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Review

Protein–lipid interactions in membrane trafficking at the Golgi complex

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Abstract

The integrated interplay between proteins and lipids drives many key cellular processes, such as signal transduction, cytoskeleton remodelling and membrane trafficking. The last of these, membrane trafficking, has the Golgi complex as its central station. Not only does this organelle orchestrates the biosynthesis, transport and intracellular distribution of many proteins and lipids, but also its own function and structure is dictated by intimate functional and physical relationships between protein-based and lipid-based machineries. These machineries are involved in the control of the fundamental events that govern membrane traffic, such as in the budding, fission and fusion of transport intermediates, in the regulation of the shape and geometry of the Golgi membranes themselves, and, finally, in the generation of "signals" that can have local actions in the secretory system, or that may affect other cellular systems. Lipid–protein interactions rely on the abilities of certain protein domains to recognize specific lipids. These interactions are mediated, in particular, through the headgroups of the phospholipids, although a few of these protein domains are able to specifically interact with the phospholipid acyl chains. Recent evidence also indicates that some proteins and/or protein domains are more sensitive to the physical environment of the membrane bilayer (such as its curvature) than to its chemical composition.

Keywords: Phosphoinositides; Golgi complex; Diacylglycerol; Lipids; Membrane traffic

Contents

Protein–lipid interactions serve many different purposes, such as: (i) recruitment of cytosolic proteins that remain peripherally associated to the membranes or may be inserted into the lipid bilayer; (ii) allosteric modulation of protein functions; (iii) modification of the geometry of the bilayer; (iv) modification of the membrane–lipid composition (as in the case of lipid-transfer proteins, flippase, phospholipases and acyltransferases); and (v) generation of lipid-bound or soluble messengers. In this review, we will analyze some of the protein–lipid interactions that occur at the Golgi complex, with particular reference to those involving proteins that possess curvature-sensing domains, and those

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that bind the phosphoinositides (PIs) and 1,2-*sn*-diacylglycerol (DAG), two lipid species with recognized roles in the physiology of this organelle. For more general information, we refer the reader to recent and comprehensive reviews on the lipid-binding modules [1–3] and the general role of the PIs in membrane trafficking [4–8].

The first evidence that DAG and the PIs might have fundamental roles at the Golgi complex derived from the observation in the early 1990s that *SEC14* (the gene encoding the major yeast PI-transfer protein; PITP) mutants have a defect in the secretory pathway at the level of the Golgi complex [9]. SEC14 is a phosphatidylinositol (PtdIns) and phosphatidylcholine (PtdCho) transfer protein; as a PtdIns transfer protein, it can increase the rate of PIs metabolism at the Golgi, and as a PtdCho transfer protein, it negatively regulates the rate of DAG consumption via the CDP–choline pathway (see below). Thus, in yeast, a defect in SEC14 induces a late secretory defect that impairs Golgi-to-plasma membrane transport [10]. In mammals, the PIs and DAG are also actively synthesized in the Golgi complex (see below), and many PI- and DAG-binding proteins localize to this organelle, and especially at the TGN, where they (co-)operate in the generation of transport intermediates directed to different final destinations.

1. Membrane-curvature-sensing proteins

Recently, two different classes of proteins that are localized at the Golgi complex have been found to associate with membranes in a curvature-dependent manner: ARF-GAP1 and the BAR-domain-containing proteins.

The small GTPase ARF1 is a key regulator of structure, function and composition of the membranes of the Golgi complex. At the Golgi, ARF1 has multiple effectors, which include different kinds of coat proteins (e.g., COPI, APs, GGAs) and phospholipid-metabolizing enzymes [e.g., phospholipase D (PLD), PI kinases], and Golgi-associated proteins (e.g., ARFaptins and FAPPs) (see below), and many others [11]. ARF itself cycles between an inactive cytosolic GDP-bound form and an active membrane- and GTP-bound form. The nature, tasks and requirement for ARF of the different effectors are disparate: for some (such as the phospholipid-metabolizing enzymes), the absolute level of active ARF directly determines their level of activity, while for others (such as the coats, which are involved in cargo sorting and/or in membrane deformation), it is the rate of turnover of the ARF cycle, and thus not the absolute level of active ARF, that determines their function. This ARF cycle relies on ARF activators (GTP-exchange factors; GEFs) and deactivators (GTPase-activating proteins; GAPs). ARF-GAP1 is a Golgi-localized GAP for ARF that resides mainly in the *cis*- and medial Golgi compartments [12]. In vitro, ARF-GAP1 binds preferentially to liposomes containing conical lipids rather than cylindrical lipids, and this marked preference for conical lipids correlates with its activity on ARF1-GTP [13]. Recently, using time-resolved assays for COPI dynamics on liposomes of controlled sizes, it has been shown that the rate of ARF-GAP1-catalysed GTP hydrolysis on ARF1 and the rate of COPI disassembly increase by over two orders of magnitude as the curvature of the lipid bilayer increases and approaches that of a typical transport vesicle (about 35-nm diameter) [14]. Thus, membrane curvature might serve as a timer for ARF1-GTP hydrolysis. Although the structural details of this process have not yet been defined, with a consideration of the preference of ARF-GAP1 for conical lipids, a reasonable hypothesis is that the conformation of ARF1-GAP1 in curved membranes favours its interaction with ARF1-GTP. Interestingly, not only ARF1, but also coatomer is released much faster as membrane curvature increases. ARF1-GAP1 and coatomer form a trimeric complex. According to the model proposed by Bigay et al. [14], while these ARF-ARF-GAP1-COPI complexes associate with flat membranes, they are relatively stable (as no GTP hydrolysis occurs on ARF1). Since coatomer can polymerize, this leads to progressive bending of the membrane, and thus provides a favourable enviroment for ARF-GAP1 to activate GTP hydrolysis on ARF, thence promoting ARF1 release. This release of ARF1 changes the conformation of the coat components, and this would induce an even more curved shape of the membrane. Afterwards, the absence of ARF1-GTP and the high level of membrane curvature would dissociate coatomer [14]. Furthermore, since membrane curvature at the periphery of a coated area is negative, a ring of ARF1-GTP molecules should be protected from ARF-GAP1, and hence may make the coat more stable, although still ready to disassemble as membrane fission occurs.

In addition to ARF-GAP1, other proteins (some of which are associated with the Golgi complex) have been characterized recently according to their abilities to recognize and/or induce membrane curvature. These proteins possess a BAR domain. The BAR domain was originally identified in amphiphysin (Bin and Rsv15 are the *Drosophila* and yeast homologues of amphiphysin, respectively), and contains around 200 amino acids in the form of two monomers, each of which contributes three α-helices to the final structure [15,16]. The BAR domain is “banana shaped”, and its curvature fits to that of membrane buds (bends) of around 30 nm in diameter. The N-BAR domain of amphiphysin is able to tubulate liposomes in a concentration-dependent manner: at low concentrations it induces small buds, at intermediate concentrations it induces tubules, and at still higher concentrations it causes the vesiculation of the liposomes [17]. In addition, the amphiphysin BAR domain is also required to recruit and polymerize clathrin into uniform patches or caps. While the best-characterized isoforms of BAR-containing proteins are localized at the plasma membrane (amphiphysin I, endophilin A), where they act in controlling the clathrin-

mediated endocytic process, some of them, such as amphiphysin IIb and endophilin B, act at the Golgi complex [15,18]. Amphiphysin IIb regulates the transport of G-protein-coupled somatostatin (SRIF) receptors from the TGN to the plasma membrane, while the role of endophilin B at the Golgi remains to be defined.

