



HAL
open science

Sorting of GPI-anchored proteins from yeast to mammals - common pathways at different sites?

Manuel Muniz, Chiara Zurzolo

► To cite this version:

Manuel Muniz, Chiara Zurzolo. Sorting of GPI-anchored proteins from yeast to mammals - common pathways at different sites?. *Journal of Cell Science*, 2014, 127 (13), pp.2793-2801. 10.1242/jcs.148056 . pasteur-01111138

HAL Id: pasteur-01111138

<https://pasteur.hal.science/pasteur-01111138>

Submitted on 29 Jan 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

COMMENTARY

Sorting of GPI-anchored proteins from yeast to mammals – common pathways at different sites?

 Manuel Muñiz^{1,*} and Chiara Zurzolo^{2,*}

ABSTRACT

Glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-APs) are luminal secretory cargos that are attached by a post-translational glycolipid modification, the GPI anchor, to the external leaflet of the plasma membrane. GPI-APs are conserved among eukaryotes and possess many diverse and vital functions for which the GPI membrane attachment appears to be essential. The presence of the GPI anchor and its subsequent modifications along the secretory pathway confer to the anchored proteins unique trafficking properties that make GPI-APs an exceptional system to study mechanisms of sorting. In this Commentary, we discuss the recent advances in the field of GPI-AP sorting focusing on the mechanisms operating at the level of the exit from the ER and from the trans-Golgi network (TGN), which take place, respectively, in yeast and in polarized mammalian cells. By considering the similarities and differences between these two sorting events, we present unifying principles that appear to work at different sorting stations and in different organisms.

KEY WORDS: GPI-anchored proteins, Membrane trafficking, Sorting, Polarized epithelial cell, Yeast, Membrane domain

Introduction

In eukaryotic cells, proteins synthesized in the endoplasmic reticulum (ER) are subsequently sorted upon vesicular transport through the secretory pathway for delivery to their proper cellular destinations (Bonifacino and Glick, 2004). Glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-APs) are a particular category of luminal secretory cargos, which contain a soluble protein attached by a conserved post-translational glycolipid modification, the GPI anchor, to the external leaflet of the plasma membrane. The GPI anchor plays crucial roles both in the transport and targeting of the protein to the plasma membrane, as well as for its function (Ferguson et al., 2009). The GPI anchor consists of a lipid moiety with a glycan backbone; it is generated by a series of sequential reactions at the ER membrane before being added *en bloc* by transamidation to the C-terminus of a newly synthesized protein. After exiting the ER, GPI-APs are transported along the secretory pathway, through the Golgi complex, to their final destination, the plasma membrane, where they are involved in a wide variety of vital physiological roles, including signal transduction, cell–cell interactions, cell adhesion, host defense and cell wall biosynthesis (Orlean and Menon, 2007).

During their transport through the secretory pathway GPI-APs are segregated and sorted from other types of cell surface proteins. In yeast, this sorting event occurs during ER exit, whereas in mammalian polarized epithelial cells, it takes place in the Golgi during export from the trans-Golgi network (TGN) towards the apical plasma membrane (Mayor and Riezman, 2004; Muñiz and Riezman, 2000). GPI-AP segregation and sorting upon vesicle transport are proposed to be driven by their clustering into special membrane domains or ‘lipid rafts’ that are enriched in saturated lipids, such as sphingolipids and sterols, which would act as selective platforms for vesicle budding (Surma et al., 2012). This model is supported by the observations that purified Golgi-derived vesicles in yeast are enriched in sphingolipids and ergosterol (Klemm et al., 2009), and that long-chain sphingomyelin is required for vesicle biogenesis at the Golgi complex in mammalian cells (Duran et al., 2012).

Several mechanisms could contribute to the clustering of GPI-APs with saturated lipids and their subsequent co-sorting in specific vesicles. During their journey along the secretory pathway, the GPI-anchor undergoes a series of structural modifications, including the acquisition of a saturated lipid moiety, which could lead the GPI-APs to associate with saturated lipids into specialized domains (Fujita and Kinoshita, 2012). Interestingly, in yeast, the lipid remodeling of the GPI anchor has been shown to be required for GPI-AP segregation and sorting upon ER exit (Castillon et al., 2009). Additionally, in mammalian polarized epithelial cells, oligomerization and *N*-glycosylation of GPI-APs have been reported to be crucial for their apical sorting (Benting et al., 1999b; Catino et al., 2008; Imjeti et al., 2011). Thus, different mechanisms might cooperate to coordinately sort and route GPI-APs through their specific pathway to the plasma membrane.

In this Commentary, we will examine the proposed mechanisms involved in sorting of GPI-APs, which operate upon the exit from the ER and from the TGN respectively in yeast and in polarized mammalian cells. By considering the similarities and differences between these sorting events, we present here unifying principles of GPI-AP sorting and propose a general mechanism.

Sorting of GPI-APs at ER – the case of yeast cells

Initial studies suggested that most secretory cargo travels together in common coat protein complex II (COPII) carrier vesicles that form at ER exit sites (ERES) for transport to the Golgi (Lee et al., 2004), and that sorting would then occur at the Golgi to route cargo to their final destinations. Subsequent studies supported a model in which secretory pathway sorting that had been previously exclusively attributed to the Golgi compartment can also take place at the ER (Muñiz et al., 2001). Pioneering work in yeast demonstrated that GPI-APs are segregated from other plasma membrane proteins at the level of the ER, and then

¹Department of Cell Biology, University of Seville, Avda. Reina Mercedes s/n 41012 Seville, Spain. ²Institut Pasteur, Unité de Trafic Membranaire et Pathogénèse, 75724 Paris CEDEX 15, France.

*Authors for correspondence (mmuniz@us.es; zurzolo@pasteur.fr)

concentrated at specific ERES from where they are subsequently incorporated into distinct COPII vesicles for Golgi delivery (Castillon et al., 2009; Muñiz et al., 2001). This indicates that following ER exit, there are at least two parallel pathways in yeast that route different secretory proteins to the Golgi. Nevertheless, it is unknown whether GPI-APs continue to travel separately from other secretory proteins through the Golgi cisternae to the plasma membrane, or if they mix in the same Golgi cisternae to be sorted again in the TGN (Harsay and Bretscher, 1995).

Lipid-dependent sorting of GPI-APs into specific ERES

Segregation of GPI-APs from transmembrane proteins and their subsequent concentration into specific ERES has been shown to be independent of the COPII machinery and to be driven by a lipid-based mechanism, which involves the structural remodeling of the lipid moiety of the GPI anchor (Castillon et al., 2009) (Fig. 1). This process begins immediately after the GPI anchor is attached to the nascent protein and, in yeast, consists of inositol deacylation followed by replacement of the unsaturated short

fatty acid diacylglycerol chains (C18) with very long and more hydrophobic fatty acid chains (C26). Then, the C26 diacylglycerol is predominantly substituted by ceramide that contains also a very long saturated fatty acid (C26) (Fujita and Kinoshita, 2012). GPI-lipid remodeling allows for the biochemical isolation of GPI-APs *in vitro* in detergent-resistant membrane (DRM) fractions, which has been proposed to reflect their clustering into ceramide-enriched domains (Bagnat et al., 2000; Castillon et al., 2009) (Fig. 1). Interestingly, ER exit of GPI-APs requires ongoing synthesis of ceramide, which in yeast also takes place in the ER (Guillas et al., 2003). Therefore, these data indicate that ceramides and lipid-remodeled GPI-APs might cooperate to be co-sorted at specific ERES, from where they can be co-transported to the Golgi by COPII vesicles. The fact that both ceramides and lipid-remodeled GPI-APs contain a very long saturated fatty acid (C26) in contrast to the ER glycerolipids (C16 and C18), might favor their selective clustering by self-assembly at specific ER membrane domains. This in turn would facilitate the sorting and concentration of GPI-APs at ERES in a

