



HAL
open science

Links between lipid homeostasis, organelle morphodynamics and protein trafficking in eukaryotic and plant secretory pathways

Su Melser, Diana Molino, Brigitte Batailler, Martine Peypelut, Maryse Laloi, Valérie Wattelet-Boyer, Yannick Bellec, Patrick Moreau, Jean Denis Faure

► To cite this version:

Su Melser, Diana Molino, Brigitte Batailler, Martine Peypelut, Maryse Laloi, et al.. Links between lipid homeostasis, organelle morphodynamics and protein trafficking in eukaryotic and plant secretory pathways. *Plant Cell Reports*, 2011, 30 (2), pp.177-193. 10.1007/s00299-010-0954-1 . hal-00715782

HAL Id: hal-00715782

<https://hal.science/hal-00715782v1>

Submitted on 11 Dec 2024

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.



Distributed under a Creative Commons Attribution 4.0 International License

Links between lipid homeostasis, organelle morphodynamics and protein trafficking in eukaryotic and plant secretory pathways

Su Melser · Diana Molino · Brigitte Batailler · Martine Peypelut · Maryse Laloi ·
Valérie Wattelet-Boyer · Yannick Bellec · Jean-Denis Faure · Patrick Moreau

Abstract The role of lipids as molecular actors of protein transport and organelle morphology in plant cells has progressed over the last years through pharmacological and genetic investigations. The manuscript is reviewing the roles of various lipid families in membrane dynamics and trafficking in eukaryotic cells, and summarizes some of the related physicochemical properties of the lipids involved. The article also focuses on the specific requirements of the sphingolipid glucosylceramide (GlcCer) in Golgi morphology and protein transport through the plant secretory pathway. The use of a specific inhibitor of plant glucosylceramide synthase and selected *Arabidopsis thaliana* RNAi lines stably expressing several markers of the plant secretory pathway, establishes specific steps sensitive to GlcCer biosynthesis. Collectively, data of the literature demonstrate the existence of links between protein

trafficking, organelle morphology, and lipid metabolism/homeostasis in eukaryotic cells including plant cells.

Keywords Secretory pathways · Membrane dynamics · Protein trafficking · Lipid homeostasis · Lipid domains · Lipid machineries

Introduction to membrane dynamics: from a protein to a lipid world

Several protein machineries have been shown to be fundamental in the functional dynamic membrane organization of the plant secretory pathway (Moreau et al. 2007; Hanton et al. 2007, 2009; Woollard and Moore 2008; Richter et al. 2009; Sparkes et al. 2009; Griffing 2009; Hwang and Robinson 2009; Hawes et al. 2010). For example, the dynamic ER network (Sparkes et al. 2009; Griffing 2009), the structural features of the ER-Golgi interface and the ER

Communicated by R. Reski.

S. Melser · V. Wattelet-Boyer · P. Moreau (✉)
Laboratoire de Biogenèse Membranaire, UMR 5200 Université
Bordeaux 2-CNRS, Université Bordeaux 2, case 92,
146 rue Léo-Saignat, 33076 Bordeaux, France
e-mail: pmoreau@biomemb.u-bordeaux2.fr

S. Melser
e-mail: su.melser@inserm.fr

V. Wattelet-Boyer
e-mail: valerie.wattelet@biomemb.u-bordeaux2.fr

D. Molino · Y. Bellec · J.-D. Faure
Laboratoire de Biologie Cellulaire, INRA UR501,
Versailles, France
e-mail: diana.molino@versailles.inra.fr

Y. Bellec
e-mail: bellec@versailles.inra.fr

J.-D. Faure
e-mail: Jean-Denis.Faure@versailles.inra.fr

B. Batailler · M. Peypelut · P. Moreau
Pôle Imagerie du Végétal du BIC (Bordeaux Imaging Center),
INRA IFR103, Villenave d'Ornon, France
e-mail: brigitte.batailler@bordeaux.inra.fr

M. Peypelut
e-mail: peypelut@bordeaux.inra.fr

M. Laloi
Physiologie Moléculaire du Transport des Sucres chez les
Végétaux, University of Poitiers, FRE3091 Poitiers, France
e-mail: maryse.laloi@univ-poitiers.fr

exit sites (ERES) of cargoes are tightly regulated by specific proteins (Moreau et al. 2007; Hanton et al. 2007, 2009; Woollard and Moore 2008; Richter et al. 2009; Sparkes et al. 2009; Griffing 2009; Hwang and Robinson 2009; Hawes et al. 2010). ERES biogenesis and possibly de novo Golgi formation were shown to be cargo induced and signal mediated (Hanton et al. 2007, 2009), and recently the COPII machinery was also suspected to regulate ER and Golgi membrane morphology and maintenance (Faso et al. 2009). Similar situations are described or can be strongly suspected at other steps (Moreau et al. 2007; Hanton et al. 2007, 2009; Woollard and Moore 2008; Richter et al. 2009; Sparkes et al. 2009; Griffing 2009; Hwang and Robinson 2009; Hawes et al. 2010).

What about lipids? Beside the protein machineries, more and more data also implicate lipids, enzymes of their metabolism and lipid modifications of proteins in the mechanisms of membrane dynamics and their regulation in the secretory pathway of eukaryotic cells (Moreau et al. 2007; Moreau 2007 and references therein). Lipids have also been shown to act as modulators of viral protein-mediated membrane fusion. For example, membrane fusion required for virus entry can be dependent on cholesterol, sphingolipids and lipid microdomains (Teissier and Pécheur 2007). Yeast screens identified all the enzymes of the ergosterol biosynthetic pathway (coded by *Erg* genes) as critical factors involved in mitochondrial morphology (Dimmer et al. 2002; Altmann and Westermann 2005). Recently, a specific mitochondrial phospholipase D in the outer membrane has also been found to be a key actor of mitochondrial fusion by generating phosphatidic acid (PA) from CL (Choi et al. 2006). In addition, the fatty acyl transferase endophilin B1 was reported to be involved in the maintenance of mitochondrial morphology (Karbowski et al. 2004). In general, it must be considered that variations of less than 1% in the amounts of a given lipid, if concentrated in a specific domain, may have critical impact on the local membrane structure, and therefore on membrane dynamics (Devaux 2000). Trafficking of lipids to their final destination has been studied in several *in vivo* and *in vitro* systems and can occur either via vesicular transfer, membrane contacts or via lipid transfer proteins (Moreau et al. 1998; Jouhet et al. 2007; Benning 2009). Whatever the means by which membrane lipids reach their final destination, they will, all along their road and/or life specifically contributes to cellular membrane morphodynamics due their physicochemical properties. In the following sections, we will describe our current knowledge on the role of various lipids in intracellular membrane dynamics and trafficking in eukaryotic cells with a special focus on phospholipids/phosphoinositides, sterols and sphingolipids in plant cells, and discuss the

physicochemical properties of the various lipid families in relation to these events.

Lipid-assisted membrane dynamics and trafficking in eukaryotes and plant cells

Phospholipids, phosphoinositides and related metabolites

A first example of the specific impact that phospholipid synthesis can have on membrane behavior is given by the depletion of phosphatidylcholine (PC) in the thermosensitive Chinese hamster ovary cell line MT58 that undergoes a 50% PC reduction. In this mutant, the structure of the plasma membrane, Golgi complex and mitochondria appears to be morphologically intact, and only part of the ER lost its reticular structure as dilated and spherical ER structures were observed (Testerink et al. 2009). Transition of a reticular to a spherical ER structure may reflect a way to preserve the integrity of the organelle when faced with a shortage of the major membrane component, due to the more efficient volume to surface ratio of the spherical structure. In addition, it is also possible that the ER serves as a “PC tank” to supply the other organelles. Interestingly, protein transport from the ER towards the Golgi was not affected in these cells whereas an inhibition from the Golgi complex towards the plasma membrane was observed (Testerink et al. 2009). Therefore, the importance of a balanced PC synthesis was probably more sensitive and critical for the Golgi membranes than the ER membranes in term of morphodynamics required for protein transport. It has been shown that both diacylglycerol (DAG) and PA, which are important PC metabolites, can be required for the vesicular transport of proteins from the Trans Golgi Network (TGN), possibly by induction of membrane bending and formation of highly curved membranes (Kearns et al. 1997; Corda et al. 2002; Mousley et al. 2007). DAG can recruit and activate proteins required for protein transport, like the Protein Kinase D (PKD). Binding of PKD to DAG was shown to be essential for its recruitment to the TGN where it acts in the formation of specific transport vesicles (Baron and Malhotra 2002), and indicated the importance of maintaining DAG concentration (Baron and Malhotra 2002). In addition, PC metabolism in eukaryotic cells might be involved in DAG homeostasis through regulations by PI-transfer proteins such as Sec-14 in yeast or Nir 2 in mammalian cells (McGee et al. 1994; Litvak et al. 2005). In the mutant MT58 cells (Testerink et al. 2009), the intracellular DAG concentration might therefore be disturbed by the inhibition of PC synthesis in the ER, and specifically perturbed the Golgi apparatus behavior.

In higher plant cells, an extensive exchange exists between plastids and the ER network for glycerolipid biosynthesis (Benning 2009). The de novo biosynthesis of fatty acids occurs in the plastids, then those fatty acids are exported as acyl-CoAs to the ER to be incorporated into various lipids including the most abundant phospholipid PC, and finally, some PC species can be transferred through different pathways (see Benning 2009 and references therein) back to the plastids to supply galactolipid biosynthesis. Although critical questions remain, the potential role of two PA phosphohydrolases (PAH1 and PAH2) in the regulation of phospholipid metabolism in *Arabidopsis* has been determined (Nakamura et al. 2009; Eastmond et al. 2010). Interestingly, the *pah1-pah2* double mutant shows unusually extended ER membranes (Eastmond et al. 2010) due to a high increase in the phospholipid content (mainly PC and phosphatidylethanolamine, PE) which are likely to be accumulated in the biosynthetic ER membranes. In agreement, the *pah1-pah2* double mutation induced an up-regulation of several genes involved in the biosynthesis of phospholipids (Eastmond et al. 2010). From a morphodynamic point of view, these results indicate that the highly regulated lipid biosynthesis in the ER is certainly a key function for the regulation of membrane extension of the ER network. At the same time, ER membrane dynamics, through the numerous potential interactions the ER can have with other organelles (Sparkes et al. 2009), serve to manage and regulate lipid biosynthesis and transport to control membrane morphogenesis of interacting organelles. For example, connections between ER and plastid envelopes have been regularly reported from ultrastructural observations (Sparkes et al. 2009), and recently an optical trapping study has confirmed the possibility of strong interactions between plastids and ER membranes (Andersson et al. 2007). In addition to direct ER-plastid contacts that may result in the exchange of lipids between the two organelles, through specific proteins such as TGD4 (Benning 2009), we have also to consider the non-exclusive possibility of a Golgi-dependent pathway to the plastids (Sparkes et al. 2009 and references therein). Whatever the different routes/processes used, these results illustrate the critical involvement of the early secretory pathway and the ER network in the regulation of organelle morphogenesis (see Sparkes et al. 2009 for more information).

Other steps of phospholipid metabolism can affect Golgi membrane dynamics and morphology. Lysophospholipids, issued from phospholipases A2 activities, can induce membrane tubulation (de Figueiredo et al. 1998), and inhibition of the reverse reaction by acyltransferases leads to Golgi membrane tubulation (Drecktrah et al. 2003). Inhibition of such hydrolytic enzymes by specific inhibitors can block endosome fusion (Mayorga et al. 1993).

Phospholipases A1-related enzymes have been shown to induce a dispersion of the Golgi complex and aggregation of ER membranes (Nakajima et al. 2002). An investigation in *Arabidopsis* roots has recently evidenced that a Golgi PLA2- α is involved in the trafficking of PIN-FORMED auxin efflux transporters to the plasma membrane of these cells (Lee et al. 2010).