The ARF-binding proteins ARFaptin1 and ARFaptin2 represent further Golgi-localized proteins that are able to recognize and/or induce membrane curvature. The ARFaptins share 80% homology and both have a domain that is structurally identical to, but has a very weak sequence homology with, the BAR domain [17,19,20]. Both the BAR domain and this BAR-like domain of ARFaptin are thus banana-shaped helix bundles with positive charges at their extremities and on their concave surface. This similarity to amphiphysin might explain why ARFaptin can bind to membranes directly, a property that is not necessarily related to its ability to bind to small GTPases (including not just ARF, but also Arl and Rac [20,21]). Full-length ARFaptin2 can bind liposomes containing PtdIns 4,5-bisphosphate (PtdIns45P2) [17]. ARFaptin also binds the PtdIns phosphates PtdIns4P and PtdIns3P on nitrocellulose filters, and in vivo it localizes to the Golgi complex. In vitro, ARFaptin tubulates liposomes. Mutations of the positive charges on the concave surface of the BAR-like domain of ARFaptin that prevent liposome tubulation in vitro also prevent ARFaptin association with the Golgi complex in vivo [17]. While ARFaptin1 has been found to negatively control PLD activity and endoplasmic reticulum (ER)-to-Golgi transport (possibly by interfering with ARF1 [22]), the role of ARFaptin2 at the Golgi, as well as the existence of Golgi-receptors for ARFaptin 2 that might specify its distribution and site of action, remains unknown.

Interestingly, ICA69, an islet cell autoantigen that is localized at the Golgi complex, has recently been identified as an ARFaptin-like molecule, possessing a domain that is structurally and compositionally very close to the BAR-like domain of ARFaptin [23]. ICA69 appears to be enriched at the TGN, but also present on immature (but not mature) secretory granules. To date, the role of ICA69 remains undefined, but it is thought that it may be involved in the generation of immature secretory granules at the TGN in insulin-secreting cells.

2. PI-binding proteins

The PIs represent an important class of cellular phospholipids; they have the unique feature of undergoing rapid phosphorylation/dephosphorylation cycles on the different positions of their inositol headgroup, thus generating distinct molecules with different properties [24]. Therefore, they constitute important spatial and temporal signals that are involved in a wide range of cellular processes, such as signal transduction, actin cytoskeleton remodelling, and membrane trafficking.

The phosphorylated PIs exert their different functions through interactions with a large number of downstream effector proteins and via specific PI-binding modules, including the PH, FYVE, PX, ENTH and ANTH domains. These lipid-binding interactions are generally of low affinity and, hence, are reversible. This allows fast cycling between the membrane-bound and cytosolic pools of such lipid-binding-module-containing proteins. For an exhaustive treatment of the structures, lipid specificities and characteristics of these PI-binding modules, we refer the reader to recent reviews [1,25,26]. Here, our aim is rather to discuss the recent advances in our understanding of the roles of PI-protein interactions at the Golgi complex.

The metabolism of the PIs is highly compartmentalized in cells, and each organelle is characterized by the presence of specific PI kinases (PIKs) and PI phosphatases, which can thus generate and maintain their characteristic PI species. The Golgi complex has been shown to contain: (i) PIKs that are able to phosphorylate the parental PI species, PtdIns (which is synthesized in the ER), in the 3′ (Vps34) and the 4′ (the PI4Ks: PI4KIIα and PI4KIIIβ) positions of the inositol ring; (ii) a PtdIns phosphate kinase (PIPK) that phosphorylates PtdIns45P2 in the 3′ position (PI3KCIIα); and (iii) PIP-phosphatases that can remove the 5′ (OCRL-1) or 3′ (PTEN1,2) phosphates of the inositol ring of PtdIns45P2 and PtdIns345P3 (Fig. 1).

Among the three yeast PI4Ks, Pik1p is the one that is localized at the Golgi. Here, it is required for the transport of secretory cargo from the Golgi complex to the plasma membrane. *Pik1* mutant strains (which have a 50% reduction in PtdIns4P levels) show accumulation/conversion of Golgi in(to) aberrant structures, known as Berkeley bodies [27–29]). Since the only yeast PtdIns4P 5-kinase (PI4P5K), Mss4p, gives no secretory phenotype when mutated, it is PtdIns4P itself that should directly control the structure and function of the Golgi complex, rather than it just acting as a precursor for PtdIns45P2. In yeast, one of the possible targets for PtdIns4P at the Golgi complex is the oxysterol-binding protein (OSBP) Kes1p; it is known that Kes1p binds to PtdIns4P and that this PtdIns4P-binding property is essential for its localization on Golgi membranes [30,31]. *KES1*, which was originally identified as a gene that elicited a "Sec14p-bypass phenotype" on its inactivation, exerts a negative regulation on *SEC14*-dependent secretory function. Recently, the mechanism of action of Kes1p at the Golgi complex has been investigated and shown to involve regulation of the activation-deactivation cycle of the small GTPase ARF [32].

As in yeast, PI4K activity is required for maintenance of the structural and functional organization of the Golgi complex in mammals. It has been shown that PI4K activity is enriched in a Golgi fraction, and that three of the mammalian PI4Ks, PI4KIIIβ, PI4KIIIα and PI4KIIα, are localized to this organelle [33–36]. PI4KIIIβ is recruited to the Golgi complex in response to ARF activation. Interfering with PI4KIIIβ activity through the use of a dominant-

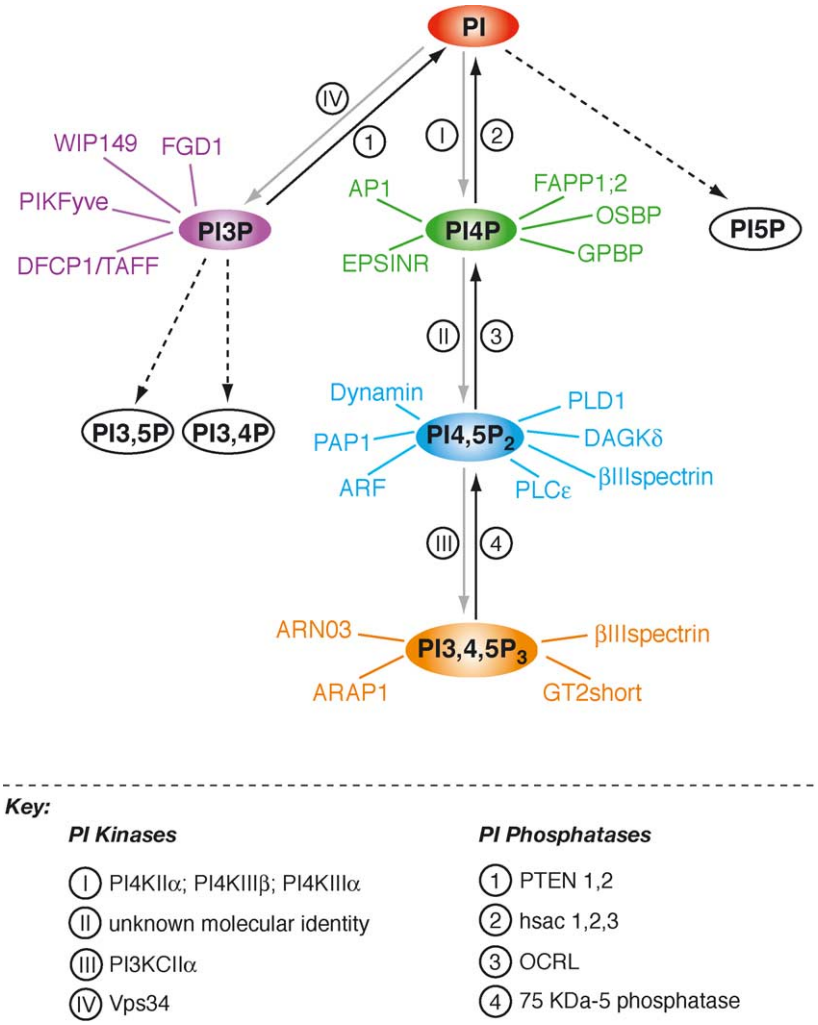

Fig. 1. The PI cycle and PI-binding proteins at the Golgi complex. Schematic representation of the phosphorylation/dephosphorylation cycles of the PIs occurring at the Golgi complex. The steps catalysed by the different PI kinases and PI phosphatases are indicated by grey and black arrows, respectively. The proteins and the PIs they bind to are in the same colour. Dotted lines indicate further potential pathways that have yet to be demonstrated for the Golgi.

negative mutant induces structural changes in the Golgi complex [33] and impairs the exit of basolateral membrane proteins from the Golgi complex in polarized cells [37]. For PI4KIIα, the knock-out inhibits transport to both endosomes and the plasma membrane [38].