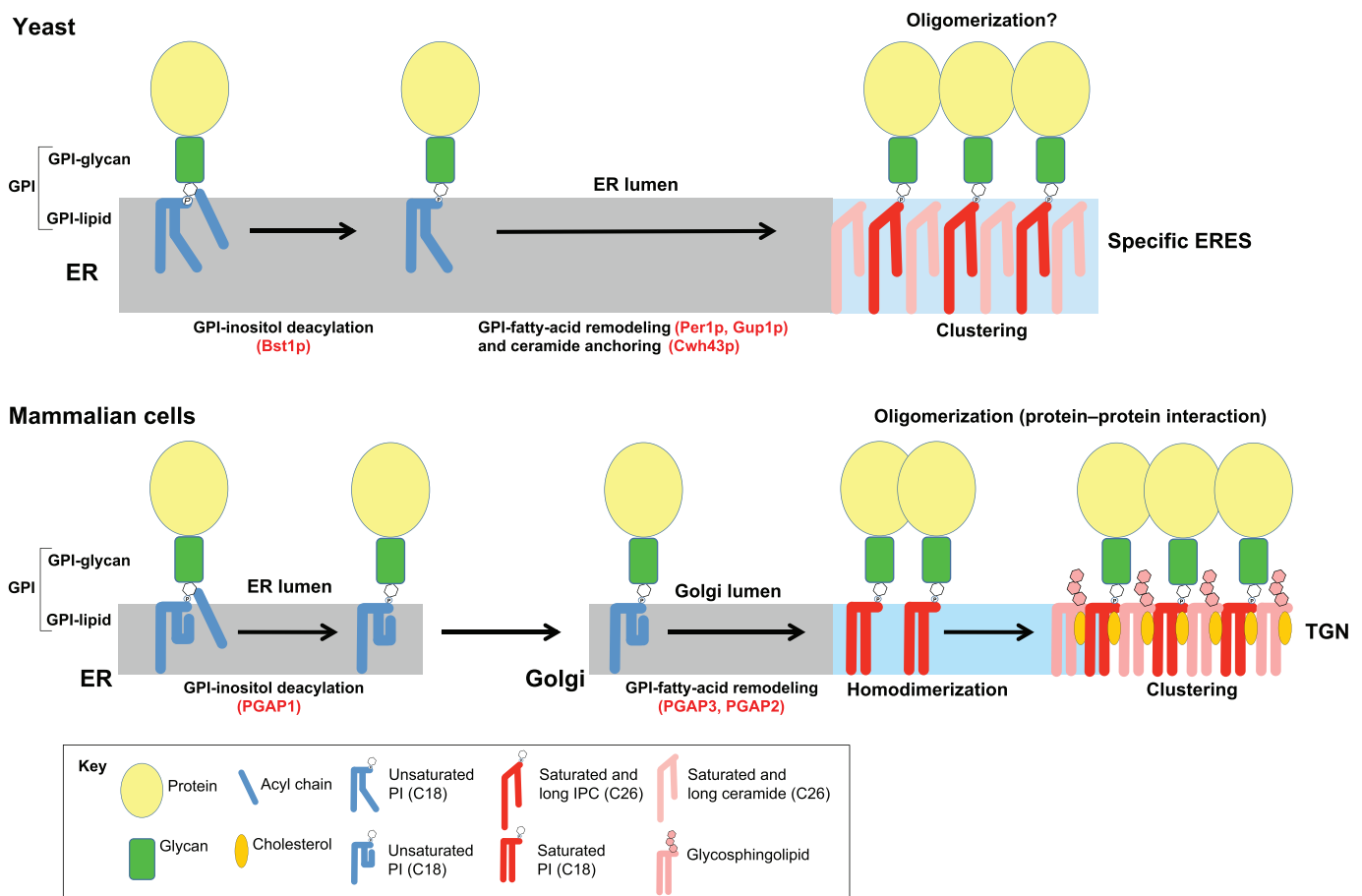


Fig. 1. GPI-lipid remodeling and clustering. After protein attachment, the GPI-lipid undergoes a structural remodeling that has the purpose of providing saturated lipids. In yeast, this process occurs entirely at the ER and consists of the inositol deacylation followed by the replacement of the primary unsaturated lipid moiety by another lipid containing a very long highly saturated fatty acid, usually ceramide (C26) (shown in the upper panel). In mammalian cells, the GPI-inositol is also deacylated at the ER, but the subsequent fatty acid remodeling to acquire a saturated fatty acid occurs at the Golgi (shown in the lower panel). This difference with regard to the site of lipid remodeling between yeast (ER) and mammals (Golgi) might be linked to the specific lipid environment. Yeast ceramides are synthesized in the ER and have the same length (C26) as GPI-lipids, which can lead to their clustering in specific membrane domains that sort them into specialized ERES. In contrast, in mammalian cells, ceramides that are made in the ER are shorter (C18–C24). Only at the Golgi, where ceramides are converted into sphingolipids and where cholesterol is enriched, a more saturated lipid environment that favours clustering of remodeled GPI-APs is generated. A possible intermediate step of homodimerization prior to oligomerization is indicated. For clarity, not all the intermediate remodeling steps are shown. Remodeling enzymes are labeled in red. GPI, glycosylphosphatidylinositol; PI, phosphatidylinositol; IPC, inositolphosphoceramide.

COPII-independent manner and provide an unfavorable membrane environment for the incorporation of transmembrane proteins. Consistently, yeast ceramides are required to stabilize the association of GPI-APs with the ER membrane (Watanabe et al., 2002).

This specific lipid-based sorting mechanism from yeast would not be expected to operate in mammalian cells as such, because mammalian GPI-APs acquire their saturated GPI-fatty acids in the Golgi instead of the ER, and mammalian ceramides are also shorter (C18–C24) (Kumagai et al., 2005). Consistently, unlike yeast, GPI-APs in mammalian cells have been found to be concentrated in the same ERES and COPII vesicles together with other secretory proteins (Rivier et al., 2010) (Fig. 2). In addition, the ER-to-Golgi transport of mammalian GPI-APs does not depend on *de novo* sphingolipid biosynthesis (Rivier et al., 2010), although, however, the transport of the longest ceramides (C24) depends on GPI-APs (Loizides-Mangold et al., 2012). Nevertheless, a minor fraction of newly synthesized mammalian GPI-APs are associated with DRMs in the ER in a cholesterol-dependent manner, suggesting a possible association of GPI-APs to cholesterol-enriched domains at this level (Bonnon et al., 2010; Sarnataro et al., 2002). However, this association would not be sufficient to sort GPI-APs into specific ERES and COPII vesicles.

Formation of GPI-AP-containing COPII vesicles

After being sorted and concentrated into specific ERES, GPI-APs must be packaged into COPII vesicles for delivery to the Golgi. Because GPI-APs do not span the ER membrane, incorporation into forming COPII vesicles would be expected to require a transmembrane cargo-coat adaptor (or cargo receptor). In yeast, as well as in mammalian cells, the COPII adaptor role for GPI-APs is fulfilled by proteins of the conserved p24 family (Castillon et al., 2011; Fujita et al., 2011) (Fig. 2). These are abundant type I transmembrane proteins with a large luminal domain and a short cytoplasmic tail, harboring binding signals for both COPII and COPI coats. Different p24 proteins assemble in a heteromeric complex that continuously cycles between ER and Golgi compartments (Dancourt and Barlowe, 2010; Strating and Martens, 2009). The yeast p24 complex is required for the incorporation of GPI-APs into COPII vesicles (Muñiz et al., 2000) (Fig. 2). Moreover, disruption of the p24-protein-binding site on Lst1p, an isoform of the Sec24p cargo-binding subunit of the COPII coat (Peng et al., 2000), specifically impairs the ER-to-Golgi transport of GPI-APs, suggesting that the yeast p24 complex functions as an adaptor that links them to the COPII coat (Castillon et al., 2011). However, this complex is not involved in cargo concentration, and recognizes preferentially GPI-APs after their lipid remodeling (Castillon et al., 2011); therefore its linker