Similarly, inhibition of phospholipase D, which decreases PA concentration, can also affect the structural integrity of the Golgi complex in animal cells (Siddhanta et al. 2000). Recently, it has been demonstrated that PA produced by the phospholipase isoform D2 is involved in the release of Golgi-derived COPI vesicles (Yang et al. 2008). The phospholipase D2 isoform was found to be concentrated to the rims of the Golgi stacks and could be involved in membrane morphodynamics driving some aspects of post-Golgi trafficking and the stability of Golgi structure (Freyberg et al. 2002). In plant cells, inhibition of phospholipase D and therefore formation of PA in the pollen tube strongly reduces the number of secretory vesicles, indicating that levels of PA may regulate pollen tube growth (Potocky et al. 2003; Monteiro et al. 2005). It has been shown in tobacco BY-2 cells that 1-butanol, which interferes with phospholipase D activity, triggered the release of the GTPase ARF1 from Golgi membranes (Langhans and Robinson 2007). However, this effect differed from that of brefeldin A (BFA, inhibitor of recruitment of coat COPI proteins) in that 1-butanol did not have the same ability as BFA to induce an interaction of Golgi membranes with the ER membranes. Interestingly, we have observed that the inhibition of phytosterol maturation by fenpropimorph (see below) was also accompanied by an increase of PA in endomembranes and particularly the Golgi (Moreau 2007), which could indicate that elevated PA levels were inducing fenestration of Golgi bodies (Hartmann et al. 2002). Recently, the *Arabidopsis* PLD ζ 2 has been shown to be involved in the regulation of root gravitropism and auxin-dependent hypocotyl elongation (Li and Xue 2007). It was shown that the internalization of an endocytic marker (the fluorescent dye FM4-64) and PIN2 cycling between the plasma membrane and endosomal compartments were positively regulated by PLD ζ 2 and its PA product (Li and Xue 2007). Therefore, it was suggested that PLD ζ 2 and PA might be required in the regulation of auxin transport through several possible mechanisms (see Li and Xue 2007 and references cited). Therefore, it should be considered that PA and DAG homeostasis (through DAG kinases, PA phosphohydrolases, and phospholipases) may be key players in the regulation of lipid metabolism and of ER and Golgi membrane morphodynamics, and as such can control the formation and fission of ER- and Golgi-derived vesicles as shown for COPI vesicles through the recruitment of the protein

ARF-GAP1 (GTPase activating protein) (Fernandez-Ulibarri et al. 2007).

Together with PA, the phosphoinositide PIP2 is another acidic phospholipid which can be critical for membrane fusion/fission (Vicogne et al. 2006 and references therein). It was observed from in vitro SNARE-reconstituted fusion assays in liposomes that the highest fusion efficiency was obtained when PA was present in the acceptor liposomes (containing the t-SNAREs syntaxin 4 and SNAP33) and the phosphoinositide PIP2 was included in the donor liposomes (containing the v-SNARE VAMP2) (Vicogne et al. 2006). These in vitro assays show that an asymmetric distribution of acidic phospholipids may act in vivo to regulate membrane fusion (Vicogne et al. 2006).

Phosphoinositide homeostasis is also critical to membrane morphodynamics and/or targeting in plant cells, and this concerns several forms of phosphoinositides. PI4P is more involved in the regulation of storage vacuole biogenesis, PI3P in that of lytic vacuole and PIP2 is chiefly related to the biogenesis of the plasma membrane. The latter has been shown to be required in the control of root hair growth (Vincent et al. 2005), and more precisely the PI(4)P 5-Kinase 3 is responsible for the enzymatic activity which produces PI(4,5)P2 in the apical region of root hair cells, and this may regulate either vesicle trafficking and/or cytoskeletal organization required for root hair growth (Stenzel et al. 2008; Kusano et al. 2008). In addition, PI(4)P 5-Kinase 3 together with several phospholipases C might control the concentration and distribution of PI(4,5)P2 at the tip-growing apex (47 and references therein). Moreover, it has also been shown that the level of PI(4)P is tightly regulated by a phosphoinositide phosphatase (Thole et al. 2008). Therefore, these overall results confirm the concept according to which the homeostasis of phosphoinositides concentration and that of their subcellular location are key parameters controlling the polarized secretion implicated in root hair growth. Lastly PIP2 synthesis may also be linked to Golgi fragmentation as suggested in animal cells (Siddhanta et al. 2003), and participate in the control of Golgi structural integrity.

Finally, beside their roles in membrane lipid asymmetry (Devaux 2000), energy-independent lipid flippases and energy-dependent lipid translocases may be required for membrane dynamics involved in membrane trafficking. For example, the aminophospholipid translocase ALA3 and ALIS1 (an ALA-interacting protein), both localized in the Golgi as a complex, have been implicated in lipid translocation and secretory vesicle formation (Poulsen et al. 2008). ALA3 activity may transfer and concentrate specific lipids in one membrane leaflet to induce the tension needed to drive vesicle formation as suggested earlier (Devaux 2000; Pomorski and Menon 2006), or serves as receptors to recruit specific proteins involved in membrane deformation

for vesicle production and release (Jürgens 2004). 12 ALA proteins have been identified in the *Arabidopsis* genome (Poulsen et al. 2008) and some may be engaged in such specific events.

Sterols and related compounds

Cholesterol has been shown to be critical in the formation of post-Golgi secretory vesicles in animal cells (Wang et al. 2000), and may be required to favor vesicle fusion (Churchward et al. 2005). In addition, it has been found that cholesterol levels in Golgi membranes are tightly regulated since an excess of this molecule can lead to a vesiculation of the Golgi complex (Grimmer et al. 2005). Such an effect also implicates the activity of a cytosolic phospholipase A2 and a GTPase dynamin (Grimmer et al. 2005). Cholesterol is required for the formation of lipid rafts at the cell surface, and depletion of this lipid induces a flattening of clathrin-coated pits and a disappearance of caveolae in animal cells (Pichler and Riezman 2004), both structures being involved in different sets of endocytic events. Another example of the involvement of sterols in controlling membrane trafficking is coming from yeast where it has been clearly established that ergosterol is also critical for endocytosis (Heese-Peck et al. 2002). Interestingly, the internalization step of endocytosis in yeast is strictly dependent on the fine structural nature of the sterol, and very small variations in this structure can have dramatic consequences on endocytosis (Munn et al. 1999; Heese-Peck et al. 2002). Therefore, these few examples in yeast and animal cells strongly argue in favor of a requirement of sterols in the regulation of exocytotic and endocytotic events in eukaryotes.

In plants, blocking phytosterol maturation in the ER by the drug fenpropimorph (a plant specific inhibitor of the cycloeucaleanol-obtusifoliol isomerase) resulted in the fenestration of the Golgi bodies (Hartmann et al. 2002). On another hand, disturbing the morphology of the Golgi bodies by a treatment with BFA, which disturbs the recruitment of COPI proteins to the Golgi, reduced the biosynthesis of phytosterols (Mérigout et al. 2002). Therefore, a relationship does exist between sterol metabolism and Golgi morphology in plant cells as in other eukaryotic cells. However, we must be aware that the morphologic modifications of the Golgi in this case may also be due to the elevation of PA amounts (Moreau 2007, see above). Similarly as ergosterol in yeast and cholesterol in animal cells, we could expect some critical role for phytosterols in membrane protein transport. This hypothesis has been verified in two different models. First, use of the inhibitor fenpropimorph resulted in the retention of sterols and sterol precursors at the Golgi level, and lipid rafts disappeared from the plasma membrane and

accumulated in the Golgi (Laloi et al. 2007). Moreover, protein transport to the plasma membrane was partly impaired, probably because of the accumulation of sterols and lipid rafts in the Golgi (Laloi et al. 2007). Such a relationship was recently confirmed by using the drug fenpropimorph on tobacco leaf epidermal cells. In this model, protein secretion was followed in vivo by a confocal microscopy approach, and the drug clearly reduced and/or delayed the delivery of proteins to the cell surface (Melser et al. 2010).

As in yeast, endocytosis in plants appears to be sensitive to the presence of minimal amounts of phytosterols. The best example has recently been given in *Arabidopsis thaliana* where the establishment of the polarity of the auxin carrier PIN2 was clearly triggered by a sterol-dependent endocytotic process (Men et al. 2008). PIN2 polarity and endocytosis, but not exocytosis, were effectively impaired in the sterol-biosynthesis mutant *cyclopropylsterol isomerase1-1* (Men et al. 2008). To perform cytokinesis, proteins of the syntaxin family mediate membrane fusion in the plane of cell division, and it has recently been demonstrated that the *Arabidopsis* KNOLLE syntaxin is maintained in the plane of cell division by a sterol-dependent endocytotic process which also involves a clathrin- and a dynamin related protein (Boutté et al. 2009). In the endocytosis-defective sterol biosynthesis mutant mentioned above, the syntaxin KNOLLE displayed a significant lateral diffusion, but the secretory trafficking of KNOLLE remained unaffected (Boutté et al. 2009). Therefore, regulation of lateral diffusion of specific proteins by sterol-dependent endocytosis may contribute to maintain the specificity of syntaxin localization during late cytokinesis.

Behind their biosynthesis, handling and mobilization of phytosterols by specific proteins such as oxysterol-binding proteins and related proteins (Boutté and Grebe 2009 and references therein), may highly contribute to sterol transport and sorting to membrane domains where their physicochemical properties act to regulate membrane structure and morphodynamics. Thus, the homeostasis of phytosterols may affect the biogenesis of numerous organelles including plastids (Boutté and Grebe 2009 and references therein).

The sphingolipid universe

The sphingolipid family may also affect the organization of endomembranes. In animal cells, ceramides dramatically increase the disassembly of the Golgi that is induced by BFA, which suggests that changes in ceramide levels affect the stability of the Golgi (Fukunaga et al. 2000). Pharmacological approaches using inhibitors of the glucosylceramide (GlcCer) synthase (GCS) argue in favor of a role of

sphingolipids in the architecture of the Golgi apparatus (Nakamura et al. 2001). In addition, high concentrations of the long chain base sphingosine derived from ceramide hydrolysis can induce the fragmentation of the Golgi complex (Hu et al. 2005). In yeast, C26-sphingolipids were shown to be involved in the transport of the Pma1 ATPase to the plasma membrane (Gaigg et al. 2005).

In plant cells, fenpropimorph treatment led to the accumulation of phytosterol precursors but also of hydroxypalmitic acid-containing GlcCer in the Golgi membranes (Laloi et al. 2007). In addition, since sterols and sphingolipids can be associated to form lipid microdomains in eukaryotic cells (Bagnat and Simons 2002; Simons and Vaz 2004; Laloi et al. 2007 and references therein), these features point to the importance of lipid domain formation may have in the efficiency of the secretory pathway (Bagnat and Simons 2002; Simons and Vaz 2004; Hancock 2006; Laloi et al. 2007 and references therein). Therefore, as sterols, sphingolipids may be critical for Golgi membrane morphodynamics in plant cells. Such an assumption has first been studied by a pharmacological approach. By using the model of tobacco leaf epidermal cells and PDMP (the ceramide analogue *d*-threo-1-phenyl-2-decanoylamino-3-morpholino-propanol which inhibits the GCS in vivo), it has been shown that the intracellular transport of soluble and membrane proteins to the cell surface is reduced (Melser et al. 2010). Moreover, membrane proteins not delivered to the plasma membrane were retained in the Golgi bodies (Melser et al. 2010). This was an encouragement to have a look on the structure of the Golgi apparatus at the level of electronic microscopy. PDMP was found to induce several morphological modifications of the Golgi bodies: misaligned stacks, decrease of stack length and cisternae width, and numerous swollen vesicles surrounding the Golgi (Melser et al. 2010). These data strongly suggested that in plant cells, the Golgi partially disaggregates into vesicular structures upon inhibition of GlcCer biosynthesis, resulting in a decrease of protein secretion. RNAi suppression of the *Arabidopsis* *LCB2a* and *LCB2b* genes (responsible for the biosynthesis of sphingolipid precursors) was shown to induce strong defects in the endomembrane system of pollen (Dietrich et al. 2008). Such a requirement of GlcCer biosynthesis for post-Golgi targeting of proteins in plant cells parallels what has been shown in animal cells where a defect in GlcCer biosynthesis induced an accumulation of transported membrane proteins in the Golgi (Sprong et al. 2001).