Recently, two important targets for PtdIns4P in the Golgi in mammals have been identified: the FAPP proteins FAPP1 and FAPP2 [39]. Both FAPP1 and FAPP2 possess a PH domain that binds PtdIns4P with high selectivity over the other PI species [40]; in addition, only FAPP2 contains a glycolipid-transfer-protein-like domain. The FAPPs localize mainly at the TGN, and more specifically, to the TGN exit sites where transport carriers destined to the plasma membrane are formed [39]. This specific localization of the FAPPs is due to their PH domain, and their PH domain by itself is sufficient to target them to the Golgi, as has been revealed using reporter proteins (such as GFP and GST). In this respect, the PH domain of FAPP (FAPP-PH) behaves in a similar way to two closely related PH domains, those of OSBP and Goodpasture antigen-binding protein (GPBP; also known as the ceramide transport protein, CERT), which also bind PtdIns4P and localize to the Golgi complex [30,41,42].

The molecular mechanisms responsible for the selective Golgi localization of FAPP-PH have been clarified recently, and involve its ability to bind the small GTPase ARF in addition to PtdIns4P. At TGN exit sites, where FAPPs localize due to the binding properties of their PH domains, FAPPs have an important role in the generation of transport carriers destined to the plasma membrane. Interfering with the endogenous FAPPs, by either displacing them or knocking them down with siRNAs, inhibits the transport of the reporter membrane protein VSVG and of soluble cargo (such as glycosaminoglycan chains) from the Golgi to the plasma membrane [39]. Interestingly, the overexpression of FAPP-PH (which displaces the endogenous proteins) induces the transformation of forming transport intermediates into long tubules that are unable to detach from the donor membrane (TGN) and to fuse with the acceptor membrane (plasma membrane). This effect is consistent

with a role for FAPPs in coupling the budding and the fission reactions during the formation of transport intermediates (see below) [39].

While FAPPs selectively control the TGN-to-plasma membrane pathway, other PtdIns4P-binding proteins are involved in other Golgi-exit pathways, mainly to the endosomal system. These include epsinR, an AP-1 accessory protein [43,44], and AP-1 itself. AP-1 association with the TGN, which is also regulated by ARF [45], requires its binding to PtdIns4P since siRNAs for PI4KIIα dramatically reduce PtdIns4P levels in cells and decrease the association of γ-adaptin (a subunit of the AP-1 complex) with Golgi membranes [38]. The region of AP-1 involved in this PtdIns4P binding has not been identified to date. EpsinR has an N-terminal ENTH domain that has been shown to bind PtdIns4P better than PtdIns5P and PtdIns35P2 in vitro [43,44]. Since, this protein localizes to the TGN in vivo, where PtdIns4P is enriched, a reasonable proposition is that the physiological substrate for ENTH binding is indeed PtdIns4P. This epsinR association with Golgi membranes is sensitive to brefeldin A and GTPγS, suggesting its regulation by ARF [43], but it does not require AP-1. Although it is not clear how ARF might control epsinR binding to membranes, it could be indirect, by controlling the PtdIns4P levels at the TGN (see above), or more direct, via binding to epsinR itself. If the latter turns out to be the case, we would again find a two-component docking mechanism (PI4K-generated docking lipids and ARF-generated docking proteins) in operation, which would resemble those used to tightly anchor many low affinity PI-binding modules to membranes [1]. In most cases, the initial docking mediated by the PIs will also increase the probability of binding between the truly specific binding site elsewhere in the protein and that in the membrane. However, an interesting, and still open, question relates to different PI-binding proteins that share the same motif and binding sites for their association with the same membrane: how are they differently regulated and recruited? The mechanisms that govern the spatial and temporal recruitment of various PI-interacting proteins with respect to transport events will be important challenges for future studies.

PtdIns4P is not the only PI present at the Golgi complex. Indeed, different enzymes involved in PI metabolism have been found associated with this organelle, and proteins with different PI-binding specificities have been identified on Golgi membranes. For instance, PtdIns45P2, which is enriched at the plasma membrane and is also present in low amounts in the Golgi complex [24,46], appears to be essential for the correct functioning of this organelle. It has been reported to be involved in ER-to-Golgi transport, in the formation of post-Golgi transport carriers, and in the maintenance of Golgi structure [47,48]. PtdIns45P2 might exert these functions through controlling, for example, the spectrin- and dynamin-based machineries (the former is required in ER-to-Golgi transport [49], and the latter in release of transport carriers from the TGN [50,51]), or by stimulating the activity of PLD, and thus controlling the levels of phosphatidic acid (PA) in the membranes [52].

Potential effectors of PtdIns3P have also been identified in the Golgi complex, where different isoforms of the PI3Ks are localized [53–55]. However, while the role of this 3′ -phosphorylated PI in Golgi-to-vacuole transport in yeast is very well established, the evidence for a functional role of PtdIns3P, PtdIns35P2 or PtdIns345P3 is less solid in mammals. Different proteins with FYVE domains have been localized both in the Golgi and the endocytic compartments (see Fig. 1), and overexpression of PI3KCIIα induces the redistribution of the mannose 6-phosphate receptor (M6PR) from the Golgi to the cell periphery, and inhibits endocytosis, suggesting that the balance of the 3′ -phosphorylated PIs is important for clathrin-dependent sorting events at the TGN [53]. In agreement with this, recent evidence reporting the identification of a novel protein, named WIPI49 (WD40 repeat protein interacting with PIs; 49 kDa; [56]), that binds preferentially to PtdIns3P (and to lesser extents to PtdIns5P and PtdIns35P2) supports a role for the 3′ -phosphorylated PI species in Golgi-to-endosome traffic. WIPI49 localizes to and cycles between *trans*-Golgi and endosomal membranes, following the recycling pathway of the M6PR. However, WIPI49 appears not just to be a passenger, but rather to be a regulator of this pathway: overexpression of WIPI49 impairs the exit of the M6PR from the TGN, and causes its accumulation in perinuclear structures, while knock-down of WIPI49 changes the structure and composition of the early endosomal compartment [56].

3. DAG-binding proteins

There are three different possible sources for DAG in the Golgi complex (see Fig. 2). DAG can be generated through (i) PA phosphatase: by dephosphorylation of PA, which is itself produced from PtdCho by PLD action; (ii) sphingomyelin (SM) synthase: a Golgi enzyme that interconverts PtdCho and SM (two phospholipids that have the same headgroup, choline, but that have different backbones—DAG in PtdCho and ceramide in SM), by consuming PtdCho and ceramide and producing SM and DAG [57]; and (iii) phospholipase C (PLC): by hydrolysis of PtdIns45P2 (or PtdIns4P), which generates both DAG and inositol 1,4,5-trisphosphate (Ins145P3) (or Ins14P2).

Three main pathways could be responsible for DAG clearance: (i) the conversion of SM to PtdCho (that consumes DAG and SM and generates ceramide and PtdCho); (ii) the phosphorylation of DAG to PA (via DAG kinase; DAGK); and (iii) the CDP-choline pathway for PtdCho biosynthesis (which also consumes one DAG for every PtdCho molecule synthesized) [57].