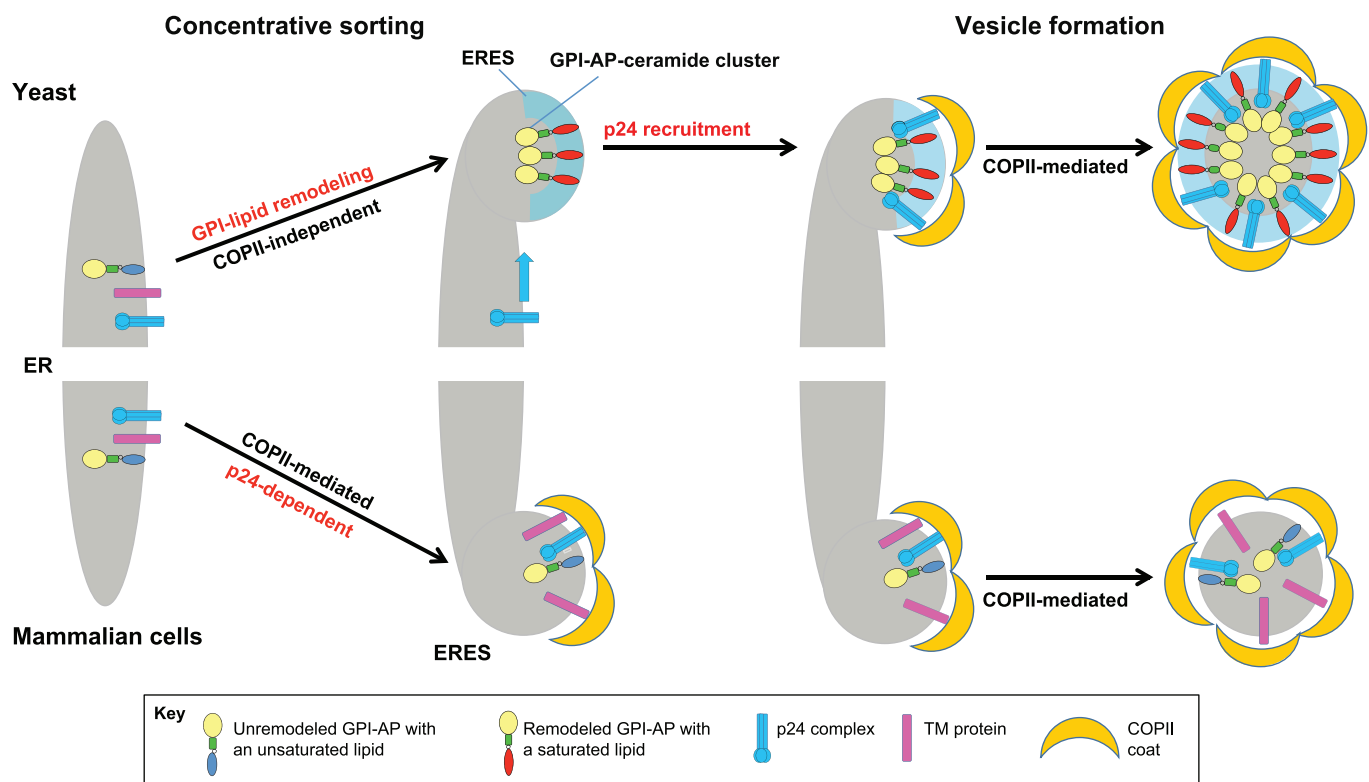


Fig. 2. GPI-AP sorting upon ER exit in yeast. A model for the ER export mechanism of GPI-APs in yeast (upper panel) and mammalian cells (lower panel) is shown. In yeast, GPI-APs are sorted from transmembrane cargo proteins and concentrated at specific ERES upon GPI-lipid remodeling. This process provides the GPI-APs with a long saturated fatty acid (C26) that might favor their selective clustering with the, also very long saturated ceramide chains (C26), through self-assembly at specific ER membrane domains. This in turn might facilitate GPI-AP sorting and their concentration at ERES in a COPII-independent manner. The p24 complex is subsequently recruited to these specific ERES by binding to the fully remodeled GPI-APs and so connects them to the cytosolic COPII coat and ensures their efficient incorporation into the nascent COPII vesicle. In contrast, in mammalian cells GPI-fatty acid remodeling does not occur in the ER and therefore GPI-APs are not concentrated at ERES by a lipid-based mechanism. Instead, they might use a COPII-mediated mechanism for their concentration in which the p24 complex recognizes GPI-APs that contain an unsaturated fatty acid and couples them with the COPII coat assembly that drives luminal cargo concentration at ERES and subsequent vesicle budding.

function must take place after remodeling and concentration at ERES. It is conceivable that the p24 complex is recruited to specific ERES that contain pre-concentrated remodeled GPI-APs in order to connect them to the cytosolic COPII coat and thus ensures their efficient incorporation into the nascent COPII vesicle (Fig. 2). In addition, in mammalian cells, the p24 proteins selectively bind and export GPI-APs from the ER (Fujita et al., 2011). However, in contrast to yeast, in mammalian cells, the p24 complex is required for concentration of GPI-APs into ERES. This is likely due to the fact that GPI-fatty acid remodeling of mammalian GPI-APs does not occur in the ER and therefore they are not concentrated at ERES by a lipid-based mechanism, but use a COPII-mediated concentration mechanism by which the COPII coat assembly drives luminal cargo concentration through the p24 proteins (Fujita et al., 2011) (Fig. 2).

Several lines of evidence indicate that the p24 proteins interact with GPI-APs through their GPI anchor (Castillon et al., 2011). Because the yeast p24 complex preferentially binds lipid-remodeled GPI-APs, it is likely that it recognizes the newly remodeled lipid. Interestingly, in mammalian cells, the transmembrane domain of the p24 protein appears to interact directly and specifically with one sphingomyelin species (N-stearoyl sphingomyelin or SM 18, a ceramide C18 with a phosphorylcholine moiety attached to position 1) at the Golgi (Contreras et al., 2012). Alternatively (or additionally), the p24 complex could interact with the GPI-glycan which, in mammalian cells, is also remodeled before ER exit by metallophosphoesterase 1 (MPPE1, also known as PGAP5) which removes the ethanolamine-phosphate (EtN-P) from its second mannose (Fujita et al., 2009). Because this remodeling step is required (like the GPI-inositol deacylation) for the binding of p24 to GPI-APs, it is possible that the p24 complex recognizes the remodeled GPI-glycan in addition to the GPI-lipid. In yeast, there are two homologs of PGAP5, Ted1p and Cdc1p, which could potentially perform the same GPI-glycan remodeling reaction (Fujita et al., 2011). Nevertheless, whether the p24 complex physically recognizes remodeled structures on the GPI anchor remains to be directly addressed. In addition, it is also unknown how the different GPI anchor remodeling processes are coordinated to allow the binding and export function of the p24 complex.

COPII vesicle formation from specific ERES with GPI-APs presents special requirements. A recent study suggests that the highly asymmetric membrane topology of both p24 and GPI-APs must influence the mechanics of COPII budding (Copic et al., 2012). Because both types of proteins have the bulk of their mass residing in the ER lumen, their localized concentration at specific ERES could create excess steric pressure on the luminal side of the ER membrane that resists the outwards membrane bending required to form a COPII vesicle. The authors of this work propose that this potential problem is overcome by the presence of Sec13p, a subunit of the outer layer of the COPII coat, which would provide the sufficient rigidity to drive membrane bending against this opposing resistance (Copic et al., 2012). In agreement with this possibility, Sec13p, which is normally essential in yeast, becomes dispensable in the absence of both p24 and remodeled GPI-APs. However, another possibility is that clustering of GPI-APs at the ERES would result in the segregation of saturated lipids and therefore create high tension at the edges of these domains that would be naturally released by the vesicle budding. In addition, it has been shown that the ER export of GPI-APs requires the specific COPII protein Lst1p, an isoform of the Sec24p cargo binding subunit (Peng et al., 2000). Because Lst1p

contains a binding site for p24 proteins (Miller et al., 2003), one specific role of the p24 complex might be to link Lst1p to GPI-APs. In line with this possibility, it has been observed that the specific interaction of p24 proteins with Lst1p is required for the efficient ER trafficking of GPI-APs (Castillon et al., 2011). Interestingly, COPII vesicles that are produced *in vitro* are larger if Lst1p is present during the budding reaction. This suggests that Lst1p can be used to ensure the packaging of potentially large cargoes, such as clusters of GPI-APs or oligomers of the plasma membrane ATPase Pma1p, whose export from the ER is also facilitated by Lst1p (Shimoni et al., 2000). Nevertheless, how Lst1p is specifically attracted to specific ERES to accommodate GPI-APs into specialized COPII vesicles remains to be explored.

During their transport or right upon arrival at the Golgi, remodeled GPI-APs dissociate from the p24 complex, most likely in a pH-dependent manner (Fujita et al., 2011). Upon their release, remodeled GPI-APs can progress through the secretory pathway and p24 proteins can be recycled to the ER in COPI vesicles. Of interest, in yeast it has been reported that the retrieval of the p24 complex is involved in a post-ER quality control system that monitors the completion of anchor remodeling and contributes to the retention of unremodeled GPI-APs in the ER (Castillon et al., 2011). It would be interesting to explore whether this post-ER quality control system also operates in mammalian cells.