In addition, it was shown that an increase in the load of secretory proteins was accompanied by an increase in the de novo biosynthesis of GlcCer, and this feature was only observed with proteins transported beyond the Golgi (Melser et al. 2010). Such a behavior is reminiscent to the de novo formation of ER export sites which is induced by

membrane cargo (Hanton et al. 2007). Moreover, it was suggested that in animal cells, GlcCer may exert specific functions at the cytosolic surface of ER and Golgi membranes in protein sorting (Halter et al. 2007 and references therein). Such functions may be strongly regulated by the glycosphingolipid transfer protein FAPP2 (Halter et al. 2007), controlling GlcCer concentration in membrane domains. Similarly, the *Arabidopsis thaliana* glycosphingolipid transfer protein (GLTP1) was shown to regulate vesicular transport to the plasma membrane/tonoplast during root hair development (West et al. 2008), probably by supplying required GlcCer amounts to specific membrane domains.

To investigate which intracellular steps of the secretory pathway are dependent on GlcCer biosynthesis, an *A. thaliana* RNAi line (*gcs2.2*) showing reduced *GCS* transcripts from 50% up to 85% of control levels, was crossed with *A. thaliana* lines expressing different plasma membrane and endomembrane markers coupled to GFP (see next chapter). In these lines, the progressive reduction in transcript levels correlated well with a progressive reduction up to 50-70% in total GlcCer content. TEM analyses also revealed apparent anomalies in root cells, notably in the morphology of the Golgi. When compared to Golgi bodies from wild type Col-0, Golgi stacks from *gcs2.2* appeared smaller (Fig. 1a, b), as the Golgi from PDMP-treated *Arabidopsis* roots (Melser et al. 2010), and the Golgi in *gcs2.2* appeared swollen, clearly confirming a strong disturbance of Golgi morphology in GlcCer-depleted cells. In addition, *gcs2.2* mutants were found more sensitive to PDMP as illustrated by the effect on root growth (Fig. 1c). Since no significant difference in root length was observed between Col-0 and *gcs2.2* mutant seedlings in the absence of PDMP (Fig. 1c), the higher inhibition of root growth by PDMP for the *gcs2.2* mutant seedlings argues for a threshold under which a decreased content of GlcCer impairs root growth. The

results with the *gcs2.2* mutant seedlings are an important confirmation of the data obtained with the inhibitor PDMP (Melser et al. 2010) and validate the specificity of the drug. The *gcs2.2* mutant line was used to determine which intracellular steps of the plant secretory pathway are dependent on GlcCer biosynthesis.

Protein marker transport defects in the glucosylceramide synthase *gcs2.2* mutant

The localization of plasma membrane and endomembrane marker proteins (under their own promoter or under 35S) was observed in 7 to 10-day-old *gcs2.2* treated or not respectively with 10 μ M PDMP, and compared to Col-0. The remaining amounts of GlcCer in *gcs2.2* were sufficient to sustain protein transport when proteins were expressed under their own promoter. In this case, *gcs2.2* lines had to be treated with PDMP, and they were found more sensitive to PDMP than the Col-0 controls since the starting amount of GlcCer was lower. This was taken as an advantage when protein markers were expressed under their endogenous promoter. On the contrary, when proteins were expressed under 35S, any effect could be detected without PDMP. The roots were also stained with the red fluorescent dye FM4-64 at a concentration of 8 μ M, applied 5–15 min before observation by confocal microscopy. This dye does not disturb integral plasma membrane proteins in *Arabidopsis* root epidermis and cortex cells (Jelinkova et al. 2010), and in the conditions used labels the plasma membrane and some endocytic compartments (not the clathrin-dependent ones) (Jelinkova et al. 2010).

As found in the model of tobacco leaf epidermal cells (Melser et al. 2010), the transport from the ER to the Golgi was insensitive to a decrease of GlcCer biosynthesis as illustrated by the Golgi marker QUA2 (a pectin methyltransferase) which was unaffected in its sub-cellular

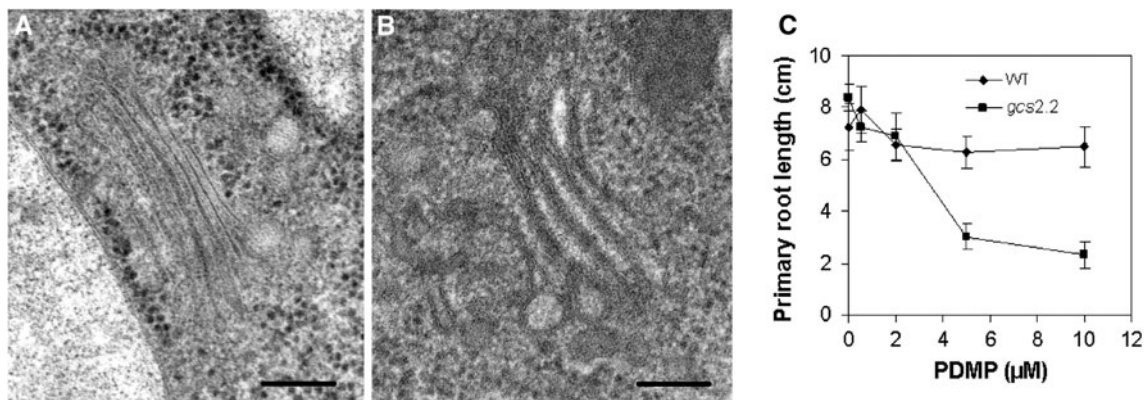


Fig. 1 GlcCer deficit affects endomembrane morphology and organ growth. **a** Typical Golgi from wild-type (WT) Col-0 root cells. **b** Golgi from mutant *gcs2.2* root cells. Bars in **a** and **b** 200 nm.

c Root growth is progressively impaired with increasing concentrations of PDMP. The mutant *gcs2.2* is highly sensitive to the GlcCer biosynthesis inhibitor PDMP

location even after PDMP treatment (Fig. 2). On the contrary, it was observed that the reverse transport from the Golgi back to the ER was sensitive to the depletion of GlcCer. The localization of GFP modified by an ER retrieval HDEL tetrapeptide signal (35S::GFP-HDEL) showed several punctuate localizations compatible with Golgi bodies in *gcs2.2* roots and exclusively the expected ER in control roots (Fig. 2). An interesting observation was that the punctuate structures labeled by GFP-HDEL in *gcs2.2* roots were not co-labeled by FM4-64, supporting a Golgi location of GFP-HDEL. In conclusion, it can be hypothesized that the delocalization of GFP-HDEL in *gcs2.2* roots may be caused by a defect in COPI vesicle function upon inhibition of GlcCer biosynthesis. Therefore, GFP-HDEL may be normally transported to the Golgi but could not be efficiently returned by the COPI system to the ER, producing an accumulation of GFP-HDEL in the Golgi in *Arabidopsis* root cells.

LTi6b, Low-temperature Induced 6b, is a small integral membrane protein originally isolated from a screen of cDNA prepared from cold-acclimated etiolated *Arabidopsis* seedlings (Capel et al. 1997). The protein is located in the plasma membrane (Cutler et al. 2000). In the wild type Col-0 roots, 35S::LTi6b was localized exclusively and uniformly in the PM (Fig. 2a). On the contrary, fluorescent labeling was found only in a few cells in *gcs2.2*, and the labeling was often found in punctuate structures (Fig. 2b). These structures appeared along the outline of the PM and were co-labeled by FM4-64 which labels the plasma membrane and additional intracellular endocytic compartments (Fig. 2c). As no co-localization was found between γ -TIP (tonoplast) and FM4-64 in the same conditions, it is likely that these structures correspond to endocytic compartments issued from the PM. Importantly, FM4-64 did not influence the behavior of LTi6b since it has been shown that FM dyes do not disturb the localization of plasma membrane proteins in *Arabidopsis* root epidermal and cortex cells (Jelinkova et al. 2010). In addition, experiments using fluorescence recovery after photobleaching (FRAP) under BFA and cycloheximide treatment (Grebe et al. 2003) suggest that LTi6b may be targeted to the PM through a Golgi-independent pathway, and therefore it can be unlikely that the localization of LTi6b in the punctuate structures observed in the *gcs2.2* mutant are only FM4-64-labeled Golgi. Although it cannot be ruled out that some LTi6b proteins are retained in the Golgi bodies, it is likely that an inhibition of GlcCer biosynthesis altered the dynamics of endosomes labeled by LTi6b in root cells.

The transmembrane auxin efflux carrier PIN1 normally localizes to the basal end of vascular root cells (Gälweiler et al. 1998). In *gcs2.2*, we detected PIN1 also at the basal end of central protophloem cells, without any visibly altered localization. Interestingly, levels of PIN1

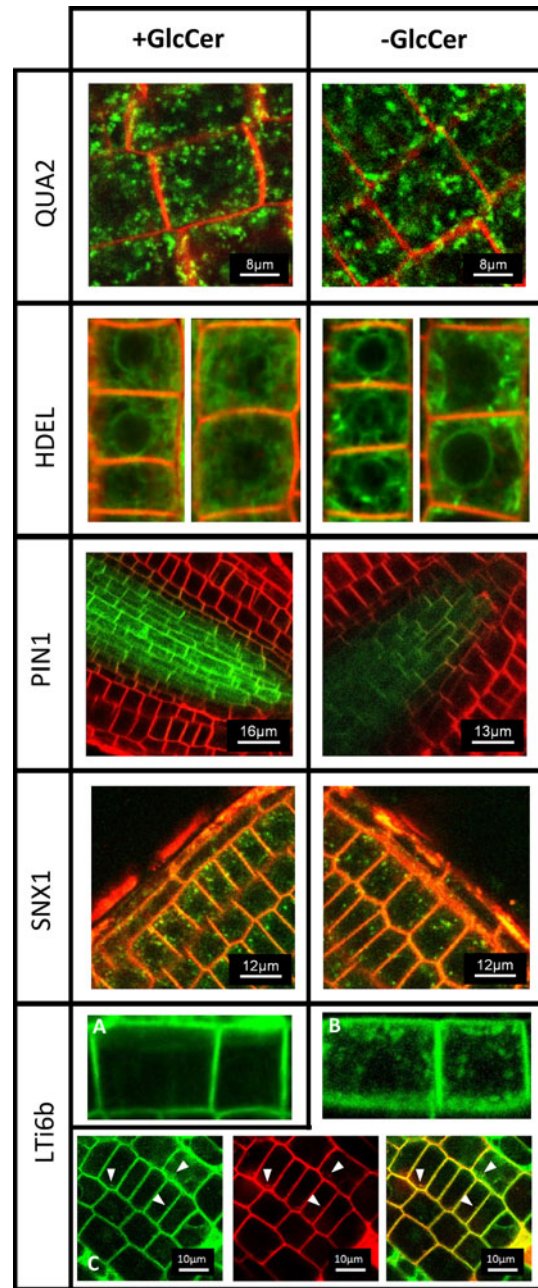


Fig. 2 Effect of GlcCer deficit (-GlcCer) compared to normal GlcCer levels (+GlcCer) on the localization of secretory proteins in *Arabidopsis* root cells. *QUAZ2* Golgi protein Quasimodo2, *left* control cells, *right* mutant cells. *HDEL* GFP coupled with an ER retention/retrieval signal, *left* control cells, *right* mutant *gcs2.2* cells. *PIN1* plasma membrane auxin efflux carrier, *left* control cells, *right* mutant *gcs2.2* cells treated with PDMP. *SNX1* prevacuole and early endosome Sortin Nexin 1, *left* control cells, *right* mutant *gcs2.2* cells. *LTi6b* plasma membrane protein Low-Temperature Induced 6b. **a** Control cells, **b** mutant *gcs2.2* cells, **c** white arrowheads indicate co-localization of LTi6b (green) and FM4-64 (red) in endosomes. A delocalization of HDEL, PIN1, and LTi6b is observed upon GlcCer deficit

fluorescence signal were systematically and consistently reduced in roots treated with PDMP (Fig. 2). In spite of the general loss in PIN1 signal, the marker did not seem

de-localized in PDMP-treated seedlings. Fluorescent signal was recovered following PDMP washout, and the reduced PIN1 levels were unlikely to be the result of a downregulation at the transcriptional level, since endogenous PIN1 transcription were found upregulated in PDMP-treated seedlings.