The balance between DAG-generating and DAG-consuming pathways is fundamental for the function of the

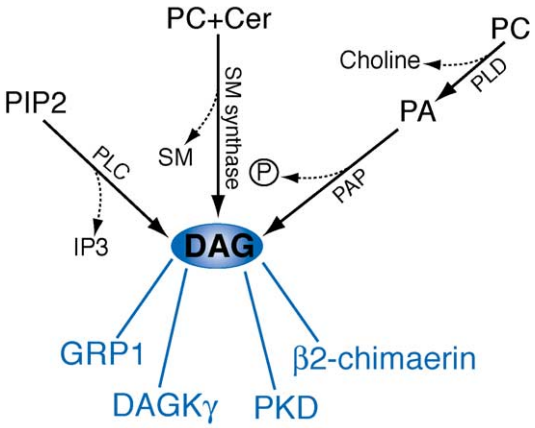

Fig. 2. DAG generation pathways and DAG-binding proteins at the Golgi complex. Schematic representation of DAG generation via the three different pathways in the Golgi complex: phospholipase C (PLC), sphingomyelin-synthase (SM synthase) and phosphatidic acid phosphatase (PAP) (see text). DAG acts as a binding site in the Golgi complex for cytosolic proteins, such as the RAS-exchange factor GRP1, the Rac-GTPase activating protein chimaerin, DAG kinase γ (DAGK γ) and PKD.

Golgi complex, and PITP is a key player in this, as highlighted by the PtdCho/DAG imbalance reported in yeast strains defective in SEC14 (the yeast PITP).

Among the different metabolic pathways of generation/consumption of DAG at the Golgi, those involving the PA/DAG cycle might, in principle, be the most important from the quantitative point of view, considering the abundance of PtdCho. Indeed, it has been shown that PA phosphatase inhibitors induce the release of DAG-binding proteins (such as PKD, see below [58]) from Golgi membranes. The PA/DAG cycle pathway is centred on the activity of PLD (and DAG kinases). While there is evidence that PA may have a functional role in the Golgi and several Golgi-localized PA-binding proteins have been identified [59], the extent of the local generation of PA through PLD as an absolute requirement for the function of the Golgi complex is still uncertain. The evidence in favour of a functional role of PLD in the Golgi relies mainly on the use of primary alcohols that divert the PLD-mediated production of PA into that of the phosphatidyl-alcohol. The primary alcohols (but not the tertiary ones, which are not substrates for PLD) induce changes in the structural organization of the Golgi complex and inhibit the generation of transport vesicles at the TGN. However, the observation that yeast strains defective in PLD show no secretory defects [60], and that PLD1 and PLD2 are localized at the Golgi complex in only some cell types, while in others they are mainly localized at the perinuclear endolysosomal compartments, on secretory granules (PLD1) or at the plasma membrane (PLD2) [61,62], casts some doubt on the importance of the local generation of PA through PLD in the Golgi.

On the other hand, since the SM content of Golgi membranes is also high (more than 10% of the total Golgi lipids), a large DAG pool may be generated through the SM synthetic pathway. Finally, the PLC-mediated generation of DAG from PIs in the Golgi complex has also to be considered, as both the substrates (PtdIns45P2 and PtdIns4P) and the enzyme (PLC) of this pathway are present in this organelle [63,64].

The best characterized DAG-binding module is the C1 domain. It is a 50/51-amino-acid domain with a cysteine-rich compact structure that contains five β strands, a short α helix, and two zinc ions [65]. C1 domains were initially identified in protein kinase Cs (PKCs) and, later, in non-kinase proteins. Indeed, the Golgi complex hosts several C1-containing proteins belonging to the kinase (such as PKD, [66,67]) and non-kinase (such as chimaerin [68], Ras GRP1 [69,70] categories, and also a still unspecified phorbol-ester-binding protein at the TGN [71]). These DAG-binding proteins are involved in different processes in the Golgi: some of them, such as PKD and the still unspecified Golgi phorbol-ester receptor, directly or indirectly control basic transport mechanisms (like the generation of transport carriers), while others, such as Ras-GRP1 and, possibly, the chimaerins, participate in signalling cascades that initiate at the plasma membrane, but that involving the participation of endomembrane signalling pathways.

The chimaerins were the first non-PKC DAG/phorbol-ester receptors to be discovered [72]. These arise from two different genes, each of which has two splice variants [65]. The regulation and function of the chimaerin isoforms are still unclear. Although they are able to accelerate the hydrolysis of the GTP on Rac in in vitro assays, the evidence in favour of their Rac-GAP activity in cellular models is so far limited. β2-Chimaerin translocates from a cytosolic compartment to the plasma membrane and Golgi membranes after phorbol-ester treatment [68,73]. This translocation is dependent on an intact C1 domain; however, it involves not only DAG, but also a protein binding site in the Golgi. This has been identified in Tmp21-I (p23), a transmembrane protein that is localized in the *cis*-Golgi network and is involved in ER–Golgi membrane trafficking [73]. The C1 domain in β2-chimaerin is required for its interaction with Tmp21-I, thereby implying a function for this domain also in protein–protein association (in addition to its role in DAG binding). The precise role of Tmp21-I binding is unclear; it might be limited to specifying the positioning of β2-chimaerin at the *cis*-Golgi, and/or it might regulate the β2-chimaerin availability for Rac, thus ultimately regulating the activation state of this GTPase.

Another GTPase, Ras, is activated by a DAG-binding protein associated with the Golgi complex (and the ER): Ras-GRP1. The subcellular localization of Ras-GRP1 is mediated by its zinc finger region, a C1-like domain, and is highly dependent on the growth status of the cell. In quiescent cells, Ras-GRP1 is mainly cytosolic, but it translocates to the Golgi upon treatment with phorbol esters or stimulation of receptor or soluble tyrosine kinases [70]. Interestingly, Ras-GRP1 not only translocates to the Golgi

in response to receptor stimulation, but it also promotes the efficient stimulation of endogenous Ras in this organelle [70,74]. The kinetics of Ras activation at the plasma membrane and in the Golgi complex is distinct: the activation at the plasma membrane is rapid (<5 min) and transient, whereas that in the Golgi complex is delayed (10–20 min) and sustained [69]. The mechanisms transducing the stimulation of receptor tyrosine kinases at the plasma membrane into the activation of Ras in the Golgi complex have been in part elucidated. They involve the Src-dependent stimulation of PLCγ (and production of DAG) and an increase in intracellular Ca^{2+} that induces the translocation of both Ras-GRP1 to the Golgi complex and a Ras-GAP, CAPRI, to the plasma membrane [74]. This would thus simultaneously lead to the activation of Ras at the Golgi complex and to its silencing at the plasma membrane.

However, other factors besides DAG/PMA are likely to contribute to the Golgi localization of Ras-GRP1 since this localization, which is found in exponentially growing cells, is not fully mimicked in quiescent cells even after long periods of stimulation with PMA. Furthermore, treatment with a PLCγ inhibitor does not abolish the reticular distribution of Ras-GRP1. Thus, it is possible that, as in the case of the chimaerins, a protein-binding site could be involved in the subcellular targeting of Ras-GRP1.

Among the DAG-binding kinases associated with the Golgi complex, including PKCα, PKCδ, PKCε, PKCθ [75,76] and PKD [66,67], only the last of these has been extensively studied in terms of its targeting mechanisms and its role in membrane trafficking. PKD possesses two cysteine-rich domains (C1a and C1b), a pleckstrin-homology (PH) domain and a catalytic domain, and it is localized in the cytosol and in several intracellular compartments, including the Golgi complex, mitochondria and the plasma membrane [67]. The first cysteine-rich domain (C1a) of PKD is required and sufficient for the binding of PKD to the Golgi, and specifically to the TGN [77]. C1a alone is also sufficient to target a reporter protein to the TGN. Moreover, compromising DAG production (with fumonisin B1, which blocks SM activity, or propanol, which inhibits PA phosphatase) prevents PKD recruitment to the TGN [58]. However, whether this Golgi-specific targeting of the PKD-C1 domain involves a Golgi-restricted DAG species or an additional non-DAG binding site remains to be determined. In favour of the former possibility, incorporation of a synthetic short-chain form of DAG into the plasma membrane results in an acute mistargeting of PKD to that organelle [58]. However, the evidence that other C1 domains are targeted to the plasma membrane and that C1 domains of other Golgi localized proteins, such as β2-chimaerin, also bind integral Golgi proteins suggests that an additional, non-DAG-binding, site could also contribute to the Golgi-targeting of the PKD-C1a domain. Despite this ability of C1 to direct PKD and reporter proteins to the TGN, interfering with the activation of PKD (e.g., by mutating its activation loop) also inhibits its localization to the TGN. Together with the observation that TGN-bound PKD is phosphorylated, this would indicate that not only C1, but also the activation state of PKD is important in its binding to the TGN.