Sorting of GPI-APs at Golgi – the case of polarized epithelial cells

In polarized epithelial cells which display (at least) two distinct plasma membrane domains (apical and basolateral) (Rodriguez-Boulau et al., 2005), sorting occurs mainly at the level of the TGN, where proteins are segregated into vesicles that undertake different routes to the plasma membrane (Weisz and Rodriguez-Boulau, 2009; Cao et al., 2012) (Fig. 3). In contrast to basolateral proteins, which contain conserved sorting sequences in their cytosolic tail (Fölsch et al., 1999; for a review, see Rodriguez-Boulau et al., 2005), different signals have been found within the tail, the transmembrane or cytosolic part of apically sorted proteins, but there is no consensus sequence (Rodriguez-Boulau et al., 2005). Furthermore, N- or O-glycans on the luminal domain can also act as apical determinants (Alfalah et al., 1999; Gut et al., 1998; Rodriguez-Boulau and Gonzalez, 1999; Yeaman et al., 1997; Scheiffele et al., 1995).

GPI-APs are predominantly sorted to the apical surface. This appears to be dependent on their inclusion into sphingolipid (more specifically glycosphingolipid, GSL) and cholesterol-rich microdomains (or rafts), which might act as apical sorting platforms at the level of the TGN (Simons and Ikonen, 1997). Pulse-chase experiments have shown that, following fatty acid remodeling of the GPI-anchor (Maeda et al., 2007; Fujita and Kinoshita, 2012), GPI-APs acquire resistance to detergent extraction during their transport through the Golgi, and co-migrate with glycosphingolipids and cholesterol on sucrose density gradients (Brown and Rose, 1992; Zurzolo et al., 1994). Furthermore, inhibitors of sphingolipid biosynthesis and/or removal of cholesterol impairs their apical sorting, supporting the original hypothesis that raft association at the TGN is involved in their apical transport (Paladino et al., 2004; Lipardi et al., 2000). However, resistance to detergents only defines a biochemical property of the GPI-AP and not an association with specific membrane domains *in vivo*. Furthermore, in different epithelial cell lines, some GPI-APs are sorted basolaterally

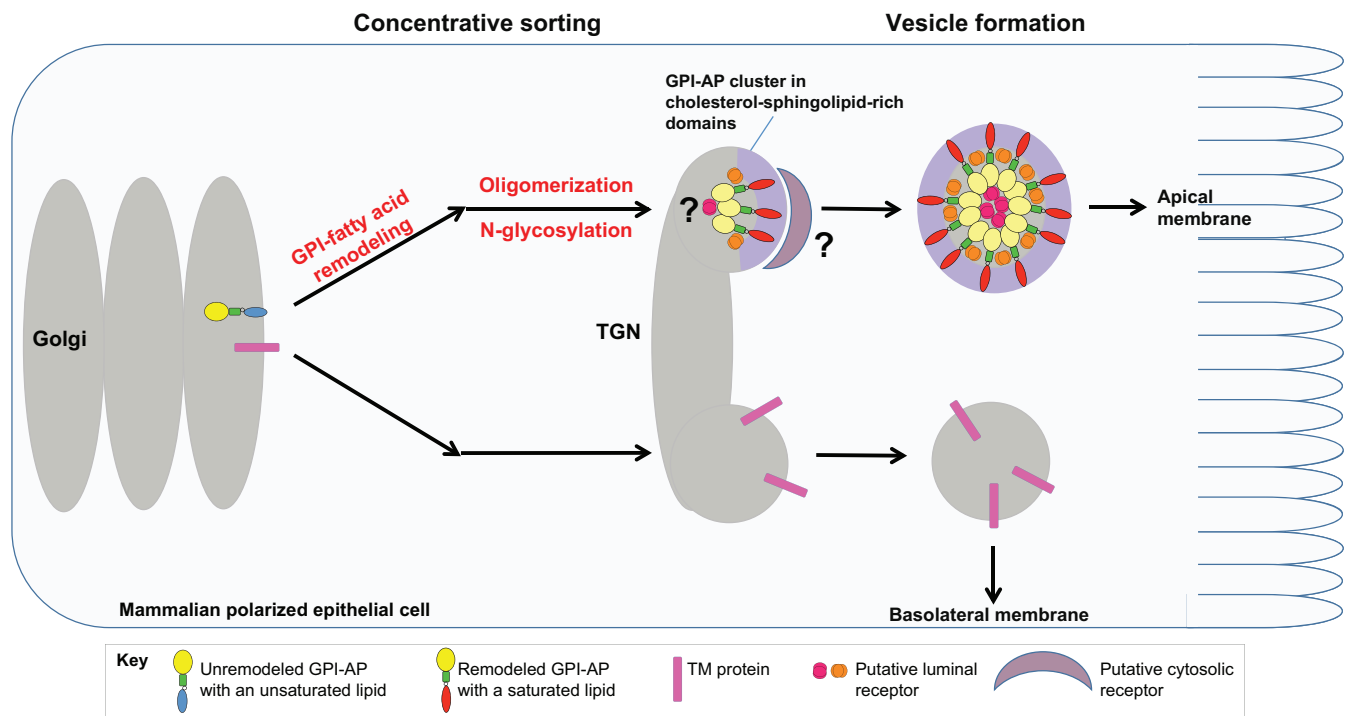


Fig. 3. GPI-AP sorting upon TGN exit in polarized epithelial cells. A schematic model for the sorting mechanism of GPI-APs at the TGN in polarized epithelial cells is shown. Upon GPI-lipid remodeling with saturated fatty acid chains in the Golgi, GPI-APs can be segregated from other transmembrane proteins (TM) into sphingolipids and cholesterol-enriched domains. Further segregation would then occur as consequence of the oligomerization process that might involve putative luminal receptors binding either the ectodomain or the lipid anchor. Vesicle formation and budding might derive from the coalescence of lipid domains that are driven by the protein oligomerization. Putative cytosolic receptors might also facilitate vesicle budding. Note that the mechanism of sorting of transmembrane proteins is not illustrated for clarity.

despite their association with DRMs (Zurzolo et al., 1993; Paladino et al., 2004; Benting et al., 1999a; Sarnataro et al., 2002) indicating that DRM association is a general property of GPI-APs, and although it might be required, it is not the primary mechanism for apical sorting (Paladino et al., 2004).

Oligomerization at the Golgi as a specific apical sorting mechanism for GPI-APs

One specific property of apical GPI-APs compared to basolateral ones is their ability to form high-molecular-mass oligomers at the Golgi (Paladino et al., 2004) (Fig. 1). This appears to be an essential step for GPI-AP apical sorting in different epithelial cells, as impairment of oligomerization results in their missorting to the basolateral domain (Paladino et al., 2007; Paladino et al., 2004). Pulse-chase experiments, followed by separation on sucrose velocity gradients, demonstrated that GPI-APs oligomerize during their passage through the Golgi concomitantly to their acquisition of detergent resistance. This suggests that oligomerization might cooperate with segregation of GPI-APs in specific membrane domains to promote apical sorting (Paladino et al., 2004). Indeed, whereas both apical and basolateral GPI-APs dynamically partition into rafts because of the chemical properties of the GPI anchor (Simons and Sampaio, 2011), it is possible that in order to be included in a raft-enriched apical vesicle GPI-APs need to be stabilized into rafts. Oligomerization might promote this process by increasing the affinity of apical GPI-APs for rafts, as previously suggested in the case of cluster induced sorting of GPI-APs in early endosomes (Fivaz et al., 2002; Howes et al., 2010).

Alternatively, GPI-APs oligomerization could lead to the clustering of small rafts into larger and more stable domains. This will increase the length of the line tension at the boundary between the raft and non-raft phases (an energy per unit length of boundary owing to differences in thickness between the rafts and the surrounding area), which could be relieved by the budding of the domain and subsequent fission of the vesicle at the boundaries (Roux et al., 2005; Allain et al., 2004; Schuck and Simons, 2004; Cao et al., 2012; Surma et al., 2012) (Fig. 3). Similarly, the multivalent binding of the pentameric shiga toxins to the glycosphingolipid GB3 at the plasma membrane has been shown to induce lipid clustering, enhancing phase separation (Römer et al., 2007) and resulting in tubular membrane formation (Ewers et al., 2010).

