Controversy fuels trafficking of GPI-anchored proteins

Sebastian Schuck and Kai Simons

Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany

The model that glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-APs) take a direct transport route to the apical membrane of epithelial cells has recently been challenged. In this issue, Paladino et al. (p. 1023) and Hua et al. (p. 1035) show that the original view nevertheless holds. This closes a chapter in the winding story of GPI-AP trafficking but opens another phase, as the controversy has stimulated the development of new methodology.

A large number of proteins in yeast, plants, and animals are attached to the cell surface by a GPI anchor. GPI-APs are functionally diverse, comprising signaling receptors, hydrolytic enzymes, and cell adhesion molecules. In addition, some proteins have GPI-anchored as well as transmembrane forms. GPI anchoring clearly does not confer a particular function, but it allows cells to control the localization of proteins by modulating their trafficking behavior. This is especially important in polarized cells, which have more than one plasma membrane domain and can selectively place the activities of GPI-APs by sending them to different parts of their surface. Thus, understanding how polarized cells handle GPI-APs has implications for many biological processes.

Initial work, the first model, and first refinements

The transport of GPI-APs in polarized cells was first investigated in the late 1980s in epithelial MDCK cells, a popular model system for polarized membrane trafficking, as they form a well-defined epithelial monolayer with apical and basolateral domains that are separated by tight junctions (Fig. 1). Two seminal papers showed that the GPI anchor serves as a signal for transport to the apical membrane (Brown et al., 1989; Lisanti et al., 1989). Soon afterward it was established that newly synthesized GPI-APs are delivered to the apical membrane of MDCK cells directly and do not make a detour to the basolateral membrane, as later observed in hepatocytes (Lisanti et al., 1990; Schell et al., 1992). This result indicated that MDCK cells sort GPI-APs at some intracellular site, but the sorting mechanism posed a puzzle. In transmembrane proteins, sorting signals typically reside in the part exposed to the cytoplasm, which enables recognition by the machinery for the generation and transport of vesicles (Matter and Mellman, 1994). GPI-APs, however, have no cytoplasmic part. A landmark study then found that a GPI-AP became associated with glycolipid-enriched detergent-resistant membranes while moving through the Golgi (Brown and Rose, 1992). This discovery provided the first experimental support for the idea that glycolipids generate apical transport platforms in the Golgi (Simons and Wandinger-Ness, 1990), a notion that later gave rise to the raft hypothesis (Simons and Ikonen, 1997). This initial work produced an appealingly simple model: correct targeting of GPI-APs is ensured by association with lipid rafts, which are destined for the apical membrane. In MDCK cells, sorting takes place intracellularly, probably at the Golgi, and apical delivery occurs along a direct transport route.

New results soon disagreed with this model. Epithelial Fisher rat thyroid cells, for instance, send most GPI-APs to the basolateral membrane (Zurzolo et al., 1993). It also became clear that some apical proteins in MDCK cells do not associate with rafts, whereas some basolateral proteins do. Thus, raft association alone is insufficient to dictate apical targeting, and additional mechanisms must be at work. Glycosylation was proposed to govern apical versus basolateral targeting of GPI-APs (Benting et al., 1999). Furthermore, oligomerization seems to be important, as apical but not basolateral GPI-APs form oligomers in the Golgi (Paladino et al., 2004). These findings have led to a refinement of the original model. It is now thought that the oligomerization or lectin-mediated cross-linking GPI-APs drive

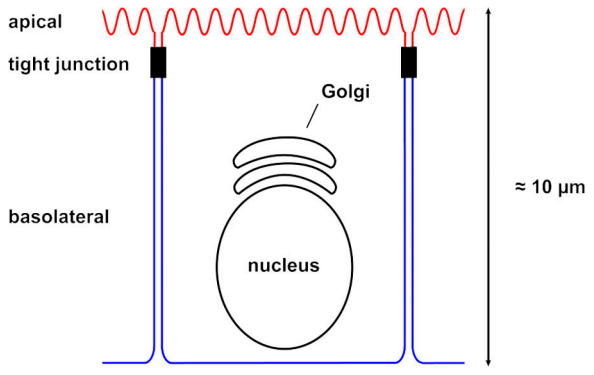

Figure 1. **MDCK cells form polarized epithelial monolayers.** The apical membrane (red) and the basolateral membrane (blue) are separated by tight junctions (black).

Correspondence to Kai Simons: simons@mpi-cbg.de

Abbreviations used in this paper: GPI, glycosylphosphatidylinositol; GPI-AP, GPI-anchored protein.