What is the role of PKD at the Golgi complex? Its main activity at the Golgi complex is related to its ability to induce membrane fission. Indeed, a vesiculating agent, such as the sponge metabolite ilimaquinone, can induce a hyperactivation of PKD [66]. Conversely, a dead-kinase variant of PKD can induce the appearance of long tubular structures emanating from the TGN [78]. These tubules can be interpreted as the results of an impaired fission process at the TGN. Interestingly, recent data indicate that PKDs are specifically involved in controlling the transport of selected basolateral-directed cargo in polarized cells. The precise mechanisms of action of PKD in membrane fission remain elusive, however. A number of different proteins have been shown to interact with PKD (in particular, with its PH domain), some of which act as upstream regulators of the kinase. This is the case with the βγ subunit of the heterotrimeric G proteins (Gβγ), which in the Golgi exert a vesiculating activity reminiscent of that of PKD). Indeed, Gβγ activity is mediated by PKD since inhibition of PKD activity through the use of pharmacological agents, synthetic peptide substrates, and, more specifically, the PH domain of PKD, prevents Gβγ-mediated Golgi breakdown [66].

PKD interactors include also PI4Ks and PI4P5Ks [79], and it has been postulated that the activation of PIKs by PKD contributes to the fission reaction. Alternatively, the PIs produced by PIKs (either PtdIns4P or PtdIns45P2) may act as substrates for PLC to produce DAG, which is required for the recruitment of PKD. In this scenario, proteins that bind PtdIns4P and that are localized at the TGN exit sites, such as the above-described FAPPs, may act as regulators of PtdIns4P metabolism.

4. Protein–lipid interactions in the formation of transport carriers at the TGN

Membrane transport from the Golgi complex to the plasma membrane is operated by carriers originating from the distal Golgi compartments, which move to the periphery along microtubules, and finally fuse with the plasma membrane. The dynamics and ultrastructure of these carriers have been defined recently [80,81], but the comprehension of the molecular machinery responsible for their formation at the TGN is still limited.

At odds with the formation of the endolysosomal-directed carriers that relies mainly on adaptor proteins [82] that can simultaneously interact with cargo, coat (clathrin) and regulatory molecules (i.e., the GTPase ARF) [83], the role of specific coats and/or adaptors for plasma-membrane-directed carriers is not clear. No specific coats for these have been identified to date, and only recently have different classes of adaptors (AP1b, AP4,

AP3) been shown to contribute to Golgi-to-plasma membrane transport [84–86]. The definition of a role for a protein coat in TGN-to-plasma membrane transport is thus still in its early days, and at present it appears to be limited to basolateral-directed cargo in polarized cells [84,86] and to only one (of several possible) pathway to the plasma membrane in non-polarized cells [85]. However, the importance of a lipid-based machinery in TGN-to-plasma membrane transport is more general and better established both in mammals and in yeast. The lipid species and lipid-binding proteins so far involved in late transport events at the TGN are depicted in Fig. 3A.

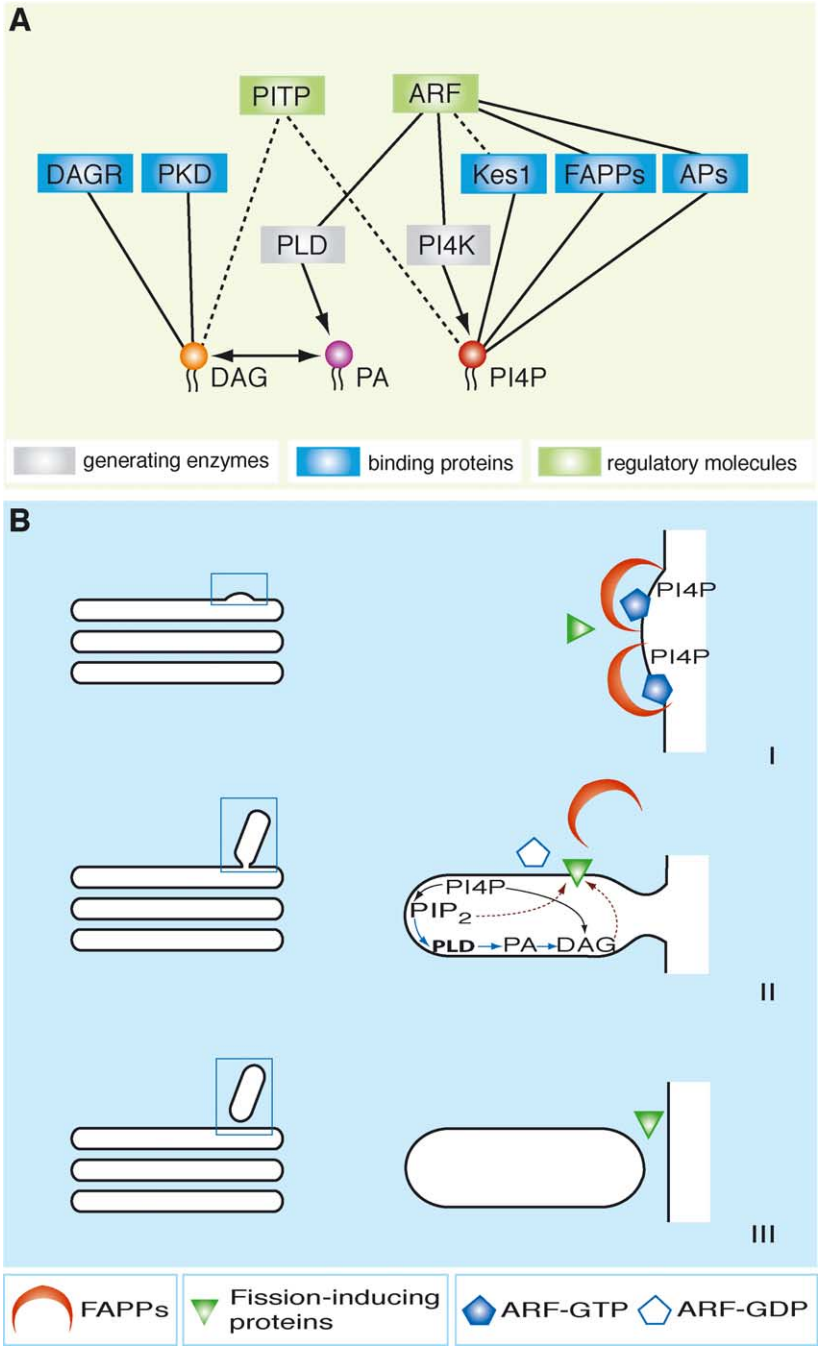

Fig. 3. Protein–lipid interactions in the formation of transport carriers at the TGN. (A) The interplay between proteins and lipids at the TGN exit sites. The three main lipid species with recognized roles in TGN-to-PM transport are indicated: DAG, phosphatidic acid (PA) and PtdIns4P (PI4P). The interactions involving some of their generating enzymes (grey; phospholipase D, PLD; PI4 kinases, PI4K) are indicated. The interactions with proteins with a recognized "binding-partner" role (blue; DAGR, the still unspecified DAG receptor, PKD, kes1p, FAPPs, and the adaptor proteins, APs) in this transport step are indicated, along with the regulatory proteins (green; PITP and the small GTPase ARF) and their target interactions. Physical interactions are indicated by solid lines, while those functional are indicated as dashed lines. Metabolic connections are indicated by arrowed lines. (B) Model of mechanism of action of PI-binding and DAG-binding proteins in the formation of constitutive transport carriers at the TGN. See text for explanation.