A growing bud could also facilitate protein sorting by recruiting curvature-preferring proteins into the lipid raft platform, further increasing the propensity to generate curvature in a feedback loop (van Meer and Vaz, 2005; Tian and Baumgart, 2009; Huttner and Zimmerberg, 2001; McMahon and Gallop, 2005; Kuzmin et al., 2005). Thus, the mechanism of oligomerization of GPI-APs could have a double role: first, it would enable GPI-APs to be segregated from the remainder of the proteins in Golgi membranes and, second, it would drive the budding of an apical vesicle, inducing coalescence of 'raft-like' domains.

Mechanism of oligomerization

One of the burning questions is what are the molecular determinants that drive oligomerization of apical GPI-APs compared to basolateral ones. Oligomerization could be driven

by interactions that involve the protein ectodomain, the GPI anchor and/or the surrounding lipid environment (Paladino et al., 2008) (Fig. 1). The evidence showing that oligomerization occurs concomitantly with association to the raft and that cholesterol depletion impairs oligomerization in the Golgi, points towards a role of the GPI anchor and the surrounding lipid environment (Paladino et al., 2004). Interestingly, a reporter GPI-AP (GFP-FR) in which GFP was fused to the GPI attachment signal of an apical GPI-AP (folate receptor, FR) oligomerizes and is apically sorted. By contrast, GFP-PrP, in which GFP is fused to the GPI attachment signal of a basolateral GPI-AP (prion protein, PrP), is sorted basolaterally and does not oligomerize (Paladino et al., 2008). Strikingly, cholesterol addition enables the basolaterally directed GPI-APs to form oligomers, results in lower diffusional mobility in the Golgi membranes and causes their partial rerouting to the apical surface (Paladino et al., 2008; Lebreton et al., 2008). One possibility is that cholesterol addition is able to stabilize the segregation of basolateral GPI-APs into lipid rafts, thus allowing their oligomerization and apical sorting. Alternatively, an increased concentration of cholesterol might change the characteristics of the Golgi lipid environment, thereby enabling the protein to oligomerize and to be sorted into apically delivered vesicles. Cholesterol might also have a more direct role, not linked to the existence of specific membrane domains, and somehow ‘force’ the clustering of an unremodeled or differently remodeled anchor. Indeed, as in the case of raft association (Fujita and Kinoshita, 2012; Maeda and Kinoshita, 2011), it might be possible that oligomerization relies in part upon the remodeling of the anchor, and that different GPI anchors would have a different sensitivity for the Golgi remodeling enzymes. Interestingly, lyso-GPI proteins (an intermediate precursor in the GPI lipid remodeling process) are apically sorted independently of raft association and are sensitive to cholesterol depletion (Castillon et al., 2013). Whether this apical sorting is mediated by protein oligomerization remains to be established. However, one should be cautious in the interpretation of this data because lyso-GPIs contain an incomplete lipid moiety (without the unsaturated acyl-chain) that might not reflect the biochemical properties of the native unremodeled GPI anchor (containing phosphatidylinositol).

In addition, the protein ectodomain might have an important role in GPI-AP oligomerization. Once formed, oligomers are insensitive to cholesterol depletion, suggesting the involvement of protein–protein interactions (Paladino et al., 2004). Indeed, a double cysteine mutation (S49C/S71C) in the GFP ectodomain of GFP-FR, which compromises its ability to dimerize, results in impaired oligomerization and missorting (Paladino et al., 2004). In this case, cholesterol addition does not reverse this phenotype, suggesting that the intrinsic ability of the protein to dimerize is necessary (Paladino et al., 2008). However, evidence exists for a role of lipid anchor remodeling in GPI-AP homodimerization (Fig. 3) (Seong et al., 2013). Consistently, recent data support the idea that the dimer is the fundamental unit for the clustered organization of GPI-APs at the plasma membrane in fibroblasts (Suzuki et al., 2012). Although fibroblasts and polarized epithelial cells might share this requirement, we have recently shown that the plasma membrane organization of GPI-APs is considerably different between fibroblasts and epithelial cells. Furthermore, we find that, in polarized epithelial cells, the oligomerization-based sorting mechanism of GPI-APs in the Golgi appears to regulate both their organization and function at the apical membrane (Paladino et al., 2014).

Non-covalent interactions between protein ectodomains that are mediated by post-translational modifications might be responsible for the formation of oligomers. Whereas the addition of N-linked glycans to the GPI-anchored form of rat growth hormone has been shown to be necessary for its apical delivery (Benting et al., 1999b), contrasting data exist regarding the role of N-glycosylation of native GPI-APs (Pang et al., 2004; Potter et al., 2004). In Madin–Darby canine kidney (MDCK) cells, mutagenesis of both N- and O-glycosylation sites does not affect oligomerization and apical sorting of PLAP (placental alkaline phosphatase, a model GPI-AP), whereas treatment with tunicamycin, which blocks N-linked glycosylation, does (Catino et al., 2008). This indicates that N-linked sugars of protein ectodomains are not directly involved in the apical sorting of GPI-APs, but that an N-glycosylated interactor might be involved (Catino et al., 2008; Rodriguez-Boulan and Gonzalez, 1999). In contrast, both in Caco2 and MDCK cells, mutagenesis of both glycosylation sites of porcine membrane dipeptidyl peptidase (MDP) impairs its apical sorting, although oligomerization of the protein was not assessed in this study (Pang et al., 2004). Finally, in Fisher rat thyroid (FRT) cells, point mutation of the N-glycosylation sites on the protein ectodomain affects PLAP oligomerization. Interestingly, in these cells, the Golgi is saturated with cholesterol compared to MDCK cells, and addition of cholesterol is not sufficient to allow oligomerization and apical sorting of basolateral GPI-APs. Therefore, these data indicate that at least two oligomerization mechanisms exist, one that is cholesterol-dependent and one that is cholesterol-independent and depends on glycosylation (Imjeti et al., 2011).

Additional factors: involvement of putative receptors

Another question is whether oligomerization is sufficient, or accessory mechanisms are at play, that result in the sorting of GPI-APs into apically delivered vesicles (Paladino et al., 2004; Paladino et al., 2007). Morphological studies indicate that GPI-APs are segregated from transmembrane proteins in the TGN, from where they exit in distinct vesicles (Keller et al., 2001; Guerriero et al., 2008). In addition, it has been reported that GPI-AP-carrying vesicles emerge from large Golgi domains with a spherical appearance, in contrast to the elongated Golgi extensions from which basolateral carriers appear to arise (Luini et al., 2005). The process of carrier generation probably does not rely exclusively on lipid clustering. Membrane-bending factors such as BAR-domain-containing proteins (Peter et al., 2004) or the insertion of a small amphipathic or hydrophobic wedge to induce membrane asymmetry and curvature (McMahon and Gallop, 2005) are likely to be required. Recently, depletion of four-phosphate-adaptor protein 2 (FAPP2), which contains a hydrophobic wedge in its PH domain, by RNA interference (Cao et al., 2009; Lenoir et al., 2010) was found to result in delayed delivery and/or intracellular accumulation of apical raft-associated proteins (either transmembrane proteins or GPI-APs) in polarized MDCK cells, whereas their basolateral transport was unaffected (Vieira et al., 2005).

It is also unclear whether specific cargo receptors and coat proteins on the cytosolic side are required for the formation of vesicles that contain raft components, including GPI-APs. GPI-AP oligomerization and, consequently, raft clustering could be promoted by a variety of raft-associated proteins (Schuck and Simons, 2004). For instance, the protein VIP17/MAL has been shown to be required for the apical delivery of some, but not all GPI-APs (Cheong et al., 1999; Martín-Belmonte et al., 2000).

Other factors, including caveolins, flotillins and stomatin, and raft-associated oligomerizing proteins have also been hypothesized to promote GPI-AP clustering (Schuck and Simons, 2004). However, it is not clear how these proteins, which are linked to the cytoplasmic leaflet of membranes through their myristoylated or palmitoylated tail, could interact with GPI-APs in the ectoplasmic leaflet. Similarly, clustering of raft lipids on the cytoplasmic side by annexin oligomers (such as of annexin II and annexin XIII), should cluster raft components in the exoplasmic leaflet (Jacob et al., 2004; Fiedler et al., 1995; Lafont et al., 1998; Lecat et al., 2000). Thus, although the mechanistic roles of these proteins have not been elucidated, it will be very interesting to explore their possible function in apical sorting of GPI-APs.