The inhibition of GlcCer biosynthesis by PDMP seems to produce similar effects as those observed in the mutant *smt1^{orc}* (Willemsen et al. 2003). Sterols and GlcCer associate in the plasma membrane to form lipid microdomains. The formation of these microdomains may provide the cell with a dynamic platform for the polar targeting and localization of proteins in the PM. Therefore, alterations in the proportion or content of sterols and GlcCer in the PM or in endomembranes may be sufficient to alter the transport and/or stability of membrane-bound proteins such as PIN1. Polarized transport routes may arise from sorting organelles during development, such as the Golgi in the biosynthetic secretory pathway, or the endosomes in the recycling pathway. GlcCer is transported along the biosynthetic secretory pathway to accumulate in the PM in lipid microdomains. Inhibition of GlcCer synthesis is sufficient to disrupt the transport flux through the Golgi, which is reflected in morphological changes of the Golgi. PIN1 does not accumulate in intracellular compartments upon a block in GlcCer synthesis but seems to be rapidly degraded. Degradation of PIN proteins is a necessary part of the regulation of root gravitropism. For example, continuous cycling between the PM and endosomes controls subcellular distribution and steady-state levels of PIN2 (Abas et al. 2006), so that a fraction of ubiquitinated endocytosed protein is not recycled but degraded by the proteasome (Abas et al. 2006). It is possible that a deregulation in PM-endosome dynamics caused by the block in GlcCer synthesis, as illustrated by *LTi6b*, may result in the rapid up-regulation of PIN1 degradation, which would help explain the disappearance of PIN1 in PDMP-treated roots. PIN1 is known to be enriched in detergent resistant membranes (DRM) through its interaction with the efflux transporter ABCB19 (Titapiwatanakun et al. 2009). When ABCB19 and PIN1 co-localize in DRM, ubiquitin-mediated degradation of PIN1 is reduced, a response modulated by MOP2 and MOP3 (Modulator of PIN) proteins (Malenica et al. 2007). It is therefore possible that the inhibition in GlcCer synthesis and its subsequent decrease in the PM is enough to increase the instability of PIN1 in the PM, thereby increasing its release for degradation. On the contrary, no effect of decreasing GlcCer amounts (even after PDMP treatment) was observed in the *gcs2.2* line expressing Sorting nexin-1 (SNX1) which regulates PIN2 endocytic sorting in distinct endosomes than those handling PIN1 (Jaillais et al. 2006). Therefore, different

endocytic routes and recycling pathways may vary in their requirement for GlcCer (and sterol) biosynthesis.

In conclusion, the biosynthesis, flow and homeostasis of GlcCer in membranes, to maintain a certain concentration in specific areas of these membranes, is likely to be required for vesicle dynamics that mediate protein transport to the cell surface, retrograde transport from the Golgi back to the ER, and in some cases recycling between PM and specific endosomal compartments. It is the synthesis of GlcCer, and not so much the total content in cells, which is critical for secretory transport dynamics. This is based on our observations of the redistribution of marker proteins in *Arabidopsis* treated with PDMP, in which the synthesis was inhibited by at least 50% whereas total GlcCer content was only reduced by 15–20%. Furthermore, the specific GlcCer concentration in different areas of the membrane may determine the formation of transport vesicles. Protein transport is particularly intensive to and from the Golgi and there is a strong interplay between GlcCer biosynthesis, protein transport and the maintenance of Golgi morphology. Coat machineries may influence lipid sorting, and lipids may be able to modify the conformation, activation and/or recruitment of proteins involved in vesicle formation. For example, the asymmetrical and unique lipid composition of the outer leaflet of the outer chloroplast envelope is essential for the correct topology of the transmembrane protein OEP7 (Schleiff et al. 2001). The interactions between GlcCer and the Golgi and endosomal compartments are not known, but we propose that GlcCer, through its ongoing synthesis, plays an active role in the regulation of several transport steps in plant cells (Fig. 3).

Lipid-protein interactions in the eukaryotic secretory pathway

Protein sorting towards the secretory pathway in eukaryotic cells may be assisted by lipid-based sorting events and also specific interactions with acidic phospholipids (Ceppi et al. 2005; Lev 2006). For example, a specific targeting of phosphatidylserine (PS) to ER-derived domains was evidenced in plant cells (Vincent et al. 2001), and PS was demonstrated to be required in the formation of ER-derived COPII vesicles in vitro (Matsuoka et al. 1998). Short chain ceramides can influence the binding of ARF to Golgi membranes and therefore affect the formation of COPI vesicles (Abousalham et al. 2002).

In yeast, the oxysterol-binding protein (OSBP) Kes1p is a lipid receptor which may regulate the formation of Golgi secretory vesicles through regulation of the ARF and Sec14 pathways (Li et al. 2002). In animal cells, OSBP-related proteins have been shown to interact with a syntaxin-like VAMP-associated protein-A, and to be involved in the

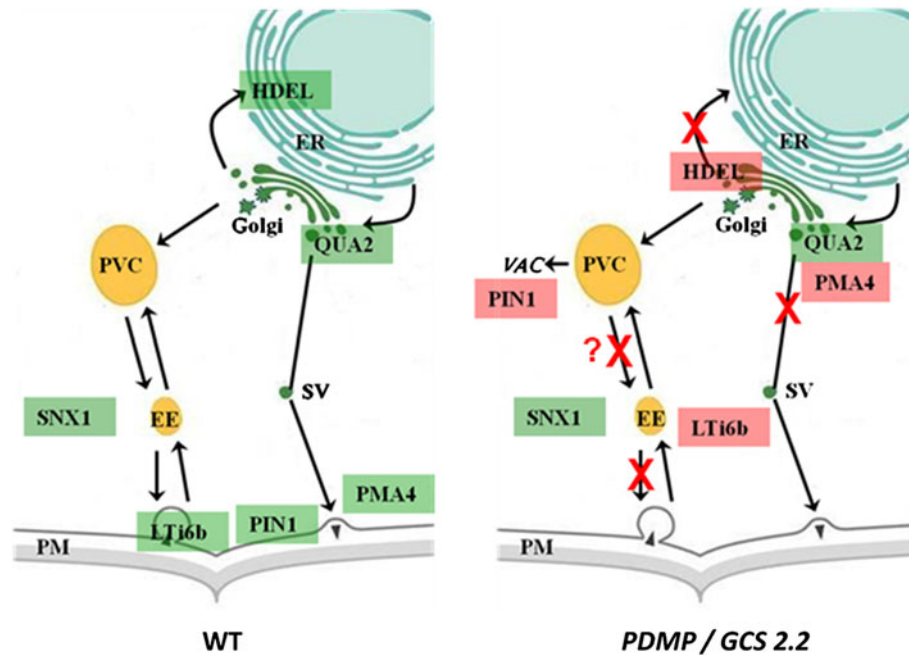


Fig. 3 Transport steps affected by inhibition of GlcCer biosynthesis in *Arabidopsis* roots. This simplified scheme presents the affected transport steps by red crosses, proteins corresponding to unaffected transport steps are shown in *green*, and proteins corresponding to the affected transport steps are shown in *red*. Arrival at the Golgi is less demanding of GlcCer than exit of proteins from the Golgi, as illustrated by QUA2 and GFP-HDEL respectively, and exit of PMA4 from the Golgi which was also affected by PDMP in tobacco leaf epidermal cells. Proteins can be internalized from the PM by endocytosis and can recycle back to the PM (PIN1, LTI6b). GlcCer

inhibition alters endosome dynamics, as illustrated by LTI6b. Endocytosis of proteins may also result in their degradation in the lytic vacuole (PIN1). However, SNX1 involved in the PIN2 endocytic pathway was not disturbed, and PIN2 was also found not affected in its recycling (not shown). Therefore, the different exocytic and endocytic transport routes may differ in their requirement of GlcCer biosynthesis and behavior when this synthesis is impaired. *EE* early endosomes, *ER* endoplasmic reticulum, *PM* plasma membrane, *PVC* pre-vacuolar compartment, *SV* secretory vesicles, *VAC* vacuole

function of the COPII-dependent ER-Golgi anterograde transport pathway required in protein and ceramide transport (Wyles et al. 2002). In *Arabidopsis*, the sterol-binding protein ORP3a localizes to ER membranes and is recycling between the ER and the Golgi, and may participate in sterol mobilization required for specific membrane dynamics related to the secretory process (Saravanan et al. 2009).

It has been observed that SNAREs can have various affinities for lipid microdomains such as sterol- and sphingolipid-rich lipid rafts, and that the degree of their interaction with these domains may regulate the efficiency of exocytosis (Salaün et al. 2005a, b). More recent work has confirmed the interactions between SNAREs and several lipids (sterols, sphingosine, phospholipids, phosphoinositides, etc.) and their very critical involvement in shaping and regulating membrane fusion (Lam et al. 2008; Tong et al. 2009; Darios et al. 2009; Mima and Wickner 2009). Phospholipases D and phospholipases A-like enzymes can interact with proteins of the COPII machinery to sustain the formation and regulation of ER export sites (Pathre et al. 2003; Shimoi et al. 2005). Moreover, the basic domain of the synaptobrevin VAMP2 strongly interacts with the phospholipid PS (De Haro et al. 2003). Acidic

phospholipids (PA, PIP2, PS) may regulate the formation of SNARE complexes and therefore the fusion events required for secretion to occur. Protein–lipid interactions can, as in the case of syntaxin1A, contribute to concentrate fusogenic lipids such as PA to the fusion sites (Lam et al. 2008). Finally, it has recently been proposed that ceramides produced from sphingolipid-rich domains contribute to the lateral segregation and sorting of protein cargos within endosomal membranes (Trajkovic et al. 2008).

Instead of the usual C-terminal isoprenylation, common to Rab5 GTPases, Ara6 is modified with N-terminal myristoylation and palmitoylation (S-acylation), both of which are essential and seem to be employed as a membrane anchor system (Ueda et al. 2001). In the case of Arf-GTPase, responsible for triggering COPI recruitment to donor Golgi membranes, myristoylation of the N-terminus is essential for membrane binding of the activated GTP form (Goldberg 1998). Ara6 co-localizes with the sterol-reactive dye filipin on a subset of early endosomes, which suggests that N-terminal myristoylation and S-acylation may enable association of proteins with DRMs in the plasma membrane. It is tempting to speculate that fatty acid modification may determine the preferential interaction of

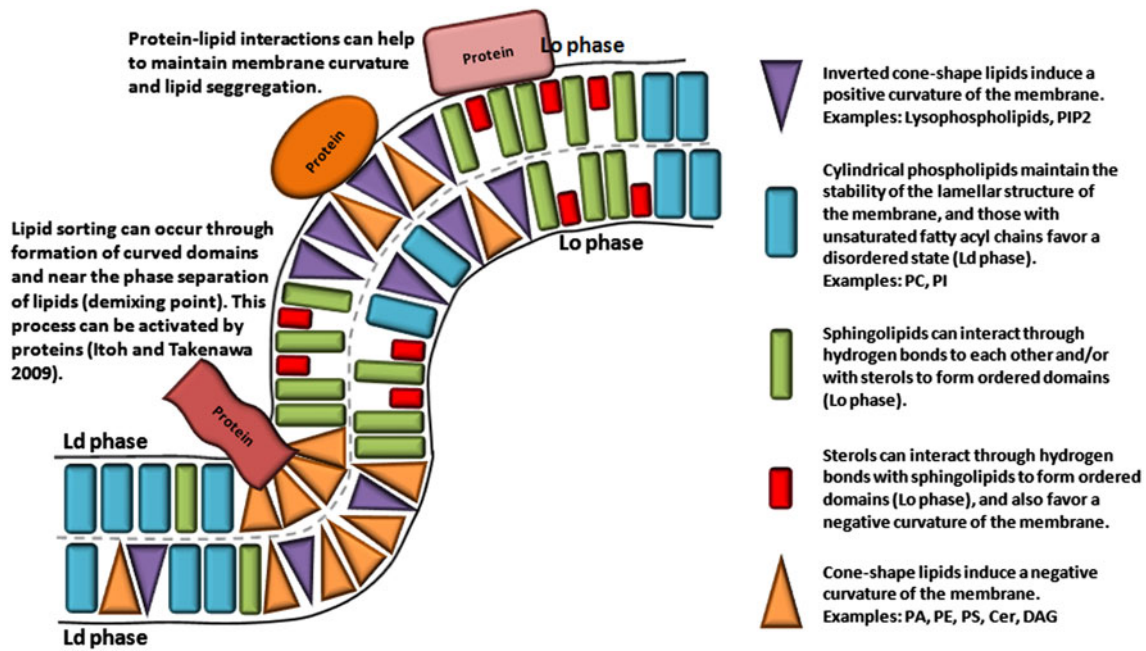


Fig. 4 Structural features of the main lipid families. Lipids which can be segregated in Ld or Lo phase domains as defined in Hammond et al. (2005) are shown. *Cone-shape* (negative membrane curvature)

proteins with GlcCer-rich membrane domains, in which GlcCer may dynamically modulate the required protein-membrane targeting, association and stability that is necessary to execute and maintain cellular responses related to growth or environmental cues.