As we have seen, proteins that have important roles in the generation of constitutive carriers include both DAG-binding proteins (e.g., PKD) and PI-binding proteins (e.g., FAPPs). FAPPs are members of a larger family of proteins that share a similar PH domain (which is able to bind PtdIns4P and ARF and to localize to the TGN), and that also possess additional and distinct lipid-binding domains. These include (i) FAPP2, which possesses a glycolipid-transfer-protein domain; (ii) GPBP, which has a STAR domain that can bind ceramide and that is responsible for the ceramide transfer activity of this protein (also known as CERT) [41]; and (iii) OSBP1, which has an oxysterol-binding domain [30]. The different members of this protein family are all TGN-located proteins, although they might act within different subdomains of the TGN. They may also all be targeted to the TGN due to the PtdIns4P and ARF enrichment in this compartment, but each of them could then be positioned to a given TGN subdomain, possibly determined by an enrichment in the lipid species that are bound by their specific lipid-binding modules (GLTP, STAR, OSB). One intriguing possibility is that each of these proteins could control the formation of a specific type of carrier.

In an attempt to build up a coherent spatio-temporal sequence within which the lipid-based machineries intervene in the process of carrier formation, one could propose two different states of the TGN exit sites (i.e., the sites where transport intermediates are formed; see Fig. 3B): (I) a PtdIns4P-characterized, budding-permissive and fission-restrictive state; and (II) a DAG/PA-characterized, fission-permissive state. The starting event in the first stage would be the activation of ARF, with the consequent recruitment of PI4Kβ and the production of PtdIns4P [87]. This would lead to the recruitment of ARF and PtdIns4P-binding proteins, such as FAPPs (OSBP, GPBP), which control the budding step. After this first step, PtdIns4P has to be metabolized (to PtdIns45P2 and/or to DAG) and FAPPs released to allow access for fission-inducing proteins, such as the PKD and/or the lysoPA/PA converting protein CtBP3/BARS [88] or the PtdIns45P2-binding protein dynamin [50,51]. Only the spatially and temporally co-ordinated sequences of these lipid-driven events, which are coupled to the GTP/GDP cycle of the small GTPase ARF, would guarantee budding and fission (i.e., formation) of transport carriers that are competent for fusion with the plasma membrane.

5. Conclusions and future directions

Membrane traffic is governed by intimate relationships between proteins and lipids. Thus, far from being just structural membrane components, the lipids are directly implicated in fundamental membrane traffic events and in the generation and propagation of signals from the endomembrane systems. Meanwhile, the proteins act both "upstream" of the lipids, through controlling their synthesis, degradation, distribution and transfer between the different membrane compartments, and "downstream" in their roles as lipid effectors, through the presence of protein modules with distinct lipid specificities. The picture as it is now, however, cannot be considered to be complete; with limited exceptions (only some of which have been specifically discussed here), the detailed functions and the mechanisms of action of the various lipid effectors remain elusive.

A great challenge for future research is therefore to reveal the molecular mechanisms by which lipid-binding proteins regulate membrane trafficking, and to provide a more integrated view of how the functions of the single molecular machineries are coordinated at the organelle and cellular levels.

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References

[1] M.A. Lemmon, Phosphoinositide recognition domains, Traffic 4 (2003) 201–213.
[2] P.J. Cullen, G.E. Cozier, G. Banting, H. Mellor, Modular phosphoinositide-binding domains—their role in signalling and membrane trafficking, Curr. Biol. 11 (2001) R882–R893.
[3] M.C. Duncan, G.S. Payne, ENTH/ANTH domains expand to the Golgi, Trends Cell Biol. 13 (2003) 211–215.
[4] V.A. Bankaitis, A.J. Morris, Lipids and the exocytotic machinery of eukaryotic cells, Curr. Opin. Cell Biol. 15 (2003) 389–395.
[5] A. Simonsen, A.E. Wurmser, S.D. Emr, H. Stenmark, The role of phosphoinositides in membrane transport, Curr. Opin. Cell Biol. 13 (2001) 485–492.
[6] J. Gruenberg, Lipids in endocytic membrane transport and sorting, Curr. Opin. Cell Biol. 15 (2003) 382–388.
[7] T.F. Martin, PI(4,5)P(2) regulation of surface membrane traffic, Curr. Opin. Cell Biol. 13 (2001) 493–499.
[8] M. De Matteis, A. Godi, PI-loting membrane traffic, Nat. Cell Biol. 6 (2004) 487–492.
[9] V.A. Bankaitis, J.R. Aitken, A.E. Cleves, W. Dowhan, An essential role for a phospholipid transfer protein in yeast Golgi function, Nature 347 (1990) 561–562.
[10] S.M. Routt, V.A. Bankaitis, Biological functions of phosphatidylinositol transfer proteins, Biochem. Cell. Biol. 82 (2004) 254–262.
[11] Z. Nie, D.S. Hirsch, P.A. Randazzo, Arf and its many interactors, Curr. Opin. Cell Biol. 15 (2003) 396–404.
[12] E. Cukierman, I. Huber, M. Rotman, D. Cassel, The ARF1 GTPase-activating protein: zinc finger motif and Golgi complex localization, Science 270 (1995) 1999–2002.