Finally galectin-4, which interacts with the sulfatides that carry long-chain-hydroxylated fatty acids and that are specifically enriched in lipid rafts, could be able to cross-link rafts and lead to the generation of lipid-raft-enriched transport carriers. Depletion of galectin-4 has been shown to affect apical trafficking of both transmembrane and GPI-anchored raft-associated proteins (Delacour et al., 2005), suggesting it has a more general involvement in the transport of all apical proteins, and is not a specific sorting receptor for GPI-APs. More recent data point towards galectin-9 as a stronger candidate to have a clustering function, owing to its binding for the Forssman glycolipid, the major apical glycosphingolipid in MDCK cells (Mishra et al., 2010). Furthermore, the GPI anchor of certain GPI-APs can be modified with the addition of branched *N*-acetylgalactosamine (GalNAc) residues to the GPI-glycan (Ferguson et al., 2008), which could be potentially recognized by multivalent galectins. Thus, it is conceivable that a joint recognition of the glycan moieties of GPI anchors and of the surrounding glycosphingolipids by galectins could promote the stabilization of GPI-AP clusters in GSL-enriched domains, thereby facilitating their sorting into apically delivered vesicles. Further studies will be necessary to test this hypothesis.

Conclusions and perspectives

The comparison of the events of GPI-AP sorting upon exit from the ER in yeast and from the TGN in polarized epithelial cells, respectively, points to several significant analogies that could be reconciled in a common mechanism. First, both sorting processes occur in a specialized saturated lipid environment: very long chain ceramides at the ER in yeast, and sphingolipids and cholesterol at the TGN in polarized epithelial cells. In addition, GPI-AP sorting appears to require the full lipid remodeling of the GPI anchor with saturated lipid as long as the ceramides in yeast or the sphingolipids in polarized epithelial cells (Fig. 1). Because in yeast GPI-APs are sorted from the ER upon GPI-lipid remodeling, it is conceivable that, in mammalian cells, GPI-APs also rely on this process for their sorting, although this remains to be shown. GPI-lipid remodeling can lead to the lateral association of GPI-APs with saturated lipids, thereby creating specialized domains at the ER or TGN membrane. These initial clusters can be further stabilized by oligomerization through interactions between the protein ectodomains of the GPI-APs to segregate them from other secretory proteins and that, subsequently, act as selective vesicle budding platforms for sorting, as proposed for apical sorting in polarized epithelial cells (Figs 2, 3). This mechanism could also operate in yeast although this has still to be investigated. Finally, sorting upon vesicle formation could be also favored if the level of GPI-AP clustering is further increased by cargo receptors that bind glycosphingolipids, such as galectin-9 in mammalian cells or GPI-APs as is the case for the p24

complex in yeast. Testing the predictions of this model in both systems represents an exciting challenge for future research.

Acknowledgements

We thank Simona Paladino and Stéphanie Lebreton for critical reading of the manuscript and the members of the Zurzolo and Muñiz laboratories for their work and helpful discussion.

Competing interests

The authors declare no competing interests.

Funding

This work was supported by grants from the Spanish Ministry of Economy and Competitiveness and Junta de Andalucía to M.M.; and by Agence nationale de la recherche and European Union FP7 to C.Z.

References

- Alfalah, M., Jacob, R., Preuss, U., Zimmer, K. P., Naim, H. and Naim, H. Y. (1999). O-linked glycans mediate apical sorting of human intestinal sucrase-isomaltase through association with lipid rafts. *Curr. Biol.* **9**, 593–596.
- Allain, J. M., Storm, C., Roux, A., Ben Amar, M. and Joanny, J. F. (2004). Fission of a multiphase membrane tube. *Phys. Rev. Lett.* **93**, 158104.
- Bagnat, M., Keränen, S., Shevchenko, A., Shevchenko, A. and Simons, K. (2000). Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast. *Proc. Natl. Acad. Sci. USA* **97**, 3254–3259.
- Benting, J., Rietveld, A., Ansoorge, I. and Simons, K. (1999a). Acyl and alkyl chain length of GPI-anchors is critical for raft association in vitro. *FEBS Lett.* **462**, 47–50.
- Benting, J. H., Rietveld, A. G. and Simons, K. (1999b). 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.
- Bonifacino, J. S. and Glick, B. S. (2004). The mechanisms of vesicle budding and fusion. *Cell* **116**, 153–166.
- Bonnon, C., Wendeler, M. W., Paccaud, J. P. and Hauri, H. P. (2010). Selective export of human GPI-anchored proteins from the endoplasmic reticulum. *J. Cell Sci.* **123**, 1705–1715.
- Brown, D. A. and Rose, J. K. (1992). Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell* **68**, 533–544.
- Cao, X., Coskun, U., Rössle, M., Buschhorn, S. B., Grzybek, M., Dafforn, T. R., Lenoir, M., Overduin, M. and Simons, K. (2009). Golgi protein FAPP2 tubulates membranes. *Proc. Natl. Acad. Sci. USA* **106**, 21121–21125.
- Cao, X., Surma, M. A. and Simons, K. (2012). Polarized sorting and trafficking in epithelial cells. *Cell Res.* **22**, 793–805.
- Castillon, G. A., Watanabe, R., Taylor, M., Schwabe, T. M. and Riezman, H. (2009). Concentration of GPI-anchored proteins upon ER exit in yeast. *Traffic* **10**, 186–200.
- Castillon, G. A., Aguilera-Romero, A., Manzano-Lopez, J., Epstein, S., Kajiwara, K., Funato, K., Watanabe, R., Riezman, H. and Muñiz, M. (2011). The yeast p24 complex regulates GPI-anchored protein transport and quality control by monitoring anchor remodeling. *Mol. Biol. Cell* **22**, 2924–2936.
- Castillon, G. A., Michon, L. and Watanabe, R. (2013). Apical sorting of lysoGPI-anchored proteins occurs independent of association with detergent-resistant membranes but dependent on their N-glycosylation. *Mol. Biol. Cell* **24**, 2021–2033.
- Catino, M. A., Paladino, S., Tivodar, S., Pocard, T. and Zurzolo, C. (2008). N- and O-glycans are not directly involved in the oligomerization and apical sorting of GPI proteins. *Traffic* **9**, 2141–2150.
- Cheong, K. H., Zaccetti, D., Schneeberger, E. E. and Simons, K. (1999). VIP17/MAL, a lipid raft-associated protein, is involved in apical transport in MDCK cells. *Proc. Natl. Acad. Sci. USA* **96**, 6241–6248.
- Contreras, F. X., Ernst, A. M., Haberkant, P., Björkholm, P., Lindahl, E., Gönen, B., Tischer, C., Elofsson, A., von Heijne, G., Thiele, C. et al. (2012). Molecular recognition of a single sphingolipid species by a protein's transmembrane domain. *Nature* **481**, 525–529.
- Copic, A., Latham, C. F., Horlbeck, M. A., D'Arcangelo, J. G. and Miller, E. A. (2012). ER cargo properties specify a requirement for COPII coat rigidity mediated by Sec13p. *Science* **335**, 1359–1362.
- Dancourt, J. and Barlowe, C. (2010). Protein sorting receptors in the early secretory pathway. *Annu. Rev. Biochem.* **79**, 777–802.
- Delacour, D., Gouyer, V., Zanetta, J. P., Drobegq, H., Leteurtre, E., Grard, G., Moreau-Hannedouche, O., Maes, E., Pons, A., André, S. et al. (2005). Galectin-4 and sulfatides in apical membrane trafficking in enterocyte-like cells. *J. Cell Biol.* **9**, 491–501.
- Duran, J. M., Campelo, F., van Galen, J., Sachsenheimer, T., Sot, J., Egorov, M. V., Rentero, C., Enrich, C., Polishchuk, R. S., Goñi, F. M. et al. (2012). Sphingomyelin organization is required for vesicle biogenesis at the Golgi complex. *EMBO J.* **31**, 4535–4546.
- Ewers, H., Römer, W., Smith, A. E., Bacia, K., Dmitrieff, S., Chai, W., Mancini, R., Kartenbeck, J., Chambon, V., Berland, L. et al. (2010). GM1 structure determines SV40-induced membrane invagination and infection. *Nat. Cell Biol.* **12**, 11–18.

