, C. G. (2000) Mol. Biol. Cell 11, 1213–1224
6. Kitagawa, Y., Sano, Y., Ueda, M., Higashiyama, K., Nairn, H., Okano, M., Matsumoto, S., and Sasaki, R. (1994) Exp. Cell Res. 213, 449–457
7. Scheiffele, P., Peranen, J., and Simons, K. (1995) Nature 378, 96–98
8. Yeaman, C., Baldwin, A. M., Baldwin, L., Le Bivic, A., and Rodriguez-Boulan, E. (1997) J. Cell Biol. 139, 929–940
9. Jacob, R., Preuss, U., Panzer, P., Alfalah, M., Quack, S., Roth, M. G., Naim, H., and Naim, H. Y. (1999) J. Biol. Chem. 274, 8061–8067
10. Lin, Z., Naim, H. Y., Rodrigues, A. C., and Roth, M. G. (1998) J. Cell Biol. 142, 51–57
11. Chuang, J. Z., and Sung, C. H. (1998) J. Cell Biol. 142, 1245–1256
12. Fiedler, K., and Simons, K. (1995) Cell 81, 309–312
13. Rodriguez-Boulan, E., and Gonzalez, A. (1999) Trends Cell Biol. 9, 291–294
14. Sun, A. Q., Ananthanarayanan, M., Soroka, C. J., Thevananther, S., Shneider, B. L., and Suchy, F. J. (1998) Am. J. Physiol. 275, G1045–G1055
15. Simons, K., and Ikonen, E. (1997) Nature 387, 569–572
16. Kundu, A., Avalos, R. T., Sanderson, C. M., and Nacy, D. P. (1996) J. Virol. 70, 6508–6515
17. Harder, T., and Simons, K. (1997) Curr. Opin. Cell Biol. 9, 534–542
18. Lipardi, L., Nitsch, L., and Zuzolo, C. (2000) Mol. Biol. Cell 11, 531–542
19. Zheng, X., Lu, D., and Saldier, J. E. (1999) J. Biol. Chem. 274, 1569–1605
20. Zuzolo, C., van’t Hof, W., van Meer, G., and Rodriguez-Boulan, E. (1994) EMBO J. 13, 42–53
21. Tienari, P. I., De Strooper, B., Ikonen, E., Simons, M., Weidemann, A., Czec, H., Hartmann, T., Ida, N., Multhaup, G., Masters, C. L., Van Leuven, F.,reyerh, K., and Dotti, C. G. (1996) EMBO J. 15, 5218–5229
22. Graichen, R., Losch, A., Appel, D., and Koch-Brandt, C. (1996) J. Biol. Chem. 271, 15854–15857
23. Marzolo, M. P., Bull, P., and Gonzalez, A. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 1834–1839
24. Jacob, R., and Naim, H. Y. (2001) Curr. Biol. 11, 1444–1450
25. Brown, D. A., and Rose, J. K. (1992) Cell 68, 533–546
26. Alfalah, M., Jacob, R., and Naim, H. Y. (2002) J. Biol. Chem. 277, 10683–10690
27. Spodsbeg, N., Alfalah, M., and Naim, H. Y. (2001) J. Biol. Chem. 276, 46597–46604
28. Hauri, H. P., Sterchi, E. E., Bienen, D., Fransen, J. A., and Marxer, A. (1985) J. Cell Biol. 101, 838–851
29. Maituri, L., Raia, V., Potier, J., Swallow, D., Ho, M. W., Fiocca, R., Finzi, G., Cornaggia, M., Capella, C., and Quaroni, A. (1991) Gastroenterology 100, 359–369
30. Naim, H. Y., Joubert, G., Alfalah, M., and Jacob, R. (1999) J. Biol. Chem. 274, 17961–17967
31. Naim, H. Y., Lacey, S. W., Sambrook, J. F., and Gething, M. J. (1991) J. Biol. Chem. 266, 12313–12320
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36. Capps, G. G., and Zuniga, M. C. (1994) J. Biol. Chem. 269, 11634–11639
37. Lodish, H. F., Kong, N., Snider, M., and Strous, G. J. (1983) Nature 304, 80–83
38. Fuhrmann, U., Bause, E., Legler, G., and Ploegh, H. (1984) Nature 307, 755–758
39. Naim, H. Y., Sterchi, E. E., and Lentze, M. J. (1988) J. Biol. Chem. 263, 7242–7253
40. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
41. Laemmli, U. K. (1970) Nature 227, 680–685
42. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Med. Sci. 37, 911–917
43. Meyer zu, D. H., Sallmann, H., Glockenthor, U., von Engelhardt, W., and Busche, R. (1999) Anal. Biochem. 269, 45–53
44. Zierath, J. R., He, L., Guma, A., Odegaard, W. E., Klip, A., and Wallberg-Henriksson, H. (1996) Diabetologia 39, 1180–1189
45. Bole, D. G., Hendershot, L. M., and Kearney, J. F. (1986) J. Cell Biol. 102, 1558–1566
46. Nakamura, N., Rabouille, C., Watson, R., Nilsson, T., Hui, N., Slusarewicz, P., Kreis, T. E., and Warren, G. (1995) J. Cell Biol. 131, 1715–1726
47. Dotti, C. G. (1999) Anat. Rec. 251, 442–447
50. Saraste, J., and Kuismanen, E. (1984) Cell 38, 535–549
51. Jekel, D., Karrenbauer, A., Birk, R., Schmidt, R. R., and Wieland, F. T. (1990) Cell 63, 259–267
52. Jacob, R., Heine, M., Alfalah, M., and Naim, H. Y. (2003) Curr. Biol. 13, 607–612
53. Jacob, R., Heine, M., Aikman, J., Hauer, H., Rescher, U., Gerke, V., and Naim, H. Y. (2004) J. Biol. Chem. 279, 3680–3684
54. Heine, M., Cramp-Behrens, C., Ansari, A., Chu, H. P., Ryazanov, A. G., Naim, H. Y., and Jacob, R. (2005) J. Biol. Chem. 280, 25637–25643
55. Vance, J. E., and Vance, D. E. (2004) Biochem. Cell Biol. 82, 113–128
56. Muniz, M., and Riezman, H. (2000) EMBO J. 19, 10–15