[13] B. Antonny, I. Huber, S. Paris, M. Chabre, D. Cassel, Activation of ADP-ribosylation factor 1 GTPase-activating protein by phosphatidylcholine-derived diacylglycerols, J. Biol. Chem. 272 (1997) 30848–30851.
[14] J. Bigay, P. Gounon, S. Robineau, B. Antonny, Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature, Nature 426 (2003) 563–566.
[15] K. Farsad, N. Ringstad, K. Takei, S.R. Floyd, K. Rose, P. De Camilli, Generation of high curvature membranes mediated by direct endophilin bilayer interactions, J. Cell Biol. 155 (2001) 193–200.
[16] B. Habermann, The BAR-domain family of proteins: a case of bending and binding? EMBO Rep. 5 (2004) 250–255.
[17] B.J. Peter, H.M. Kent, I.G. Mills, Y. Vallis, P.J. Butler, P.R. Evans, H.T. McMahon, BAR domains as sensors of membrane curvature: the amphiphysin BAR structure, Science 303 (2004) 495–499.
[18] P. Sarret, M.J. Esdaile, P.S. McPherson, A. Schonbrunn, H.J. Kreienkamp, A. Beaudet, Role of amphiphysin II in somatostatin receptor trafficking in neuroendocrine cells, J. Biol. Chem. 279 (2004) 8029–8037.
[19] H. Kanoh, B.T. Williger, J.H. Exton, Arfaptin 1, a putative cytosolic target protein of ADP-ribosylation factor, is recruited to Golgi membranes, J. Biol. Chem. 272 (1997) 5421–5429.
[20] C. Tarricone, B. Xiao, N. Justin, P.A. Walker, K. Rittinger, S.J. Gamblin, S.J. Smerdon, The structural basis of Arfaptin-mediated cross-talk between Rac and Arf signalling pathways, Nature 411 (2001) 215–219.
[21] H. Van Valkenburgh, J.F. Shern, J.D. Sharer, X. Zhu, R.A. Kahn, ADP-ribosylation factors (ARFs) and ARF-like 1 (ARL1) have both specific and shared effectors: characterizing ARL1-binding proteins, J. Biol. Chem. 276 (2001) 22826–22837.
[22] B.T. Williger, J. Ostermann, J.H. Exton, Arfaptin 1, an ARF-binding protein, inhibits phospholipase D and endoplasmic reticulum/Golgi protein transport, FEBS Lett. 443 (1999) 197–200.
[23] F. Spitzenberger, S. Pietropaolo, P. Verkade, B. Habermann, S. Lacas-Gervais, H. Mziaut, M. Pietropaolo, M. Solimena, Islet cell autoantigen of 69 kDa is an arfaptin-related protein associated with the Golgi complex of insulinoma INS-1 cells, J. Biol. Chem. 278 (2003) 26166–26173.
[24] M.A. De Matteis, A. Godi, D. Corda, Phosphoinositides and the golgi complex, Curr. Opin. Cell Biol. 14 (2002) 434–447.
[25] J.P. DiNitto, T.C. Cronin, D.G. Lambright, Membrane recognition and targeting by lipid-binding domains, Sci. STKE 2003 (2003) re16.
[26] T. Itoh, T. Takenawa, Phosphoinositide-binding domains: functional units for temporal and spatial regulation of intracellular signalling, Cell. Signal. 14 (2002) 733–743.
[27] H. Hama, E.A. Schnieders, J. Thorner, J.Y. Takemoto, D.B. DeWald, Direct involvement of phosphatidylinositol 4-phosphate in secretion in the yeast *Saccharomyces cerevisiae*, J. Biol. Chem. 274 (1999) 34294–34300.
[28] C. Walch-Solimena, P. Novick, The yeast phosphatidylinositol-4-OH kinase pik1 regulates secretion at the Golgi, Nat. Cell Biol. 1 (1999) 523–525.
[29] A. Audhya, M. Foti, S.D. Emr, Distinct roles for the yeast phosphatidylinositol 4-kinases, Stt4p and Pik1p, in secretion, cell growth, and organelle membrane dynamics, Mol. Biol. Cell 11 (2000) 2673–2689.
[30] T.P. Levine, S. Munro, Targeting of Golgi-specific pleckstrin homology domains involves both PtdIns 4-kinase-dependent and -independent components, Curr. Biol. 12 (2002) 695–704.
[31] X. Li, M.P. Rivas, M. Fang, J. Marchena, B. Mehrotra, A. Chaudhary, L. Feng, G.D. Prestwich, V.A. Bankaitis, Analysis of oxysterol binding protein homologue Kes1p function in regulation of Sec14p-dependent protein transport from the yeast Golgi complex, J. Cell Biol. 157 (2002) 63–77.
[32] L.L. Yanagisawa, J. Marchena, Z. Xie, X. Li, P.P. Poon, R.A. Singer, G.C. Johnston, P.A. Randazzo, V.A. Bankaitis, Activity of specific lipid-regulated ADP ribosylation factor-GTPase-activating proteins is required for Sec14p-dependent Golgi secretory function in yeast, Mol. Biol. Cell 13 (2002) 2193–2206.
[33] A. Godi, P. Pertile, R. Meyers, P. Marra, G. Di Tullio, C. Iurisci, A. Luini, D. Corda, M.A. De Matteis, ARF mediates recruitment of PtdIns-4-OH kinase-beta and stimulates synthesis of PtdIns(4,5)P2 on the Golgi complex, Nat. Cell Biol. 1 (1999) 280–287.
[34] K. Wong, R. Meyers dd, L.C. Cantley, Subcellular locations of phosphatidylinositol 4-kinase isoforms, J. Biol. Chem. 272 (1997) 13236–13241.
[35] Y.J. Wei, H.Q. Sun, M. Yamamoto, P. Wlodarski, K. Kunii, M. Martinez, B. Barylko, J.P. Albanesi, H.L. Yin, Type II phosphatidylinositol 4-kinase beta is a cytosolic and peripheral membrane protein that is recruited to the plasma membrane and activated by Rac-GTP, J. Biol. Chem. 277 (2002) 46586–46593.
[36] A. Balla, G. Tuymetova, M. Barshishat, M. Geiszt, T. Balla, Characterization of type II phosphatidylinositol 4-kinase isoforms reveals association of the enzymes with endosomal vesicular compartments, J. Biol. Chem. 277 (2002) 20041–20050.
[37] O.A. Weisz, G.A. Gibson, S.M. Leung, J. Roder, A. Jeromin, Overexpression of frequenin, a modulator of phosphatidylinositol 4-kinase, inhibits biosynthetic delivery of an apical protein in polarized Madin–Darby canine kidney cells, J. Biol. Chem. 275 (2000) 24341–24347.
[38] Y.J. Wang, J. Wang, H.Q. Sun, M. Martinez, Y.X. Sun, E. Macia, T. Kirchhausen, J.P. Albanesi, M.G. Roth, H.L. Yin, Phosphatidylinositol 4 phosphate regulates targeting of clathrin adaptor AP-1 complexes to the Golgi, Cell 114 (2003) 299–310.
[39] A. Godi, A. Di Campli, A. Konstantakopoulos, G. Di Tullio, D. Alessi, G.S. Kular, T. Daniele, P. Marra, J.M. Lucocq, M.A De Matteis, FAPPs control Golgi-to-cell-surface membrane traffic by binding ARF and PI4P, Nat. Cell Biol. 6 (2004) 393–404.
[40] S. Dowler, R.A. Currie, D.G. Campbell, M. Deak, G. Kular, C.P. Downes, D.R. Alessi, Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities, Biochem. J. 351 (2000) 19–31.
[41] K. Hanada, K. Kumagai, S. Yasuda, Y. Miura, M. Kawano, M. Fukasawa, M. Nishijima, Molecular machinery for non-vesicular trafficking of ceramide, Nature 426 (2003) 803–809.
[42] T.P. Levine, S. Munro, The pleckstrin homology domain of oxysterol-binding protein recognises a determinant specific to Golgi membranes, Curr. Biol. 8 (1998) 729–739.
[43] J. Hirst, A. Motley, K. Harasaki, S.Y. Peak Chew, M.S. Robinson, EpsinR: an ENTH domain-containing protein that interacts with AP-1, Mol. Biol. Cell 14 (2) (2003) 625–641.
[44] I.G. Mills, G.J. Praefcke, Y. Vallis, B.J. Peter, L.E. Olesen, J.L. Gallop, P.J. Butler, P.R. Evans, H.T. McMahon, EpsinR: an AP1/clathrin interacting protein involved in vesicle trafficking, J. Cell Biol. 160 (2003) 213–222.
[45] M.A. Stamnes, J.E. Rothman, The binding of AP-1 clathrin adaptor particles to Golgi membranes requires ADP-ribosylation factor, a small GTP-binding protein, Cell 73 (1993) 999–1005.
[46] S.A. Watt, G. Kular, I.N. Fleming, C.P. Downes, J.M. Lucocq, Subcellular localization of phosphatidylinositol 4,5-bisphosphate using the pleckstrin homology domain of phospholipase C delta1, Biochem. J. 363 (2002) 657–666.
[47] A. Siddhanta, J.M. Backer, D. Shields, Inhibition of phosphatidic acid synthesis alters the structure of the Golgi apparatus and inhibits secretion in endocrine cells, J. Biol. Chem. 275 (2000) 12023–12031.
[48] D.A. Sweeney, A. Siddhanta, D. Shields, Fragmentation and re-assembly of the Golgi apparatus in vitro. A requirement for phosphatidic acid and phosphatidylinositol 4,5-bisphosphate synthesis, J. Biol. Chem. 277 (2002) 3030–3039.
[49] M.A. De Matteis, J.S. Morrow, The role of ankyrin and spectrin in membrane transport and domain formation, Curr. Opin. Cell Biol. 10 (1998) 542–549.