- Ferguson, M. A., Kinoshita, T. and Hart, G. W. (2008). Glycosylphosphatidylinositol anchors. In *Essentials of Glycobiology* (ed. A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart and M. E. Etzler), pp. 143–161. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Ferguson, M. A. J., Kinoshita, T. and Hart, G. W. (2009). Glycosylphosphatidylinositol anchors. In *Essentials of Glycobiology* (ed. A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart and M. E. Etzler), Chapter 11. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Fiedler, K., Lafont, F., Parton, R. G. and Simons, K. (1995). Annexin XIIIb: a novel epithelial specific annexin is implicated in vesicular traffic to the apical plasma membrane. *J. Cell Biol.* **128**, 1043–1053.
- Fivaz, M., Vilbois, F., Thurnheer, S., Pasquali, C., Abrami, L., Bickel, P. E., Parton, R. G. and van der Goot, F. G. (2002). Differential sorting and fate of endocytosed GPI-anchored proteins. *EMBO J.* **1**, 3989–4000.
- Fölsch, H., Ohno, H., Bonifacino, J. S. and Mellman, I. (1999). A novel clathrin adaptor complex mediates basolateral targeting in polarized epithelial cells. *Cell* **99**, 189–198.
- Fujita, M. and Kinoshita, T. (2012). GPI-anchor remodeling: potential functions of GPI-anchors in intracellular trafficking and membrane dynamics. *Biochim. Biophys. Acta* **1821**, 1050–1058.
- Fujita, M., Maeda, Y., Ra, M., Yamaguchi, Y., Taguchi, R. and Kinoshita, T. (2009). GPI glycan remodeling by PGAP5 regulates transport of GPI-anchored proteins from the ER to the Golgi. *Cell* **139**, 352–365.
- Fujita, M., Watanabe, R., Jaensch, N., Romanova-Michaelides, M., Satoh, T., Kato, M., Riezman, H., Yamaguchi, Y., Maeda, Y. and Kinoshita, T. (2011). Sorting of GPI-anchored proteins into ER exit sites by p24 proteins is dependent on remodeled GPI. *J. Cell Biol.* **194**, 61–75.
- Guerrero, C. J., Lai, Y. and Weisz, O. A. (2008). Differential sorting and Golgi export requirements for raft-associated and raft-independent apical proteins along the biosynthetic pathway. *J. Biol. Chem.* **27**, 18040–18047.
- Guillas, I., Jiang, J. C., Vionnet, C., Roubaty, C., Uldry, D., Chuard, R., Wang, J., Jazwinski, S. M. and Celzermann, A. (2003). Human homologues of LAG1 reconstitute Acyl-CoA-dependent ceramide synthesis in yeast. *J. Biol. Chem.* **278**, 37083–37091.
- Gut, A., Kappeler, F., Hyka, N., Balda, M. S. and Hauri, H. P. and Matter, K. (1998). Carbohydrate-mediated Golgi to cell surface transport and apical targeting of membrane proteins. *EMBO J.* **1**, 1919–1929.
- Harsay, E. and Bretscher, A. (1995). Parallel secretory pathways to the cell surface in yeast. *J. Cell Biol.* **131**, 297–310.
- Howes, M. T., Mayor, S. and Parton, R. G. (2010). Molecules, mechanisms, and cellular roles of clathrin-independent endocytosis. *Curr. Opin. Cell Biol.* **22**, 519–527.
- Huttner, W. B. and Zimmerberg, J. (2001). Implications of lipid microdomains for membrane curvature, budding and fission. *Curr. Opin. Cell Biol.* **13**, 478–484.
- Imjeti, N. S., Lebreton, S., Paladino, S., de la Fuente, E., Gonzalez, A. and Zurzolo, C. (2011). N-Glycosylation instead of cholesterol mediates oligomerization and apical sorting of GPI-APs in FRT cells. *Mol. Biol. Cell* **22**, 4621–4634.
- Jacob, R., Heine, M., Eikemeyer, J., Frerker, N., Zimmer, K. P., Rescher, U., Gerke, V. and Naim, H. Y. (2004). Annexin II is required for apical transport in polarized epithelial cells. *J. Biol. Chem.* **279**, 3680–3684.
- Keller, P., Toomre, D., Diaz, E., White, J. and Simons, K. (2001). Multicolour imaging of post-Golgi sorting and trafficking in live cells. *Nat. Cell Biol.* **3**, 140–149.
- Klemm, R. W., Ejsing, C. S., Surma, M. A., Kaiser, H. J., Gerl, M. J., Sampaio, J. L., de Robillard, Q., Ferguson, C., Proszynski, T. J., Shevchenko, A. et al. (2009). Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. *J. Cell Biol.* **185**, 601–612.
- Kumagai, K., Yasuda, S., Okemoto, K., Nishijima, M., Kobayashi, S. and Hanada, K. (2005). CERT mediates intermembrane transfer of various molecular species of ceramides. *J. Biol. Chem.* **280**, 6488–6495.
- Kuzmin, P. I., Akimov, S. A., Chizmadzhev, Y. A., Zimmerberg, J. and Cohen, F. S. (2005). Line tension and interaction energies of membrane rafts calculated from lipid splay and tilt. *Biophys. J.* **88**, 1120–1133.
- Lafont, F., Lecat, S., Verkade, P. and Simons, K. (1998). Annexin XIIIb associates with lipid microdomains to function in apical delivery. *J. Cell Biol.* **142**, 1413–1427.
- Lebreton, S., Paladino, S. and Zurzolo, C. (2008). Selective roles for cholesterol and actin in compartmentalization of different proteins in the Golgi and plasma membrane of polarized cells. *J. Biol. Chem.* **283**, 29545–29553.
- Lecat, S., Verkade, P., Thiele, C., Fiedler, K., Simons, K. and Lafont, F. (2000). Different properties of two isoforms of annexin XIII in MDCK cells. *J. Cell Sci.* **113**, 2607–2618.
- Lee, M. C., Miller, E. A., Goldberg, J., Orci, L. and Schekman, R. (2004). Bidirectional protein transport between the ER and Golgi. *Annu. Rev. Cell Dev. Biol.* **20**, 87–123.
- Lenoir, M., Coskun, U., Grzybek, M., Cao, X., Buschhorn, S. B., James, J., Simons, K. and Overduin, M. (2010). Structural basis of wedging the Golgi membrane by FAPP pleckstrin homology domains. *EMBO Rep.* **11**, 279–284.
- Lipardi, C., Nitsch, L. and Zurzolo, C. (2000). Detergent-insoluble GPI-anchored proteins are apically sorted in fischer rat thyroid cells, but interference with cholesterol or sphingolipids differentially affects detergent insolubility and apical sorting. *Mol. Biol. Cell* **11**, 531–542.
- Loizides-Mangold, U., David, F. P., Nesatyy, V. J., Kinoshita, T. and Riezman, H. (2012). Glycosylphosphatidylinositol anchors regulate glycosphingolipid levels. *J. Lipid Res.* **53**, 1522–1534.
- Luini, A., Ragnini-Wilson, A., Polishchuck, R. S. and De Matteis, M. A. (2005). Large pleiomorphic traffic intermediates in the secretory pathway. *Curr. Opin. Cell Biol.* **17**, 353–361.
- Maeda, Y. and Kinoshita, T. (2011). Structural remodeling, trafficking and functions of glycosylphosphatidylinositol-anchored proteins. *Prog. Lipid Res.* **50**, 411–424.
- Maeda, Y., Tashima, Y., Houjou, T., Fujita, M., Yoko-o, T., Jigami, Y., Taguchi, R. and Kinoshita, T. (2007). Fatty acid remodeling of GPI-anchored proteins is required for their raft association. *Mol. Biol. Cell* **18**, 1497–1506.
- Martin-Belmonte, F., Puertollano, R., Millán, J. and Alonso, M. A. (2000). The MAL proteolipid is necessary for the overall apical delivery of membrane proteins in the polarized epithelial Madin-Darby canine kidney and fischer rat thyroid cell lines. *Mol. Biol. Cell* **11**, 2033–2045.
- Mayor, S. and Riezman, H. (2004). Sorting GPI-anchored proteins. *Nat. Rev. Mol. Cell Biol.* **5**, 110–120.
- McMahon, H. T. and Gallop, J. L. (2005). Membrane curvature and mechanisms of dynamic cell membrane remodelling. *Nature* **438**, 590–596.
- Miller, E. A., Beilharz, T. H., Malkus, P. N., Lee, M. C., Hamamoto, S., Orci, L. and Schekman, R. (2003). Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. *Cell* **114**, 497–509.
- Mishra, R., Grzybek, M., Niki, T., Hirashima, M. and Simons, K. (2010). Galectin-9 trafficking regulates apical-basal polarity in Madin-Darby canine kidney epithelial cells. *Proc. Natl. Acad. Sci. USA* **107**, 17633–17638.
- Muñiz, M. and Riezman, H. (2000). Intracellular transport of GPI-anchored proteins. *EMBO J* **19**, 10–15.
- Muñiz, M., Nuoffer, C., Hauri, H. P. and Riezman, H. (2000). The Emp24 complex recruits a specific cargo molecule into endoplasmic reticulum-derived vesicles. *J. Cell Biol.* **148**, 925–930.
- Muñiz, M., Morsomme, P. and Riezman, H. (2001). Protein sorting upon exit from the endoplasmic reticulum. *Cell* **104**, 313–320.
- Orlean, P. and Menon, A. K. (2007). Thematic review series: lipid posttranslational modifications. GPI anchoring of protein in yeast and mammalian cells, or: how we learned to stop worrying and love glycosphospholipids. *J. Lipid Res.* **48**, 993–1011.
- Paladino, S., Sarnataro, D., Pillich, R., Tivodar, S., Nitsch, L. and Zurzolo, C. (2004). Protein oligomerization modulates raft partitioning and apical sorting of GPI-anchored proteins. *J. Cell Biol.* **167**, 699–709.
- Paladino, S., Sarnataro, D., Tivodar, S. and Zurzolo, C. (2007). Oligomerization is a specific requirement for apical sorting of glycosyl-phosphatidylinositol-anchored proteins but not for non-raft-associated apical proteins. *Traffic* **8**, 251–258.
- Paladino, S., Lebreton, S., Tivodar, S., Campana, V., Tempere, R. and Zurzolo, C. (2008). Different GPI-attachment signals affect the oligomerization of GPI-anchored proteins and their apical sorting. *J. Cell Sci.* **121**, 4001–4007.
- Paladino, S., Lebreton, S., Tivodar, S., Formiggini, F., Ossato, G., Gratton, E., Tramier, M., Coppey-Moisand, M. and Zurzolo, C. (2014). Golgi sorting regulates organization and activity of GPI proteins at apical membranes. *Nature Chem. Biol.* **10**, 350–357.
- Pang, S., Urquhart, P. and Hooper, N. M. (2004). N-glycans, not the GPI anchor, mediate the apical targeting of a naturally glycosylated, GPI-anchored protein in polarized epithelial cells. *J. Cell Sci.* **117**, 5079–5086.
- Peng, R., De Antoni, A. and Gallwitz, D. (2000). Evidence for overlapping and distinct functions in protein transport of coat protein Sec24p family members. *J. Biol. Chem.* **275**, 11521–11528.
- Peter, B. J., Kent, H. M., Mills, I. G., Vallis, Y., Butler, P. J., Evans, P. R. and McMahon, H. T. (2004). BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science* **303**, 495–499.
- Potter, B. A., Ihrke, G., Bruns, J. R., Weixel, K. M. and Weisz, O. A. (2004). Specific N-glycans direct apical delivery of transmembrane, but not soluble or glycosylphosphatidylinositol-anchored forms of endolyn in Madin-Darby canine kidney cells. *Mol. Biol. Cell* **15**, 1407–1416.
- Rivier, A. S., Castillon, G. A., Michon, L., Fukasawa, M., Romanova-Michaelides, M., Jaensch, N., Hanada, K. and Watanabe, R. (2010). Exit of GPI-anchored proteins from the ER differs in yeast and mammalian cells. *Traffic* **11**, 1017–1033.
- Rodriguez-Boulant, E. and Gonzalez, A. (1999). Glycans in post-Golgi apical targeting: sorting signals or structural props? *Trends Cell Biol.* **9**, 291–294.
- Rodriguez-Boulant, E., Kreitzer, G. and Müsch, A. (2005). Organization of vesicular trafficking in epithelia. *Nat. Rev. Mol. Cell Biol.* **6**, 233–247.
- Römer, W., Berland, L., Chambon, V., Gaus, K., Windschiagl, B., Tenza, D., Aly, M. R., Fraiser, V., Florent, J. C., Perrais, D. et al. (2007). Shiga toxin induces tubular membrane invaginations for its uptake into cells. *Nature* **450**, 670–675.
- Roux, A., Cuvelier, D., Nassoy, P., Prost, J., Bassereau, P. and Goud, B. (2005). Role of curvature and phase transition in lipid sorting and fission of membrane tubules. *EMBO J.* **24**, 1537–1545.
- Sarnataro, D., Paladino, S., Campana, V., Grassi, J., Nitsch, L. and Zurzolo, C. (2002). PrPC is sorted to the basolateral membrane of epithelial cells independently of its association with rafts. *Traffic* **3**, 810–821.
- Scheiffele, P., Peränen, J. and Simons, K. (1995). N-glycans as apical sorting signals in epithelial cells. *Nature* **378**, 96–98.
- Schuck, S. and Simons, K. (2004). Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. *J. Cell Sci.* **117**, 5955–5964.