The interactions between lipids and proteins of the intracellular transport machineries may contribute to regulate the specific targeting and function of these proteins. It must be considered that at the time a lipid is synthesized, its physicochemical properties will spontaneously allow or avoid specific interactions with other partners (other lipids or proteins) that will govern its interaction within a microdomain or another, and according to this environment, will target the lipid and its partners to a specific transport pathway.

Therefore, lipids, lipid-modifying enzymes and lipid modification of proteins must be considered as key regulators of membrane domain formation and membrane/organelle homeostasis. With protein-based machineries, lipid-based machineries are also crucial regulatory forces related to protein trafficking and sorting through the secretory pathway.

Physicochemical properties of lipids in membrane dynamics

As described above, lipids can serve as protein receptors through ionic interactions and contribute to the recruitment

and *inverted cone-shape* lipids (positive membrane curvature) are indicated. Proteins able to interact with specific lipids to form/maintain specific membrane domains are also illustrated

of cytosolic proteins of the machineries, they can modify proteins for their targeting and/or function, they can help the lateral segregation of protein partners into specific domains (Haucke and di Paolo 2007; Lang et al. 2008), but they contribute directly, through their biophysical properties (non-lamellar intermediary phases, membrane curvature, membrane elasticity, etc.) to help protein machineries to shape/distort membranes for membrane bending, budding and fusion processes (Janmey and Kinnunen 2006; Wickner and Schekman 2006; Chernomordik and Kozlov 2008; Martens and McMahon 2008; Phillips et al. 2009; Itoh and Takenawa 2009). Lipid-induced membrane curvature can condition the recruitment and activity of protein partners (Bigay et al. 2005; Mesmin et al. 2007). Some features of the physicochemical properties of lipids are summarized in Fig. 4.

It is now mostly accepted that membrane fusion proceeds through a hemifusion intermediate also called “hemifusion stalk”, and progress then via a fusion pore (Lang et al. 2008; Chernomordik and Kozlov 2008; Martens and McMahon 2008). There is also compelling evidence that lipids directly participate in the different steps of the fusion process. For fusion to occur, membranes have to be into close proximity, and to be destabilized. It has been suggested that both events require the induction of membrane curvature at the fusion site (Martens and McMahon 2008). Membrane curvature can be managed by specific proteins and peptidic sequences such as the C2 domains (Chernomordik and Kozlov 2008; Martens and

McMahon 2008; Hu et al. 2008), which are Ca^{2+} and lipid-binding domains (Meijer and Munnik 2003), but is also highly provoked and regulated by lipids and their metabolism. The spontaneous curvature of lipids is related to their structure. PC and SM, which are cylindrical molecules, favor flat bilayer structures, lysophospholipids such as LPC and polyphosphoinositides such as PIP2 (inverted cone shaped lipid) induce membranes with a positive curvature, whereas PA, PE, PS, DAG, ceramides (cone shaped lipids) and fatty acids and cholesterol induce membranes with a negative curvature (Janmey and Kinnunen 2006; Chernomordik and Kozlov 2008). The stalk hypothesis considers that the formation of the hemifusion stalk intermediate is favored by lipids inducing negative curvature (Lang et al. 2008; Chernomordik and Kozlov 2008). Therefore, lipids inducing positive curvature such as lysophosphatidylcholine (LPC) are inhibiting the early stages of membrane fusion whereas lipids, which induce negative curvature such as PE, are stimulating these steps (Lang et al. 2008; Chernomordik and Kozlov 2008). Then the reverse is observed for the formation of the fusion pore, LPC favors and PE inhibits when they are added to the distal leaflets of the fusing membranes, suggesting that a positive curvature of the inner leaflets is required for full fusion to be achieved (Chernomordik and Kozlov 2008). It has been suggested for example that PA stimulates the formation of the hemifusion state and PIP2 is required to complete fusion in the case of SNARE-driven liposome fusion (Vicogne et al. 2006). The sterol structure can affect membrane curvature and prepare a membrane for budding or fusion events (Bacia et al. 2005). Therefore, lipid metabolizing enzymes such as phospholipases, sphingomyelinase, acyltransferases, and the overall lipid metabolism strongly contribute to membrane fusion and its regulation by providing lipids with specific structures compatible with the different steps of the fusion process. More examples are illustrating the requirement of such specific lipids in various intracellular fusion events (Janmey and Kinnunen 2006; Wickner and Schekman 2006; Lang et al. 2008; Chernomordik and Kozlov 2008; Martens and McMahon 2008; Brown et al. 2008; Phillips et al. 2009; Itoh and Takenawa 2009 and references therein).

In addition, it has also to be taken into account that lipid phase separation, which may be handled by specific proteins, can trigger membrane fission (Roux et al. 2005). Recently, lipid sorting without any protein machinery was found to proceed through curved membranes structures, and that it occurs near a demixing point (i.e. close to the phase separation state between curved and non curved domains or may be also between Ld and Lo phases, see Fig. 4) (Sorre et al. 2009). Therefore, physiological membranes being mostly close to such phase separation (Roux et al. 2005 and references therein), it is easy to deduce how

even small variations in lipid composition and/or content in specific domains can modulate membrane morphodynamics. We have also to take into account that lipid metabolism, through phospholipases, acyltransferases, kinases, phosphatases, and the various metabolic pathways, can rapidly (from seconds to minutes) modify the membrane lipid composition in small or wider membrane domains. In addition, several proteins (Itoh and Takenawa 2009), through their lipid-binding domains (ALPS, BAR, I-BAR, F-BAR/EFC, ENTH, etc.) not only will bind to such specific membrane domains but will help shaping these domains for specific purposes (fusion, fission, tubulation, etc.). Therefore, there are synergistic effects (lipids on proteins and proteins on lipids) to govern the subtle membrane lipid composition and structure changes.

ARFGAP1 activity toward ARF1 is repressed by low membrane curvature to ensure ARF1-mediated recruitment of COPI to the membrane (Bigay et al. 2005). Recently, ARFGAP1 was shown to be hypersensitive to positive membrane curvature for binding, to be restricted to curved regions of the membrane whereas ARF1 binds to flat regions, and that in the model proposed, membrane fission (COPI vesicle release) is the governing step in coat disassembly (Ambroggio et al. 2009). Therefore, ARFGAP1 activity is sensitive to membrane curvature and ARFGAP1 accelerates deactivation and release of ARF1 with increasing positive curvature of the membrane as the vesicle is formed. This implies that different factors regulate COPII and COPI vesicle formation in the ER and Golgi, respectively. Differences in the lipid composition of Golgi and ER membranes may be a key factor in the regulation of COPII and COPI coat dynamics and cargo sorting during vesicle formation in the Golgi and the ER.

CtBP/BARS (BARS-50) has been identified as an important factor for COPI-mediated transport. It has been shown that CtBP/BARS play a key role in COPI vesicle fission. Although CtBP/BARS is not essential for cargo recruitment, CtBP/BARS association with ARF1-directed ARFGAP1 promotes vesicle fission (Yang et al. 2008). Membrane lipids are important elements in the function of CtBP/BARS, as it requires PA, which is generated by phospholipase D2 (PLD2) (Yang et al. 2008). In addition, DAG synthesis from PA is required in membrane vesicle formation in the Golgi apparatus in animal cells (Asp et al. 2009). An orthologue of the human CtBP/BARS has been identified in plants (Kim et al. 2002).

It has been found that PDMP partially blocks the ADP-ribosylation of BARS-50 (CtBP/BARS) induced by BFA (128) and inhibits the disassembly of the Golgi complex and its merging with the ER compartment in BFA-treated cells (De Matteis et al. 1999). The effect of PDMP on BARS-50 is an event that occurs after the binding of COPI

components to Golgi membranes as PDMP did not alter the effect of BFA on ARF or β -COP binding to isolated Golgi membranes (De Matteis et al. 1999). These data, combined with the delocalization of GFP-HDEL in punctuate structures in the *gcs2.2* mutant (Fig. 2) suggest a possible role for GlcCer in the fission of COPI vesicles through CtBP/BARS function.

This hypothesis could be challenged by a parallel study, as PDMP inhibition of BFA-induced Golgi dispersal could not be counteracted by addition of C6-GlcCer (Kok et al. 1998). The study suggested instead that the PDMP-mediated inhibition of Golgi dispersal by BFA was accomplished via a specific modulation of calcium homeostasis. However, although calcium is important in COPI-mediated retrograde transport, the need for calcium occurs at late stages in the COPI system, notably in the timing of COPI vesicle uncoating, well after ADP-ribosylation factor binding to the membrane (Ahluwalia et al. 2001). The results by Kok et al. (1998) may also be confounded by the observation that short-chain ceramides are able to inhibit BFA-induced Golgi disassembly whereas long-chain ceramides do not seem to have any effect (Fukunaga et al. 2000). Glucosylceramides include a large number of molecular species in plant membranes (Warnecke and Heinz 2003), which makes them a varied group of lipids. One main features of GlcCer is the presence of an alpha-hydroxylated long chain fatty acid (LCFA, up to 18 carbon atoms) or very long chain fatty acid (VLCFA, more than 18 carbon atoms). The VLCFA are inherently essential components of sphingolipids, and it is possible that the effects of PDMP in BFA-treated cells simply could not be bypassed by the addition of GlcCer with a short six-carbon acyl chain.

More precise analysis of the requirement for sphingolipids in surface transport and stabilization of Pma1 subsequently revealed that all mutations that affect the synthesis of VLCFA (C26) result in the rapid turnover of newly synthesized Pma1 in yeast (Gaigg et al. 2005). The synthesis of C26-containing lipids, rather than ceramide or sphingolipids per se, is important for raft association of newly synthesized Pma1 and for its stable delivery to the cell surface (Gaigg et al. 2005). It has been observed that C26-containing PI can replace the essential function of sphingolipids and structurally and functionally mimic sphingolipids (Schneiter et al. 2004). Shortening the C26 fatty acid on these suppressor lipids by means of an *elo3 Δ* mutation, however, neutralized the suppressor activity of these lipids and resulted in the rapid turnover of the newly synthesized Pma1 (Gaigg et al. 2006). These results thus strongly indicate that lipids containing C26 fatty acids (VLCFA), either bound to ceramide or glycerophospholipids, are important for the stable biogenesis of Pma1. VLCFA were recently shown to be critical in plants since

VLCFA synthesized by the fatty acid elongase complex in the ER are required for polar auxin transport, cell expansion and tissue patterning during *Arabidopsis* development (Zheng et al. 2005; Roudier et al. 2010). Targeting of VLCFA-containing phospholipids and especially VLCFA-PS to ER-derived domains was also observed as critical events of the early plant secretory pathway (Moreau et al. 1998; Vincent et al. 2001; Moreau 2007). PS was shown to be required in the formation of ER-derived COPII vesicles (Matsuoka et al. 1998), and GlcCer was found to be involved in several steps of post-Golgi trafficking (Melser et al. 2010, Figs. 2, 3). We can therefore speculate that VLCFA-containing lipids (glycerophospholipids and sphingolipids) may be crucial in some instances to membrane dynamics through their tendency to manage membrane curvature (Zheng et al. 2005).

