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Unravelling protein sorting

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Abstract

Protein sorting to distinct plasma membrane domains in polarized epithelial cells is thought to occur in the Golgi complex, and to be mediated in part by lipid rafts. Now, analysis of protein trafficking in live cells has revealed unexpected sorting pathways that raise new questions about the specificity and sites of action of sorting mechanisms.

Many cell types display an asymmetric or polarized organization of proteins in different domains of the plasma membrane. This organization reflects a requirement for asymmetric growth or division, directional migration, or vectorial transport and delivery of solutes/signals. Understanding the mechanisms that specify protein sorting to different membrane domains is a major goal in cell biology.

Polarized epithelial cells display distinct patterns of protein distribution on their apical and basolateral surfaces (Fig. 1a). In addition, several cell lines retain the ability to form polarized membrane domains in tissue culture and provide experimentally tractable systems to examine mechanisms of protein sorting¹. Three sites of protein sorting have been identified — the *trans*-Golgi network (TGN), endosomes and the plasma membrane — all with potentially similar mechanisms for sorting different classes of membrane protein (Fig. 1b–d). For example, apical proteins may be sorted by clustering into lipid rafts enriched in glycosphingolipids and cholesterol, or by the addition of *N*- or *O*-linked glycosylation signals. Basolateral proteins may be clustered by specific clathrin adaptor proteins (for example, AP1B) that recognize cytoplasmic sorting determinants (reviewed in ref. 1). However, it remains unclear which of these sites is the major station for sorting of newly synthesized proteins to different membrane domains, and whether sorting pathways are cell-type-specific (Fig. 1b–d). For example, it is currently thought that the TGN is the major sorting station during exocytosis in MDCK cells (Fig. 1b), and that some basolateral proteins may make a detour to the endosome on their way to the plasma membrane (Fig. 1c). In liver cells, however, most proteins (both apical and basolateral) are delivered to the basolateral membrane and a subset are sorted there or in adjacent endosomes for delivery to the apical surface (Fig. 1c). On page 297 of this issue, Polishchuk *et al.*² analyse protein sorting pathways in polarized MDCK cells by imaging apical and basolateral cargo proteins as they are delivered from the Golgi complex to the plasma membrane. They suggest that in MDCK cells, glycosylphosphatidyl inositol (GPI)-linked proteins (a subgroup of apical proteins) are packaged together with basal lateral proteins in post-Golgi carrier vesicles for delivery to the basolateral membrane. From there, GPI-linked proteins are endocytosed and delivered to the apical surface. These conclusions are provocative, and indicate that some rethinking of protein sorting pathways may be required.

Polishchuk *et al.* used well-characterized cargo proteins tagged with different green fluorescent proteins (GFP, YFP and CFP) as markers for specific sorting pathways. At steady state, these proteins localized to the expected membrane domains. However, direct analysis of trafficking of GPI-YFP (an apical cargo protein that is thought to be sorted via lipid rafts) and VSVG-tsO45-CFP (a basolateral cargo protein that is sorted by a cytoplasmic determinant) revealed that both co-localize in tubulovesicular carriers emanating from the TGN, rather than separate carriers. These post-TGN tubulovesicular carriers fused with the plasma membrane. Inhibition of vesicle fusion with the plasma membrane using a mutant α -SNAP protein or tannic acid (see below) resulted in the accumulation of vesicular carriers containing both cargo proteins adjacent to the plasma membrane. These results indicate that a protein sorting step may not occur *en route* to the plasma membrane; for example, in the endosome, as suggested previously⁴.

The finding that apical and basolateral cargo exit the TGN in the same carrier seems to be inconsistent with previous analysis, showing that apical and basolateral membrane proteins are sorted away from each other into separate carriers in the TGN³. However, close inspection of the images produced by Polishchuk *et al.* reveals some separation of GPI-YFP and VSVG-tsO45-CFP within the same tubulovesicular structures leaving the TGN. This separation was particularly evident when fission of these carriers was blocked (for example, through expression of a kinase-dead mutant of protein kinase D). Thus, some degree of sorting of these different cargoes does occur in the TGN, although they are ultimately packaged together into the same post-TGN carrier.