© The Rockefeller University Press $8.00
The Journal of Cell Biology, Vol. 172, No. 7, March 27, 2006 963–965
http://www.jcb.org/cgi/doi/10.1083/jcb.200603015

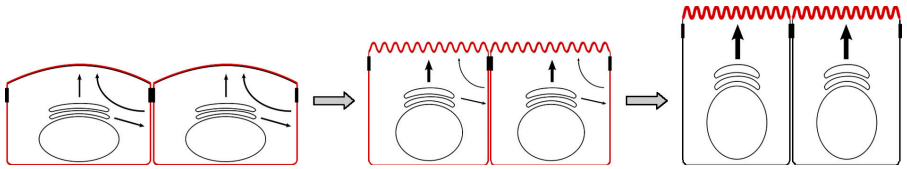

Figure 2. **GPI-AP trafficking can be reprogrammed during differentiation.** In polarizing MDCK cells, GPI-APs (red) are increasingly targeted to the apical membrane. This could be accompanied by a down-regulation of transcytotic apical delivery.

their inclusion into, and perhaps also the generation of, clustered rafts, which then facilitate apical transport of their constituents (Paladino et al., 2004; Schuck and Simons, 2004).

The model challenged

The original model was questioned even more fundamentally by a recent high-profile publication from the group of Jennifer Lippincott-Schwartz (Polishchuk et al., 2004). Using live-cell imaging of nonpolarized cells, the authors first provided evidence that GPI-APs and basolateral proteins leave the Golgi in the same transport carriers. They then treated polarized MDCK cells with tannic acid, a fixative used for leather production but here applied for the first time to study polarized membrane trafficking. Striking images showed that GPI-anchored YFP failed to reach the apical membrane after tannic acid had inactivated transport from the basolateral domain. This startling result indicated that GPI-APs need to traverse the basolateral membrane. Finally, the authors demonstrated that GPI-anchored GFP undergoes transcytosis, i.e., that it can be endocytosed from the basolateral membrane and then travel to the apical side. Although it was not shown that this occurs for newly synthesized GFP-GPI, Polishchuk et al. (2004) concluded that GPI-APs reach the apical membrane via the basolateral domain. They proposed that GPI-APs are sorted at the basolateral membrane rather than at the Golgi.

These results contradicted many previous studies. Earlier live-cell imaging of nonpolarized cells had shown that apical and basolateral proteins leave the Golgi in separate transport carriers (Keller et al., 2001). Biochemical experiments had never detected the bulk of newly synthesized GPI-APs passing through the basolateral domain in polarized MDCK cells. Polishchuk et al. (2004) suggested that endocytosis was rapid, so that only few GPI-AP molecules would be present at the basolateral membrane at any given time and might have been missed. This explanation was hard to reconcile with the slow basolateral endocytosis of GPI-APs in MDCK cells (Lisanti et al., 1990). Polishchuk et al. (2004) showed that the transcytosis of GPI-APs has a half-time of >60 min, consistent with their slow exit from recycling endosomes and their slow transcytosis in hepatocytes (Schell et al., 1992; Chatterjee et al., 2001). However, GPI-APs typically appear at the apical membrane within 15 min of leaving the Golgi, making it difficult to see how they could complete the circuitous journey via the basolateral membrane quickly enough. These discrepancies emphasized the need for more incisive assays and more quantitative data. The contradicting imaging results in particular highlighted that live-cell imaging had to be extended to fully polarized cells, a difficult task given that MDCK monolayers are ~10 μm thick.

New results back up old conclusions

The groups of Chiara Zurzolo and Ira Mellman have now independently revisited the issue of the routes taken by newly synthesized GPI-APs (Paladino et al., p. 1023, and Hua et al., p. 1035, this issue). Paladino et al. (2006) first improved the biochemical analysis of GPI-AP transport in polarized MDCK cells using the same GFP-GPI construct as Polishchuk et al. (2004). To ensure that rapid passage through the basolateral domain would be detected, they treated the basolateral side with low concentrations of trypsin during transport. In this way, GFP-GPI appearing at the basolateral membrane would be cleaved. Cleavage did indeed occur, but only for the small fraction of GFP-GPI missorted to the basolateral side. The vast majority of newly synthesized GFP-GPI reached the apical membrane uncleaved, indicating that it travels along a direct pathway. Interestingly, the authors noticed that the accuracy of targeting increases during polarization. At early stages, the delivery of GPI-APs showed little preference for either surface domain but was restricted mostly to the apical membrane in fully polarized cells. Next, the authors reexamined the results obtained with tannic acid. When transport from the basolateral membrane was inhibited by a brief tannic acid treatment, GFP-GPI still reached the apical membrane. Importantly, when the conditions of Polishchuk et al. (2004) were replicated by prolonging the exposure to tannic acid, tight junction integrity was compromised and the segregation of apical and basolateral membranes was abolished. These observations stress that tannic acid should be used with utmost caution. Finally, the authors used spinning-disc confocal microscopy to achieve live-cell imaging of polarized MDCK cells. This allowed them to show that GFP-GPI accumulates only at the apical side after exit from the Golgi, reinforcing the conclusion that GPI-APs take a direct route.