Concluding remarks and perspectives

A tremendous amount of data implicate lipids, lipid-modifying enzymes and lipid-modified proteins as determinant partners of the functional machineries acting in the secretory pathway of eukaryotic and plant cells and also in cytokinesis (Montagnac and Chavrier 2010). One key feature in these events is also an incredible co-regulation of the lipid biosynthetic pathways involved between themselves so that each step/event undergoes several levels of regulation (Venable et al. 1996; van Blitterswijk et al. 2003; Tóth et al. 2006; Kajiwara et al. 2008; Aerts et al. 2008; Domonkos et al. 2008). A big challenge for the future will be to inter-connect all these lipid biosynthetic pathways and their regulations in an overall model of lipid-assisted mechanisms involved in secretory and endocytic pathways and in diverse cell functions (cytokinesis, apoptotic and cell death events, ageing, photosynthesis, energy management, etc.). In addition, modeling mathematically such different events as recently shown for vesicle trafficking in pollen tubes (Kato et al. 2010) will be other perspectives. Finally, behind genetic, biochemical and biophysical approaches of lipid-dependent phenomena, another challenge in plant cells will be the monitoring of lipid dynamics in vivo with specific tools (Vielhaber et al. 2001; Kuerschner et al. 2005, 2008; Men et al. 2008; Babiychuk et al. 2008; Stöckl et al. 2008; Boutté et al. 2009; Vermeer et al. 2009; Stöckl and Herrmann 2010).

Acknowledgments We thank the IFR 103 (IBVM, INRA Bordeaux) and the Bordeaux Imaging Centre (BIC), University Victor Segalen Bordeaux 2, for the confocal microscope facilities at the Plant Imaging Platform and the Photonic Imaging Platform. We also thank C. Cheniclet and V. Rouyere (Plant Imaging Platform of BIC) for their help with transmission electron microscopy (TEM). This

work was financed by the ANR SphingopolaR grant (ANR-07-BLAN-0202), University Victor Segalen Bordeaux 2, CNRS, INRA, and the Conseil Régional d'Aquitaine.

References

- Abas L, Benjamins R, Malenica N, Paciorek T, Wiśniewska J, Moulinier-Anzola JC, Sieberer T, Friml J, Luschnig C (2006) Intracellular trafficking and proteolysis of the Arabidopsis auxin-efflux facilitator PIN2 are involved in root gravitropism. *Nat Cell Biol* 8:249–256
- Abousalham A, Hobman TC, Dewald J, Garbutt M, Brindley DN (2002) Cell-permeable ceramides preferentially inhibit coated vesicle formation and exocytosis in Chinese hamster ovary compared with Madin-darby canine kidney cells by preventing the membrane association of ADP-ribosylation factor. *Biochem J* 361:653–661
- Aerts AM, Zabrocki P, François IE, Carmona-Gutierrez D, Govaert G, Mao C, Smets B, Madeo F, Winderickx J, Cammue BP, Thevissen K (2008) Ydc1p ceramidase triggers organelle fragmentation, apoptosis and accelerated ageing in yeast. *Cell Mol Life Sci* 65:1933–1942
- Ahluwalia JP, Topp JD, Weirather K, Zimmerman M, Stamnes M (2001) A role for calcium in stabilizing transport vesicle coats. *J Biol Chem* 276:34148–34155
- Altmann K, Westermann B (2005) Role of essential genes in mitochondrial morphogenesis in *Saccharomyces cerevisiae*. *Mol Biol Cell* 16:5410–5417
- Ambroggio E, Sorre B, Bassereau P, Goud B, Manneville JB, Antonny B (2009) ArfGAP1 generates an Arf1 gradient on continuous lipid membranes displaying flat and curved regions. *EMBO J* 9:292–303
- Andersson MX, Goksor M, Sandelius AS (2007) Optical manipulation reveals strong attracting forces at membrane contact sites between endoplasmic reticulum and chloroplasts. *J Biol Chem* 282:1170–1174
- Asp L, Kartberg F, Fernandez-Rodriguez J, Smedh M, Elsner M, Laporte F, Bárcena M, Jansen KA, Valentijn JA, Koster AJ, Bergeron JJ, Nilsson T (2009) Early stages of Golgi vesicle and tubule formation require diacylglycerol. *Mol Biol Cell* 20:780–790
- Babiychuk EB, Monastyrskaya K, Draeger A (2008) Fluorescent annexin A1 reveals dynamics of ceramide platforms in living cells. *Traffic* 9:1757–1775
- Bacia K, Schwille P, Kurzchalia T (2005) Sterol structure determines the separation of phases and the curvature of the liquid-ordered phase in model membranes. *Proc Natl Acad Sci USA* 102:3272–3277
- Bagnat M, Simons K (2002) Cell surface polarization during yeast mating. *J Biol Chem* 277:1475–1480
- Baron CL, Malhotra V (2002) Role of diacylglycerol in PKD recruitment to the TGN and protein transport to the plasma membrane. *Science* 295:325–338
- Benning C (2009) Mechanisms of lipid transport involved in organelle biogenesis in plant cells. *Annu Rev Cell Dev Biol* 25:71–91
- Bigay J, Casella JF, Drin G, Mesmin B, Antonny B (2005) ArfGAP1 responds to membrane curvature through the folding of a lipid packing sensor motif. *EMBO J* 24:2244–2253
- Boutté Y, Grebe M (2009) Cellular processes relying on sterol function in plants. *Curr Opin Plant Biol* 12:705–713
- Boutté Y, Frescatada-Rosa M, Men S, Chow CM, Ebine K, Gustavsson A, Johansson L, Ueda T, Moore I, Jürgens G, Grebe M (2009) Endocytosis restricts Arabidopsis KNOLLE syntaxin to the cell division plane during late cytokinesis. *EMBO J* 29:546–558
- Brown WJ, Plutner H, Drecktrah D, Judson BL, Balch WE (2008) The lysophospholipid acyltransferase antagonist CI-976 inhibits a late step in COPII vesicle budding. *Traffic* 9:786–797
- Capel J, Jarillo JA, Salinas J, Martínez-Zapater JM (1997) Two homologous low-temperature-inducible genes from Arabidopsis encode highly hydrophobic proteins. *Plant Physiol* 115:569–576
- Ceppi P, Colombo S, Francolini M, Raimondo F, Borgese N, Masserini M (2005) Two tail-anchored protein variants, differing in transmembrane domain length and intracellular sorting, interact differently with lipids. *Proc Natl Acad Sci USA* 102:16269–16274
- Chernomordik LV, Kozlov MM (2008) Mechanisms of membrane fusion. *Nat Struct Mol Biol* 15:675–683
- Choi SY, Huang P, Jenkins GM, Chan DC, Schiller J, Frohman MA (2006) A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. *Nat Cell Biol* 8:1255–1262
- Churchward MA, Rogasevskaia T, Höfgen J, Bau J, Coorssen JR (2005) Cholesterol facilitates the native mechanism of Ca²⁺-triggered membrane fusion. *J Cell Sci* 118:4833–4848
- Cordeiro D, Hidalgo Carcedo C, Bonazzi M, Luini A, Spano S (2002) Molecular aspects of membrane fission in the secretory pathway. *Cell Mol Life Sci* 59:1819–1832
- Cutler SR, Ehrhardt DW, Griffiths JS, Somerville CR (2000) Random GFP:cDNA fusions enable visualization of subcellular structures in cells of Arabidopsis at a high frequency. *Proc Natl Acad Sci USA* 97:3718–3723
- Darios F, Wasser C, Shakirzyanova A, Giniatullin A, Goodman K, Munoz-bravo JL, Raingo J, Jorgacevski J, Kreft M, Zorec R, Rosa JM, gandia L, Gutierrez LM, Binz T, Giniatullin R, Kavalali ET, Davletov B (2009) Sphingosine facilitates SNARE complex assembly and activates synaptic vesicle exocytosis. *Neuron* 62:683–694
- De Figueiredo P, Drecktrah D, Katzenellenbogen JA, Strang M, Brown WJ (1998) Evidence that phospholipase A2 activity is required for Golgi complex and trans Golgi network membrane tubulation. *Proc Natl Acad Sci USA* 95:8642–8647
- De Haro L, Quetglas S, Iborra C, Leveque C, Seagar M (2003) Calmodulin-dependent regulation of a lipid binding domain in the v-SNARE synaptobrevin and its role in vesicular fusion. *Biol Cell* 95:459–464
- De Matteis MA, Luna A, Di Tullio G, Corda D, Kok JW, Luini A, Egea G (1999) PDMP blocks the BFA-induced ADP-ribosylation of BARS-50 in isolated Golgi membranes. *FEBS Lett* 459:310–312
- Devaux PF (2000) Is lipid translocation involved during endo- and exocytosis? *Biochimie* 82:497–509
- Dietrich CR, Han G, Chen M, Berg RH, Dunn TM, Cahoon EB (2008) Loss-of-function mutations and inducible RNAi suppression of Arabidopsis LCB2 genes reveal the critical role of sphingolipids in gametophytic and sporophytic cell viability. *Plant J* 54:284–298
- Dimmer KS, Fritz S, Fuchs F, Messerschmitt M, Weinbach N, Neupert W, Westermann B (2002) Genetic basis of mitochondrial function and morphology in *Saccharomyces cerevisiae*. *Mol Biol Cell* 13:847–853
- Domonkos I, Laczko-Dobos H, Gombos Z (2008) Lipid-assisted protein-protein interactions that support photosynthetic and other cellular activities. *Prog Lipid Res* 47:422–435
- Drecktrah D, Chambers K, Racoosin EL, Cluett EB, Gucwa A, Jackson B, Brown WJ (2003) Inhibition of a Golgi complex lysophospholipid acyltransferase induces membrane tubule formation and retrograde trafficking. *Mol Biol Cell* 14:3459–3469