The authors also noted that tannic acid — a cell-impermeable fixative that cross-links cell-surface carbohydrate groups — does not diffuse across the tight junction, a structural barrier that separates apical and basolateral membrane domains (Fig. 1). Therefore, they reasoned that if tannic acid was applied selectively to only one of these membrane domains, vesicle delivery to just that single domain would be inhibited. These experiments provided clear and dramatic results: incubation of the basolateral membrane with tannic acid resulted in intracellular accumulation of both GPI-YFP and VSVG-tsO45-CFP in the same tubulovesicular carriers (Fig. 2a). Thus, these post-TGN carriers were destined for the basolateral surface, and not the apical membrane. These carriers accumulated immediately adjacent to the tight junction, a sub-domain of the lateral membrane shown previously to be the site for vesicle delivery⁵ and to contain the Sec6/8-exocyst protein complex⁶. Incubation of the apical membrane domain with tannic acid did not perturb delivery of VSVG-tsO45-CFP to the basolateral membrane (Fig. 2b). However, GPI-YFP was not localized to the apical membrane, but instead accumulated in intracellular vesicular carriers. These results led the authors to suggest that after being delivered to the basolateral membrane, GPI-YFP is then selectively internalized for re-sorting to the apical domain. Co-localization of GPI-YFP with endocytic tracers provided further evidence for endocytosis of GPI-GFP from the basolateral membrane. Electron micrographs revealed that GPI-YFP was clustered into non-clathrin-coated membrane buds with 'grape-like' profiles, which Polishchuk *et al.* suggest might be caveolae. Because caveolae are found predominantly on the basolateral surface of polarized MDCK cells and are enriched in lipid raft components that cluster GPI-anchored proteins⁷, the authors propose that GPI-linked proteins may be endocytosed via caveolae.

So, are all apical membrane proteins sorted in the same way as GPI-YFP? The answer seems to be no, at least for p75, an apical protein that is not dependent on lipid rafts for sorting⁸. Polishchuk *et al.* show that the apical delivery of p75 is not affected by the addition of tannic acid to the basal surface (Fig. 2c). This confirms the existence of a direct apical route from the TGN to the apical membrane domain, as previously demonstrated by quantitative imaging of post-Golgi vesicle fusion⁵ and by abundant biochemical data (reviewed in ref. 1). However, whether p75 and GPI-YFP are sorted into different

tubulovesicular carriers in the TGN was not addressed in this study, and the nature of the different pathways taken by these apical proteins remains unclear.

Why was this mode for sorting GPI-anchored proteins such as GPI-YFP missed in previous targeting studies with MDCK cells? Protocols used in the past may not have been sufficiently sensitive to detect the transient appearance of GPI-anchored proteins in the basolateral membrane, perhaps because the sorting and internalization efficiency of GPI-anchored proteins may be particularly high at the basolateral surface of epithelial cells.

What are the implications of these findings for sorting pathways in polarized epithelial cells (Fig. 2d)? Clearly, sorting of proteins occurs in the TGN, but the general nature of this process in different epithelial cells needs to be re-evaluated as powerful imaging techniques and procedures to interfere with specific steps in the pathway become available. Another issue is the precise site of fusion: the authors found that delivery of carriers containing GFP-GPI was restricted to the apex of the lateral membrane, a region previously shown to contain the Sec6/8-exocyst complex and to be a site for docking and fusion of basolateral vesicles with the plasma membrane^{5,6}. It remains unknown whether vesicle delivery — for example, of p75 — to the apical membrane also occurs at this site. An earlier study showed that delivery of low-density lipoprotein receptor and VSV-G, but not p75, was inhibited by anti-Sec6 antibodies⁶, although further studies are needed to fully evaluate the specificity and role of this complex in vesicle docking and fusion.

The results from Polishchuk *et al.* suggest that GPI-anchored proteins and VSV-G exit the TGN through a similar mechanism. It will be important to test whether peptides that selectively inhibit VSV-G exit from the Golgi⁹ also inhibit the exit of GPI-anchored proteins or other raft proteins; or conversely, whether inhibition of sorting of GPI-anchored proteins affects sorting and exit of VSV-G from the Golgi. It is worth noting that previous experiments in which cholesterol depletion selectively affected the delivery of influenza hemagglutinin (another lipid-raft-associated protein) to the apical surface, but not the delivery of VSV-G to the basolateral membrane¹⁰, are now subject to the alternative interpretation that sorting via lipid rafts might not occur at the TGN but during internalization from the basolateral membrane.