- Seong, J., Wang, Y., Kinoshita, T. and Maeda, Y.** (2013). Implications of lipid moiety in oligomerization and immunoreactivities of GPI-anchored proteins. *J. Lipid Res.* **54**, 1077–1091.
- Shimoni, Y., Kurihara, T., Ravazzola, M., Amherdt, M., Orci, L. and Schekman, R.** (2000). Lst1p and Sec24p cooperate in sorting of the plasma membrane ATPase into COPII vesicles in *Saccharomyces cerevisiae*. *J. Cell Biol.* **151**, 973–984.
- Simons, K. and Ikonen, E.** (1997). Functional rafts in cell membranes. *Nature.* **387**, 569–572.
- Simons, K. and Sampaio, J. L.** (2011). Membrane organization and lipid rafts. *Cold Spring Harb. Perspect Biol.* **3**, a004697.
- Strating, J. R. and Martens, G. J.** (2009). The p24 family and selective transport processes at the ER-Golgi interface. *Biol. Cell* **101**, 495–509.
- Surma, M. A., Klose, C. and Simons, K.** (2012). Lipid-dependent protein sorting at the trans-Golgi network. *Biochim. Biophys. Acta* **1821**, 1059–1067.
- Suzuki, K. G., Kasai, R. S., Hirose, K. M., Nemoto, Y. L., Ishibashi, M., Miwa, Y., Fujiwara, T. K. and Kusumi, A.** (2012). Transient GPI-anchored protein homodimers are units for raft organization and function. *Nat. Chem. Biol.* **8**, 774–783.
- Tian, A. and Baumgart, T.** (2009). Sorting of lipids and proteins in membrane curvature gradients. *Biophys. J.* **96**, 2676–2688.
- van Meer, G. and Vaz, W. L.** (2005). Membrane curvature sorts lipids. Stabilized lipid rafts in membrane transport. *EMBO Rep.* **6**, 418–419.
- Vieira, O. V., Verkade, P., Manninen, A. and Simons, K.** (2005). FAPP2 is involved in the transport of apical cargo in polarized MDCK cells. *J. Cell Biol.* **170**, 521–526.
- Watanabe, R., Funato, K., Venkataraman, K., Futerman, A. H. and Riezman, H.** (2002). Sphingolipids are required for the stable membrane association of glycosylphosphatidylinositol-anchored proteins in yeast. *J. Biol. Chem.* **277**, 49538–49544.
- Weisz, O. A. and Rodriguez-Boulan, E.** (2009). Apical trafficking in epithelial cells: signals, clusters and motors. *J. Cell Sci.* **122**, 4253–4266.
- Yeaman, C., Le Gall, A. H., Baldwin, A. N., Monlauzeur, L., Le Bivic, A. and Rodriguez-Boulan, E.** (1997). The O-glycosylated stalk domain is required for apical sorting of neurotrophin receptors in polarized MDCK cells. *J. Cell Biol.* **139**, 929–940.
- Zurzolo, C., Lisanti, M. P., Caras, I. W., Nitsch, L. and Rodriguez-Boulan, E.** (1993). Glycosylphosphatidylinositol-anchored proteins are preferentially targeted to the basolateral surface in Fischer rat thyroid epithelial cells. *J. Cell Biol.* **121**, 1031–1039.
- Zurzolo, C., van't Hof, W., van Meer, G. and Rodriguez-Boulan, E.** (1994). VIP21/caveolin, glycosphingolipid clusters and the sorting of glycosylphosphatidylinositol-anchored proteins in epithelial cells. *EMBO J.* **13**, 42–53.