- Eastmond PJ, Quettier AL, Kroon JTM, Craddock C, Adams N, Slabas AR (2010) Phosphatidic acid phosphohydrolase 1 and 2 regulate phospholipid synthesis at the Endoplasmic Reticulum in *Arabidopsis*. *Plant Cell* 22:2796–2811
- Faso C, Chen YN, Tamura K, held M, Zemelis S, Marti L, Saravanan R, Hummel E, Kung L, Miller E, Hawes C, Brandizzi F (2009) A missense mutation in the Arabidopsis COPII coat protein Sec24A induces the formation of clusters of the endoplasmic reticulum and Golgi apparatus. *Plant Cell* 21:3655–3671
- Fernandez-Ulibarri I, Vilella M, Lazaro-Dieguez F, Sarri E, Martinez SE, Jimenez N, Claro E, Merida I, Burger KNJ, Egea G (2007) Diacylglycerol is required for the formation of COPI vesicles in the Golgi-to-ER transport pathway. *Mol Biol Cell* 18:3250–3263
- Freyberg Z, Bourgoin S, Shields D (2002) Phospholipase D2 is localized to the rims of the Golgi apparatus in mammalian cells. *Mol Biol Cell* 13:3930–3942
- Fukunaga T, Nagahama M, Hatsuzawa K, Tani K, Yamamoto A, Tagaya M (2000) Implication of sphingolipid metabolism in the stability of the Golgi apparatus. *J Cell Sci* 113:3299–3307
- Gaigg B, Timischl B, Corbino L, Schneiter R (2005) Synthesis of sphingolipids with very long chain fatty acids but not ergosterol is required for routing of newly synthesized plasma membrane ATPase to the cell surface of yeast. *J Biol Chem* 280:22515–22522
- Gaigg B, Toulmay A, Schneiter R (2006) Very long-chain fatty acid-containing lipids rather than sphingolipids per se are required for raft association and stable surface transport of newly synthesized plasma membrane ATPase in yeast. *J Biol Chem* 281:34135–34145
- Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science* 282:2226–2230
- Goldberg J (1998) Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. *Cell* 95:237–248
- Grebe M, Xu J, Möbius W, Ueda T, Nakano A, Geuze HJ, Rook MB, Scheres B (2003) Arabidopsis sterol endocytosis involves actin-mediated trafficking via ARA6-positive early endosomes. *Curr Biol* 13:1378–1387
- Griffing LR (2009) Networking in the endoplasmic reticulum. *Biochem Soc Trans* 38:747–753
- Grimmer S, Ying M, Wälchli S, van Deurs B, Sandvig K (2005) Golgi vesiculation induced by cholesterol occurs by a dynamin- and cPLA2-dependent mechanism. *Traffic* 6:144–156
- Halter D, Neumann S, van Dijk SM, Wolthoorn J, de Mazière AM, Vieira OV, Mattjus P, Klumperman J, van Meer G, Sprong H (2007) Pre- and post-Golgi translocation of glucosylceramide in glycosphingolipid synthesis. *J Cell Biol* 179:101–115
- Hammond AT, Heberle FA, Baumgart T, Holowka D, Baird B, Feigenson DW (2005) Crosslinking a lipid raft component triggers liquid ordered–liquid disordered phase separation in model plasma membranes. *Proc Natl Acad Sci USA* 102:6320–6325
- Hancock JF (2006) Lipid rafts: contentious only from simplistic standpoints. *Nat Rev Mol Cell Biol* 7:456–462
- Hanton S, Chatre L, Renna L, Matheson LA, Brandizzi F (2007) De novo formation of plant endoplasmic reticulum export sites is membrane cargo induced and signal mediated. *Plant Physiol* 143:1640–1650
- Hanton S, Matheson LA, Chatre L, Brandizzi F (2009) Dynamic organization of COPII coat proteins at endoplasmic reticulum export sites in plant cells. *Plant J* 57:963–974
- Hartmann MA, Perret AM, Carde JP, Cassagne C, Moreau P (2002) Inhibition of the sterol pathway in leek seedlings impairs phosphatidylserine and glucosylceramide synthesis but triggers an accumulation of triacylglycerols. *Biochim Biophys Acta* 1583:285–296
- Haucke V, Di Paolo G (2007) Lipids and lipid modifications in the regulation of membrane traffic. *Curr Opin Cell Biol* 19:426–435
- Hawes C, Schoberer J, Hummel E, Osterrieder A (2010) Biogenesis of the plant Golgi apparatus. *Biochem Soc Trans* 38:761–767
- Heese-Peck A, Pichler H, Zanolari B, Watanabe R, Daum G, Riezman H (2002) Multiple functions of sterols in yeast endocytosis. *Mol Biol Cell* 13:2664–2680
- Hu W, Xu R, Zhang G, Jin J, Szulc ZM, Bielawski J, Hannun YA, Obeid LM, Mao C (2005) Golgi fragmentation is associated with ceramide-induced cellular effects. *Mol Biol Cell* 16:1555–1567
- Hu J, Shibata Y, Voss C, Shemesh T, Li Z, Coughlin M, Kozlov MM, Rapoport TA, Prinz WA (2008) Membrane proteins of the endoplasmic reticulum induce high-curvature tubules. *Science* 319:1247–1250
- Hwang I, Robinson DG (2009) Transport vesicle formation in plant cells. *Curr Opin Plant Biol* 12:660–669
- Itoh T, Takenawa T (2009) Mechanisms of membrane deformation by lipid-binding domains. *Prog Lipid Res* 48:298–305
- Jaillais AY, Fobis-Loisy I, Miège C, Rollin C, Gaude T (2006) tSNX1 defines an endosome for auxin-carrier trafficking in Arabidopsis. *Nature* 443:106–109
- Janmey PA, Kinnunen PKJ (2006) Biophysical properties of lipids and dynamic membranes. *Trends Cell Biol* 16:538–546
- Jelinkova A, Malinska K, Simon S, Kleine-Vehn J, Parezova M, Pejchar P, Kubes M, Martinec J, Friml J, Zazimalova E, Petrasek J (2010) Probing plant membranes with FM dyes: tracking, dragging or blocking? *Plant J* 61:883–892
- Jouhet J, Maréchal E, Block MA (2007) Glycerolipid transfer for the building of membranes in plant cells. *Prog Lipid Res* 46:37–55
- Jürgens G (2004) Membrane trafficking in plants. *Annu Rev Cell Dev Biol* 20:481–504
- Kajiwara K, Watanabe R, Pichler H, Ihara K, Murakami S, Riezman H, Funato K (2008) Yeast ARV1 is required for efficient delivery of an early GPI intermediate to the first mannosyltransferase during GPI assembly and controls lipid flow from the endoplasmic reticulum. *Mol Biol Cell* 19:2069–2082
- Karbowski M, Jeong SY, Youle RJ (2004) Endophilin B1 is required for the maintenance of mitochondrial morphology. *J Cell Biol* 166:1027–1039
- Kato N, He H, Steger AP (2010) A systems model of vesicle trafficking in Arabidopsis pollen tubes. *Plant Physiol* 152:590–601
- Kearns BG, McGee TP, Mayinger P, Gedvilaite A, Phillips SE, Kagiwada S, Bankaitis VA (1997) Essential role for diacylglycerol in protein transport from the yeast Golgi complex. *Nature* 387:101–105
- Kim GT, Shoda K, Tsuge T, Cho KH, Uchimiya H, Yokoyama R, Nishitani K, Tsukaya H (2002) The ANGUSTIFOLIA gene of Arabidopsis, a plant CtBP gene, regulates leaf-cell expansion, the arrangement of cortical microtubules in leaf cells and expression of a gene involved in cell-wall formation. *EMBO J* 21:1267–1279
- Kok JW, Babia T, Filipeanu CM, Nelemans A, Egea G, Hoekstra D (1998) PDMP blocks brefeldin A-induced retrograde membrane transport from Golgi to ER: evidence for involvement of calcium homeostasis and dissociation from sphingolipid metabolism. *J Cell Biol* 142:25–38
- Kuerschner L, Ejsing CS, Ekroos K, Shevchenko A, Anderson KL, Thiele C (2005) Polyene-lipids: a new tool to image lipids. *Nat Methods* 2:39–45
- Kuerschner L, Moessinger C, Thiele C (2008) Imaging of lipid biosynthesis: how a neutral lipid enters lipid droplets. *Traffic* 9:338–352

- Kusano H, Testerink C, Vermeer JEM, Tsuge T, Shimada H, Oka A, Munnik T, Aoyama T (2008) The *Arabidopsis* phosphatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth. *Plant Cell* 20:367–380
- Laloi M, Perret AM, Chatre L, Melsner S, Cantrel C, Vaultier MN, Zachowski A, Bathany K, Schmitter JM, Vallet M, Lessire R, Hartmann MA, Moreau P (2007) Insights into the role of specific lipids in the formation and delivery of lipid microdomains to the plasma membrane of plant cells. *Plant Physiol* 143:1–12
- Lam AD, Tryoen-Toth P, Tsai B, Vitale N, Stuenkel EL (2008) SNARE-catalyzed fusion events are regulated by syntaxin1A-lipid interactions. *Mol Biol Cell* 19:485–497
- Lang T, Halemani ND, Rammner B (2008) Interplay between lipids and the proteinaceous membrane fusion machinery. *Prog Lipid Res* 47:461–469
- Langhans M, Robinson DG (2007) 1-Butanol targets the Golgi apparatus in tobacco BY-2 cells, but in a different way to Brefeldin A. *J Exp Bot* 58:3439–3447
- Lee OR, Kim SJ, Kim HJ, Hong JK, Ryu SB, Lee SH, Ganguly A, Cho HT (2010) Phospholipase A₂ is required for PIN-FORMED protein trafficking to the plasma membrane in the *Arabidopsis* root. *Plant Cell* 22:1812–1825
- Lev S (2006) Lipid homeostasis and Golgi secretory function. *Biochem Soc Trans* 34:363–366
- Li G, Xue HW (2007) *Arabidopsis* PLD ζ 2 regulates vesicle trafficking and is required for auxin response. *Plant Cell* 19:281–295
- Li X, Rivas MP, Fang M, Marchena J, Mehrotra B, Chaudhary A, Feng L, Prestwich GD, Bankaitis VA (2002) Analysis of oxysterol binding protein homologue Kes1p function in regulation of Sec14p-dependent protein transport from the yeast Golgi complex. *J Cell Biol* 157:63–78
- Litvak V, Dahan N, Ramachandran S, Sabanay H, Lev S (2005) Maintenance of the diacylglycerol level in the Golgi apparatus by the Nir2 protein is critical for Golgi secretory function. *Nat Cell Biol* 7:225–234
- Malenica N, Abas L, Benjamins R, Kitakura S, Sigmund HF, Jun KS, Hauser MT, Friml J, Luschnig C (2007) MODULATOR OF PIN genes control steady-state levels of *Arabidopsis* PIN proteins. *Plant J* 51:537–550
- Martens S, McMahon HT (2008) Mechanisms of membrane fusion: disparate players and common principles. *Nat Rev Mol Cell Biol* 9:543–556
- Matsuoka K, Orci L, Amherdt M, Bednarek SY, Hamamoto S, Schekman R, Yeung T (1998) COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes. *Cell* 93:263–275
- Mayorga LS, Colombo MI, Lennartz M, Brown EJ, Rahman KH, Weiss R, Lennon PJ, Stahl PD (1993) Inhibition of endosome fusion by phospholipase A₂ (PLA₂) inhibitors points to a role for PLA₂ in endocytosis. *Proc Natl Acad Sci USA* 90:10255–10259
- McGee TP, Skinner HB, Whitters EA, Henry SA, Bankaitis VA (1994) A phosphatidylinositol transfer protein controls the phosphatidylcholine content of yeast Golgi membranes. *J Cell Biol* 124:273–287
- Meijer HJ, Munnik T (2003) Phospholipid-based signaling in plants. *Annu Rev Plant Biol* 54:265–306
- Melsner S, Batailler B, Peypelut M, Poujol C, Bellec Y, Wattlelet-Boyer V, Maneta-Peyret L, Faure JD, Moreau P (2010) Glucosylceramide biosynthesis is involved in Golgi morphology and protein secretion in plant cells. *Traffic* 11:479–490
- Men S, Boutté Y, Ikeda Y, Li X, Palme K, Stierhof YD, Hartmann MA, Moritz T, Grebe M (2008) Sterol-dependent endocytosis mediates post-cytokinetic acquisition of PIN2 auxin efflux carrier polarity. *Nat Cell Biol* 10:237–244
- Mérigout P, Képès F, Perret AM, Satiat-Jeunemaitre B, Moreau P (2002) Effects of brefeldin A and nordihydroguaiaretic acid on endomembrane dynamics and lipid synthesis in plant cells. *FEBS Lett* 518:88–92
- Mesmin B, Drin G, Levi S, Rawet M, Cassel D, Bigay L, Antonny B (2007) Two lipid-packing sensor motifs contribute to the sensitivity of ArfGAP1 to membrane curvature. *Biochemistry* 46:1779–1790
- Mima J, Wickner W (2009) Phosphoinositides and SNARE chaperones synergistically assemble and remodel SNARE complexes for membrane fusion. *Proc Natl Acad Sci USA* 106:16191–16196
- Montagnac G, Chavrier P (2010) Abscission accomplished by PtdIns(3)P. *Nat Cell Biol* 12:308–310
- Monteiro D, Liu Q, Lisboa S, Scherer GEF, Quader H, Malho R (2005) Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of (Ca²⁺) and membrane secretion. *J Exp Bot* 416:1665–1674
- Moreau P (2007) Lipids: architects and regulators of membrane dynamics and trafficking. *Plant Signal Behav* 2:157–159
- Moreau P, Bessoule JJ, Mongrand S, Testet E, Vincent P, Cassagne C (1998) Lipid trafficking in plant cells. *Prog Lipid Res* 37:371–391
- Moreau P, Brandizzi F, Hanton S, Chatre C, Melsner S, Hawes C, Satiat-Jeunemaitre B (2007) The plant ER-Golgi interface: a highly structured and dynamic membrane complex. *J Exp Bot* 58:49–64
- Mousley CJ, Tyeryar KR, Vincent-Pope P, Bankaitis VA (2007) The Sec14-superfamily and the regulatory interface between phospholipid metabolism and membrane trafficking. *Biochim Biophys Acta* 1771:727–736
- Munn AL, Heese-Peck A, Stevenson BJ, Pichler H, Riezman H (1999) Specific sterols required for the internalization step of endocytosis in yeast. *Mol Biol Cell* 10:3943–3957
- Nakajima KI, Sonoda H, Mizoguchi T, Aoki J, Arai H, Nagahama M, Tagaya M, Tani K (2002) A novel phospholipase A1 with sequence homology to a mammalian Sec23p-interacting protein, p125. *J Biol Chem* 277:11329–11335
- Nakamura M, Kuroiwa N, Kono Y, Takatsuki A (2001) Glucosylceramide synthesis inhibitors block pharmacologically induced dispersal of the Golgi and anterograde membrane flow from the endoplasmic reticulum: implication of sphingolipid metabolism in maintenance of the Golgi architecture and anterograde membrane flow. *Biosci Biotech Biochem* 65:1369–1378
- Nakamura Y, Koizumi R, Shui G, Shimojima M, Wenk MR, Ito T, Ohta H (2009) *Arabidopsis* lipins mediate eukaryotic pathway of lipid metabolism and cope critically with phosphate starvation. *Proc Natl Acad Sci USA* 106:20978–20983
- Pathre P, Shome K, Blumental-Perry A, Bielli A, Haney CJ, Alber S, Watkins SC, Romero G, Aridor M (2003) Activation of phospholipase D by the small GTPase Sar1p is required to support COPII assembly and ER export. *EMBO J* 22:4059–4069
- Phillips R, Ursell T, Wiggins P, Sens P (2009) Emerging roles for lipids in shaping membrane-protein function. *Nature* 459:379–385
- Pichler H, Riezman H (2004) Where sterols are required for endocytosis. *Biochim Biophys Acta* 1666:51–61
- Pomorski T, Menon AK (2006) Lipid flippases and their biological functions. *Cell Mol Life Sci* 63:2908–2921
- Potocky M, Elias M, Profotova B, Novotna Z, Valentova O, Zarsky V (2003) Phosphatidic acid produced by phospholipase D is required for tobacco pollen tube growth. *Planta* 217:122–130
- Poulsen LR, Lopez-Marquez RL, McDowell SC, Okkeri J, Licht D, Schulz A, Pomorski T, Harper JF, Palmgren MG (2008) The *Arabidopsis* P₄-ATPase ALA3 localizes to the Golgi and requires a β -subunit to function in lipid translocation and secretory vesicle formation. *Plant Cell* 20:658–676