Finally, the authors' suggestion that GPI-anchored proteins are selectively endocytosed from the basolateral membrane via caveolae is difficult to reconcile with the available evidence. For example, the epithelial cell line FRT lacks morphological caveolae, its main structural component caveolin and mis-sorts some GPI-anchored proteins to the basolateral membrane. However, although transfection of caveolin 1 promotes the assembly of basolateral caveolae, it does not restore correct sorting of GPI-anchored proteins in these cells¹¹. Furthermore, it is not yet clear whether caveolae facilitate or retard the internalization of GPI-anchored proteins¹².

One of the oldest debates about how proteins are sorted in polarized epithelial cells has centred around the idea of cell-type-specific sorting pathways¹³. The study by Polishchuk *et al.* provides further evidence that some apical sorting pathways may, in fact, be more conserved between different cell types than previously thought. It is clear that some of these variations reflect physiological requirements for different sorting pathways in different cells. In addition, cell-type-specific differences in the expression of sorting machinery components are only now becoming apparent and may also account for the cell-type specificity of sorting pathways¹⁴. It is clear that there is still much more to be discovered about protein sorting pathways.

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Figure 1.

Models for polarized sorting of membrane proteins in epithelial cells. **(a)** Polarized epithelial cells form tubes in which the apical membrane (Ap) faces the lumen, and the basolateral membrane (BL) faces the interstitium. Membrane domains are separated by a tight junction (TJ). Differences in protein distribution between these membrane domains allow vectorial solute transport (red arrow). **(b–d)** Possible sorting pathways for newly synthesized apical (p75, light green; GPI-linked proteins, dark green) and basolateral (VSV-G; blue) proteins. **(b)** Apical and basolateral proteins are sorted at the *trans*-Golgi network (TGN) into distinct carriers that are delivered directly to the respective domains. This model represents the current data for MDCK cells, with the exception of polymeric IgA receptor, which is first delivered basolaterally, internalized into endosomes (E) and then transcytosed to the apical membrane¹ (dotted line). **(c)** Apical proteins are delivered directly, whereas basolateral proteins are delivered via the endosome (E). **(d)** Both basolateral and apical proteins are delivered first to the basal lateral plasma membrane, from where apical proteins are endocytosed and subsequently targeted to the apical surface; some basolateral proteins are constantly recycling via endosomes. This model represents the current data on protein trafficking in liver cells¹³. Some combinations of these pathways are also possible. Vesicles carrying basolateral proteins are delivered to the plasma membrane near the tight junction at the location of the Sec6/8–exocyst complex (yellow star); this has been shown only for MDCK cells.

Figure 2.

Effects of selective inhibition of vesicle delivery to the apical or basolateral membrane. Tannic acid, a cell-impermeable fixative, was applied to either the basolateral (**a**, **c**) or apical (**b**) surface of polarized MDCK cells to selectively inhibit vesicle fusion on that surface. The delivery of GPI-YFP (green), p75-YFP (light green) and VSV-G-CFP (purple) was examined. (**a**) After application of tannic acid to the basolateral membrane, both GPI-YFP and VSV-G-CFP co-localize and accumulate in vesicles close to the tight junction where the Sec6/8-exocyst complex is located (yellow star), and neither protein is found on the plasma membrane. (**b**) After application of tannic acid to the apical membrane, VSV-G-CFP is delivered to the basolateral membrane via post-TGN vesicle carriers. However, GPI-CFP-containing vesicles are present below the apical surface, because of internalization from the basolateral surface and inhibition of fusion with the apical membrane. (**c**) After application of tannic acid to the basolateral membrane, p75-YFP is delivered directly to the apical membrane from the TGN. (**d**) On the basis of the results of Polishchuk *et al.*, apical proteins such as GPI-YFP may be delivered to the basolateral surface from where they are internalized and re-targeted to the apical membrane. Other apical proteins, such as p75, are delivered directly to the apical membrane.