Hua et al. (2006) approached the problem of visualizing surface transport in polarized MDCK cells more generally and focused on imaging Golgi to plasma membrane trafficking quantitatively. Using laser-scanning confocal microscopy, they were able to follow the transport of fluorescent apical and basolateral marker proteins for ~30 min. They then derived rate constants for Golgi exit and for passage through the cytosol by quantifying the amount of marker protein present at the Golgi, at the apical or basolateral membrane, and in the intervening cytosol at 1-min intervals. As one way of demonstrating the utility of their system, the authors used GPI-anchored YFP as an apical marker, a construct also analyzed by Polishchuk et al. (2004). The rate-limiting step for YFP-GPI transport was exit from the Golgi, the marker accumulated at the apical but never at the basolateral membrane, and surface arrival was largely

complete within 20 min. These data support direct apical transport. Interestingly, the authors noted that YFP-GPI seemed to pass through a subapical kinetic intermediate en route to the apical membrane. The live-cell imaging results were confirmed by antibody uptake experiments. An antibody against YFP was added to the basolateral side during transport so that YFP-GPI passing through the basolateral domain would be labeled. Only a small fraction of the YFP-GPI that appeared on the apical side was antibody bound. A known transcytotic protein, on the other hand, efficiently picked up the antibody during transport to the apical membrane. Also, these observations argue against transcytosis being a major pathway during the biosynthetic delivery of GPI-APs.

Case closed?

These reports leave little room for biosynthetic apical delivery of GPI-APs by a transcytotic route in fully polarized MDCK cells. It is not entirely clear why the data of Polishchuk et al. (2004) differ so much from all other studies, but the evidence against their conclusions seems overwhelming. So, was this a fairly unremarkable affair after all? We do not think so. Rather, we believe that the debate sparked by Polishchuk et al. (2004) has been quite productive in both a technical and a conceptual sense.

First, the controversy has stimulated the development of new methodology. Live-cell imaging of polarized MDCK cells holds great promise for resolving other open issues. For example, the question of exactly when and where apical and basolateral proteins separate may now be answered by tracking individual transport carriers. The tracking of early endosomes was recently achieved by fast live-cell imaging of nonpolarized cells (Rink et al., 2005). If imaging were restricted to the supranuclear Golgi region of polarized MDCK cells, multicolor tracking of Golgi-derived vesicles might now be feasible.

Second, the new results herald yet more refinements of the original model for GPI-AP trafficking. The observation that polarizing MDCK cells increasingly restrict the delivery of GPI-APs to the apical membrane is reminiscent of the situation in hippocampal neurons, in which the GPI-anchored prion protein is present in all neurites early during differentiation but localizes only to the axon later on (Galvan et al., 2005). In the developing *Drosophila melanogaster* embryo, GPI-APs are basolateral in the surface ectoderm but apical in ectoderm-derived internal epithelia (Shiel and Caplan, 1995). Such shifts could be achieved by a reorganization of intracellular trafficking. For instance, Fisher rat thyroid cells use a transcytotic mode of apical delivery at early stages of polarization but then switch to direct targeting (Zurzolo et al., 1992). Perhaps epithelial cells more generally abandon the transcytotic mode during differentiation (Fig. 2).

Future directions

Many questions remain. There is great variety in the structure of the GPI anchor itself, but we have little clue why. Maybe different anchors determine to what degree a GPI-AP can be sorted by inclusion into clustered rafts. The possible switching between transport routes during differentiation is very intriguing, but what could it be good for? To extend the list, neither do we understand how GPI-APs are endocytosed by different pathways. We are confident that the trafficking of GPI-APs will continue to provide ample opportunity for debate.

Submitted: 3 March 2006
Accepted: 6 March 2006

References

Benting, J.H., A.G. Rietveld, and K. Simons. 1999. N-glycans mediate the apical sorting of a GPI-anchored, raft-associated protein in Madin-Darby canine kidney cells. *J. Cell Biol.* 146:313–320.