- Richter S, Voss U, Jürgens G (2009) Post-Golgi traffic in plants. *Traffic* 10:819–828
- Roudier F, Gissot L, Beaudoin F, Haslam R, Michaelson L, Marion J, Molino D, Lima A, Bach L, Morin H, Tellier F, Palauqui JC, Bellec Y, Renne C, Miquel M, Dacosta M, Vignard J, Rochat C, Markham JE, Moreau P, Napier J, Faure JD (2010) Very-long-chain fatty acids are involved in polar auxin transport and developmental patterning in *Arabidopsis*. *Plant Cell* 22:364–375
- Roux A, Cuvelier D, Nassoy P, Prost J, Bassereau P, Goud B (2005) Role of curvature and phase transition in lipid sorting and fission of membrane tubules. *EMBO J* 24:1537–1545
- Salaün C, Gould GW, Chamberlain LH (2005a) The SNARE proteins SNAP-25 and SNAP-23 display different affinities for lipid rafts in PC12 cells. *J Biol Chem* 280:1236–1240
- Salaün C, Gould GW, Chamberlain LH (2005b) Lipid raft association of SNARE proteins regulates exocytosis in PC12 cells. *J Biol Chem* 280:19449–19453
- Saravanan RS, Slabaugh E, Singh VR, Lapidus LJ, Haas T, Brandizzi F (2009) The targeting of the oxysterol-binding protein ORP3a to the endoplasmic reticulum relies on the plant VAP33 homolog PVA12. *Plant J* 58:817–830
- Schleiff E, Tien R, Salomon M, Soll J (2001) Lipid composition of outer leaflet of chloroplast outer envelope determines topology of OEP7. *Mol Biol Cell* 12:4090–4102
- Schneider R, Brügger B, Amann CM, Prestwich GD, Epan RF, Zellnig G, Wieland FT, Epan RM (2004) Identification and biophysical characterization of a very-long-chain-fatty-acid-substituted phosphatidylinositol in yeast subcellular membranes. *Biochem J* 381:941–949
- Shimoi W, Ezawa I, Nakamoto K, Uesaki S, Gabreski G, Aridor M, Yamamoto A, Nagahama M, Agaya M, Tani K (2005) p125 is localized in endoplasmic reticulum exit sites and involved in their organization. *J Biol Chem* 280:10141–10148
- Siddhanta A, Backer JM, Shields D (2000) Inhibition of phosphatidic acid synthesis alters the structure of the Golgi apparatus and inhibits secretion in endocrine cells. *J Biol Chem* 275:12023–12031
- Siddhanta A, Radulescu A, Stankewich MC, Morrow JS, Shields D (2003) Fragmentation of the Golgi apparatus: a role for β III spectrin and synthesis of phosphatidylinositol 4, 5-bisphosphate. *J Biol Chem* 278:1957–1965
- Simons K, Vaz WL (2004) Model systems, lipid rafts, and cell membranes. *Annu Rev Biophys Biomol Struct* 33:269–295
- Sorre B, Callan-Jones A, Manneville JB, Nassoy P, Joanny JF, Prost J, Goud B, Bassereau P (2009) Curvature-driven lipid sorting needs proximity to a demixing point and is aided by proteins. *Proc Natl Acad Sci USA* 106:5622–5626
- Sparkes IA, Frigerio L, Tolley N, Hawes C (2009) The plant endoplasmic reticulum: a cell-wide web. *Biochem J* 423:145–155
- Sprong H, Degroote S, Claessens T, van Drunen J, Oorschot V, Westerink BH, Hirabayashi Y, Klumperman J, van der Sluijs P, van Meer G (2001) Glycosphingolipids are required for sorting melanosomal proteins in the Golgi complex. *J Cell Biol* 155:369–380
- Stenzel I, Ischebeck T, König S, Holubowska A, Sporysz M, Hause B, Heilmann I (2008) The type B phosphatidylinositol-4-phosphate 5-kinase 3 is essential for root hair formation in *Arabidopsis thaliana*. *Plant Cell* 20:124–141
- Stöckl MT, Herrmann A (2010) Detection of lipid domains in model and cell membranes by fluorescence lifetime imaging microscopy. *Biochim Biophys Acta* 1798:1444–1456
- Stöckl M, Plazzo AP, Korte T, Herrmann A (2008) Detection of lipid domains in model and cell membranes by fluorescence lifetime imaging microscopy of fluorescent lipid analogues. *J Biol Chem* 283:30828–30837
- Teissier E, Pécheur EI (2007) Lipids as modulators of membrane fusion mediated by viral fusion proteins. *Eur Biophys J* 36:887–899
- Testerink N, van der Sanden MHM, Houweling M, Helms JB, Vaandrager AB (2009) Depletion of phosphatidylcholine affects endoplasmic reticulum morphology and protein traffic at the Golgi complex. *J Lipid Res* 50:2182–2192
- Thole JM, Vermeer JEM, Zhang Y, Gadella TWJ Jr, Nielsen E (2008) *Root Hair Defective4* encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in *Arabidopsis thaliana*. *Plant Cell* 20:381–395
- Titapiwatanakun B, Blakeslee JJ, Bandyopadhyay A, Yang H, Mravec J, Sauer M, Cheng Y, Adamec J, Nagashima A, Geisler M, Sakai T, Friml J, Peer WA, Murphy AS (2009) ABCB19/PGP19 stabilises PIN1 in membrane microdomains in *Arabidopsis*. *Plant J* 57:27–44
- Tong J, Borbat PP, Freed JH, Shin YK (2009) A scissors mechanism for stimulation of SNARE-mediated lipid mixing by cholesterol. *Proc Natl Acad Sci USA* 106:5141–5146
- Tóth B, Balla A, Ma H, Knight ZA, Shokat KM, Balla T (2006) Phosphatidylinositol 4-kinase III β regulates the transport of ceramide between the endoplasmic reticulum and Golgi. *J Biol Chem* 281:36369–36377
- Trajkovic K, Hsu C, Chianta S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brügger B, Simons M (2008) Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* 319:1244–1247
- Ueda T, Yamaguchi M, Uchimiya H, Nakano A (2001) Ara6, a plant-unique novel type Rab GTPase, functions in the endocytic pathway of *Arabidopsis thaliana*. *EMBO J* 20:4730–4741
- van Blitterswijk WJ, van der Luit AH, Veldman RJ, Verheij M, Borst J (2003) Ceramide: second messenger or modulator of membrane structure and dynamics? *Biochem J* 369:199–211
- Venable ME, Bielawska A, Obeid LM (1996) Ceramide inhibits phospholipase D in a cell free system. *J Biol Chem* 271:24800–24805
- Vermeer JE, Thole JM, Goedhart J, Nielsen E, Munnik T, Gadella TW Jr (2009) Imaging phosphatidylinositol 4-phosphate dynamics in living plant cells. *Plant J* 57:356–372
- Vicogne J, Vollenweider D, Smith JR, Huang P, Frohman MA, Pessin JE (2006) Asymmetric phospholipids distribution drives in vitro reconstituted SNARE-dependent membrane fusion. *Proc Natl Acad Sci USA* 103:14761–14766
- Vielhaber G, Pfeiffer S, Brade L, Lindner B, Goldmann T, Vollmer E, Hintze U, Wittern KP, Wepf R (2001) Localization of ceramide and glucosylceramide in human epidermis by immunogold electron microscopy. *J Invest Dermatol* 117:1126–1136
- Vincent P, Maneta-Peyret L, Cassagne C, Moreau P (2001) Phosphatidylserine delivery to endoplasmic reticulum-derived vesicles of plant cells depends on two biosynthetic pathways. *FEBS Lett* 498:32–36
- Vincent P, Chua M, Nogue F, Fairbrother A, Mekeel H, Xu Y, Allen M, Bibikova TN, Gilroy S, bankaitis VA (2005) Sec14-nodulin domain phosphatidylinositol transfer protein polarizes membrane growth of *Arabidopsis thaliana* root hairs. *J Cell Biol* 168:801–812
- Wang Y, Thiele C, Huttner WB (2000) Cholesterol is required for the formation of regulated and constitutive secretory vesicles from the trans-Golgi network. *Traffic* 1:952–962
- Warnecke D, Heinz E (2003) Recently discovered functions of glucosylceramides in plants and fungi. *Cell Mol Life Sci* 60:919–941
- West G, Viitanen L, Alm C, Mattjus P, Salminen TA, Edqvist J (2008) Identification of a glycosphingolipid transfer protein GLTP1 in *Arabidopsis thaliana*. *FEBS J* 275:3421–3437
- Wickner W, Schekman R (2006) Membrane fusion. *Nat Struct Mol Biol* 7:658–664

- Willemsen V, Friml J, Grebe M, van den Toorn A, Palme K, Scheres B (2003) Cell polarity and PIN protein positioning in Arabidopsis require STEROL METHYLTRANSFERASE1 function. *Plant Cell* 15:612–625
- Woollard AA, Moore I (2008) The functions of Rab GTPases in plant membrane traffic. *Curr Opin Plant Biol* 11:610–619
- Wyles JP, McMaster CR, Ridgway ND (2002) VAMP-associated protein-A (VAP-A) interacts with the oxysterol binding protein (OSBP) to modify export from the endoplasmic reticulum. *J Biol Chem* 277:29908–29918
- Yang JS, Gas H, Lee SY, Mironov A, Zhang L, beznoussenko GV, Valente C, Turacchio G, Bonsra AN, Du G, Baldanzi G, Graziani A, Bourgoïn S, Frohman MA, Luini A, Hsu VW (2008) A role for phosphatidic acid in COPI vesicle fission yields insights into Golgi maintenance. *Nat Cell Biol* 10:1146–1153
- Zheng H, Rowland O, Kunst L (2005) Disruptions of the Arabidopsis Enoyl-CoA reductase gene reveal an essential role for very-long-chain fatty acid synthesis in cell expansion during plant morphogenesis. *Plant Cell* 17:1467–1481