Brown, D.A., and J.K. Rose. 1992. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell.* 68:533–544.

Brown, D.A., B. Crise, and J.K. Rose. 1989. Mechanism of membrane anchoring affects polarized expression of two proteins in MDCK cells. *Science.* 245:1499–1501.

Chatterjee, S., E.R. Smith, K. Hanada, V.L. Stevens, and S. Mayor. 2001. GPI anchoring leads to sphingolipid-dependent retention of endocytosed proteins in the recycling endosomal compartment. *EMBO J.* 20:1583–1592.

Galvan, C., P.G. Camoletto, C.G. Dotti, A. Aguzzi, and M.D. Ledesma. 2005. Proper axonal distribution of PrP(C) depends on cholesterol-sphingomyelin-enriched membrane domains and is developmentally regulated in hippocampal neurons. *Mol. Cell. Neurosci.* 30:304–315.

Hua, W., D. Sheff, D. Toomre, and I. Mellman. 2006. Vectorial insertion of apical and basolateral membrane proteins in polarized epithelial cells revealed by quantitative 3D live cell imaging. *J. Cell Biol.* 172:1035–1044.

Keller, P., D. Toomre, E. Diaz, J. White, and K. Simons. 2001. Multicolour imaging of post-Golgi sorting and trafficking in live cells. *Nat. Cell Biol.* 3:140–149.

Lisanti, M.P., I.W. Caras, M.A. Davitz, and E. Rodriguez-Boulan. 1989. A glycophospholipid membrane anchor acts as an apical targeting signal in polarized epithelial cells. *J. Cell Biol.* 109:2145–2156.

Lisanti, M.P., I.W. Caras, T. Gilbert, D. Hanzel, and E. Rodriguez-Boulan. 1990. Vectorial apical delivery and slow endocytosis of a glycolipid-anchored fusion protein in transfected MDCK cells. *Proc. Natl. Acad. Sci. USA.* 87:7419–7423.

Matter, K., and I. Mellman. 1994. Mechanisms of cell polarity: sorting and transport in epithelial cells. *Curr. Opin. Cell Biol.* 6:545–554.

Paladino, S., D. Sarnataro, R. Pillich, S. Tivodar, L. Nitsch, and C. Zurzolo. 2004. Protein oligomerization modulates raft partitioning and apical sorting of GPI-anchored proteins. *J. Cell Biol.* 167:699–709.

Paladino, S., T. Pocard, M.A. Catino, and C. Zurzolo. 2006. GPI-anchored proteins are directly targeted to the apical surface in fully polarized MDCK cells. *J. Cell Biol.* 172:1023–1034.

Polishchuk, R., A. Di Pentima, and J. Lippincott-Schwartz. 2004. Delivery of raft-associated, GPI-anchored proteins to the apical surface of polarized MDCK cells by a transcytotic pathway. *Nat. Cell Biol.* 6:297–307.

Rink, J., E. Ghigo, Y. Kalaidzidis, and M. Zerial. 2005. Rab conversion as a mechanism of progression from early to late endosomes. *Cell.* 122:735–749.

Schell, M.J., M. Maurice, B. Stieger, and A.L. Hubbard. 1992. 5′ nucleotidase is sorted to the apical domain of hepatocytes via an indirect route. *J. Cell Biol.* 119:1173–1182.

Schuck, S., and K. Simons. 2004. Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. *J. Cell Sci.* 117:5955–5964.

Shiel, M.J., and M.J. Caplan. 1995. Developmental regulation of membrane protein sorting in *Drosophila* embryos. *Am. J. Physiol.* 269:C207–C216.

Simons, K., and A. Wandinger-Ness. 1990. Polarized sorting in epithelia. *Cell.* 62:207–210.

Simons, K., and E. Ikonen. 1997. Functional rafts in cell membranes. *Nature.* 387:569–572.

Zurzolo, C., A. Le Bivic, A. Quaroni, L. Nitsch, and E. Rodriguez-Boulan. 1992. Modulation of transcytotic and direct targeting pathways in a polarized thyroid cell line. *EMBO J.* 11:2337–2344.

Zurzolo, C., M.P. Lisanti, I.W. Caras, L. Nitsch, and E. Rodriguez-Boulan. 1993. Glycosylphosphatidylinositol-anchored proteins are preferentially targeted to the basolateral surface in Fischer rat thyroid epithelial cells. *J. Cell Biol.* 121:1031–1039.