[50] G. Kreitzer, A. Marmorstein, P. Okamoto, R. Vallee, E. Rodriguez-Boulan, Kinesin and dynamin are required for post-Golgi transport of a plasma-membrane protein, Nat. Cell Biol. 2 (2000) 125–127.
[51] S.M. Jones, K.E. Howell, J.R. Henley, H. Cao, M.A. McNiven, Role of dynamin in the formation of transport vesicles from the *trans*-Golgi network, Science 279 (1998) 573–577.
[52] K. Bi, M.G. Roth, N.T. Ktistakis, Phosphatidic acid formation by phospholipase D is required for transport from the endoplasmic reticulum to the Golgi complex, Curr. Biol. 7 (1997) 301–307.
[53] I. Gaidarov, M.E. Smith, J. Domin, J.H. Keen, The class II phosphoinositide 3-kinase C2alpha is activated by clathrin and regulates clathrin-mediated membrane trafficking, Mol. Cell 7 (2001) 443–449.
[54] J. Domin, I. Gaidarov, M.E. Smith, J.H. Keen, M.D. Waterfield, The class II phosphoinositide 3-kinase PI3K-C2alpha is concentrated in the *trans*-Golgi network and present in clathrin-coated vesicles, J. Biol. Chem. 275 (2000) 11943–11950.
[55] A. Kihara, Y. Kabeya, Y. Ohsumi, T. Yoshimori, Beclin-phosphatidylinositol 3-kinase complex functions at the *trans*-Golgi network, EMBO Rep. 2 (2001) 330–335.
[56] T.R. Jeffries, S.K. Dove, R.H. Michell, P.J. Parker, PtdIns-specific MPR Pathway association of a novel WD40 repeat protein, WIPI49, Mol. Biol. Cell (2004).
[57] M. Fang, M.P. Rivas, V.A. Bankaitis, The contribution of lipids and lipid metabolism to cellular functions of the Golgi complex, Biochim. Biophys. Acta 1404 (1998) 85–100.
[58] C.L. Baron, V. Malhotra, Role of diacylglycerol in PKD recruitment to the TGN and protein transport to the plasma membrane, Science 295 (2002) 325–328.
[59] Z. Freyberg, A. Siddhanta, D. Shields, "Slip, sliding away": phospholipase D and the Golgi apparatus, Trends Cell Biol. 13 (2003) 540–546.
[60] Z. Xie, M. Fang, M.P. Rivas, A.J. Faulkner, P.C. Sternweis, J.A. Engebrecht, V.A. Bankaitis, Phospholipase D activity is required for suppression of yeast phosphatidylinositol transfer protein defects, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 12346–12351.
[61] G. Du, P. Huang, B.T. Liang, M.A. Frohman, Phospholipase D2 localizes to the plasma membrane and regulates angiotensin II receptor endocytosis, Mol. Biol. Cell 15 (2004) 1024–1030.
[62] Z. Freyberg, S. Bourgoin, D. Shields, Phospholipase D2 is localized to the rims of the Golgi apparatus in mammalian cells, Mol. Biol. Cell 13 (2002) 3930–3942.
[63] T.G. Jin, T. Satoh, Y. Liao, C. Song, X. Gao, K. Kariya, C.D. Hu, T. Kataoka, Role of the CDC25 homology domain of phospholipase Cepsilon in amplification of Rap1-dependent signaling, J. Biol. Chem. 276 (2001) 30301–30307.
[64] S.A. Barker, K.K. Caldwell, J.R. Pfeiffer, B.S. Wilson, Wortmannin-sensitive phosphorylation, translocation, and activation of PLCgamma1, but not PLCgamma2, in antigen-stimulated RBL-2H3 mast cells, Mol. Biol. Cell 9 (1998) 483–496.
[65] W. Cho, Membrane targeting by C1 and C2 domains, J. Biol. Chem. 276 (2001) 32407–32410.
[66] C. Jamora, N. Yamanouye, J. Van Lint, J. Laudenslager, J.R. Vandenheede, D.J. Faulkner, V. Malhotra, Gbetagamma-mediated regulation of Golgi organization is through the direct activation of protein kinase D, Cell 98 (1999) 59–68.
[67] J. Van Lint, A. Rykx, Y. Maeda, T. Vantus, S. Sturany, V. Malhotra, J.R. Vandenheede, T. Seufferlein, Protein kinase D: an intracellular traffic regulator on the move, Trends Cell Biol. 12 (2002) 193–200.
[68] M.J. Caloca, H. Wang, A. Delemos, S. Wang, M.G. Kazanietz, Phorbol esters and related analogs regulate the subcellular localization of beta 2-chimaerin, a non-protein kinase C phorbol ester receptor, J. Biol. Chem. 276 (2001) 18303–18312.
[69] V.K. Chiu, T. Bivona, A. Hach, J.B. Sajous, J. Silletti, H. Wiener, R.L. Johnson II, A.D. Cox, M.R. Philips, Ras signalling on the endoplasmic reticulum and the Golgi, Nat. Cell Biol. 4 (2002) 343–350.
[70] M.J. Caloca, J.L. Zugaza, X.R. Bustelo, Exchange factors of the RasGRP family mediate Ras activation in the Golgi, J. Biol. Chem. 278 (2003) 33465–33473.
[71] J.P. Simon, I.E. Ivanov, B. Shopsin, D. Hersh, M. Adesnik, D.D. Sabatini, The in vitro generation of post-Golgi vesicles carrying viral envelope glycoproteins requires an ARF-like GTP-binding protein and a protein kinase C associated with the Golgi apparatus, J. Biol. Chem. 271 (1996) 16952–16961.
[72] S. Ahmed, R. Kozma, C. Monfries, C. Hall, H.H. Lim, P. Smith, L. Lim, Human brain n-chimaerin cDNA encodes a novel phorbol ester receptor, Biochem. J. 272 (1990) 767–773.
[73] H. Wang, M.G. Kazanietz, Chimaerins, novel non-protein kinase C phorbol ester receptors, associate with Tmp21-I (p23): evidence for a novel anchoring mechanism involving the chimaerin C1 domain, J. Biol. Chem. 277 (2002) 4541–4550.
[74] T.G. Bivona, I. Perez De Castro, I.M. Ahearn, T.M. Grana, V.K. Chiu, P.J. Lockyer, P.J. Cullen, A. Pellicer, A.D. Cox, M.R. Philips, Phospholipase Cgamma activates Ras on the Golgi apparatus by means of RasGRP1, Nature 424 (2003) 694–698.
[75] T. Kajimoto, Y. Shirai, N. Sakai, T. Yamamoto, H. Matsuzaki, U. Kikkawa, N. Saito, Ceramide-induced apoptosis by translocation, phosphorylation, and activation of protein kinase Cdelta in the Golgi complex, J. Biol. Chem. 279 (2004) 12668–12676.
[76] B. Sparatore, M. Passalacqua, M. Pedrazzi, S. Ledda, M. Patrone, D. Gaggero, S. Pontremoli, E. Melloni, Role of the kinase activation loop on protein kinase C theta activity and intracellular localisation, FEBS Lett. 554 (2003) 35–40.
[77] Y. Maeda, G.V. Beznoussenko, J. Van Lint, A.A. Mironov, V. Malhotra, Recruitment of protein kinase D to the *trans*-Golgi network via the first cysteine-rich domain, EMBO J. 20 (2001) 5982–5990.
[78] M. Liljedahl, Y. Maeda, A. Colanzi, I. Ayala, J. Van Lint, V. Malhotra, Protein kinase D regulates the fission of cell surface destined transport carriers from the *trans*-Golgi network, Cell 104 (2001) 409–420.
[79] K. Nishikawa, A. Toker, K. Wong, P.A. Marignani, F.J. Johannes, L.C. Cantley, Association of protein kinase Cmu with type II phosphatidylinositol 4-kinase and type I phosphatidylinositol-4-phosphate 5-kinase, J. Biol. Chem. 273 (1998) 23126–23133.
[80] R.S. Polishchuk, E.V. Polishchuk, P. Marra, S. Alberti, R. Buccione, A. Luini, A.A. Mironov, Correlative light-electron microscopy reveals the tubular-saccular ultrastructure of carriers operating between Golgi apparatus and plasma membrane, J. Cell Biol. 148 (2000) 45–58.
[81] G. Kreitzer, J. Schmoranzer, S.H. Low, X. Li, Y. Gan, T. Weimbs, S.M. Simon, E. Rodriguez-Boulan, Three-dimensional analysis of post-Golgi carrier exocytosis in epithelial cells, Nat. Cell Biol. 5 (2003) 126–136.
[82] M.S. Robinson, J.S. Bonifacino, Adaptor-related proteins, Curr. Opin. Cell Biol. 13 (2001) 444–453.
[83] J.S. Bonifacino, J. Lippincott-Schwartz, Coat proteins: shaping membrane transport, Nat. Rev., Mol. Cell Biol. 4 (2003) 409–414.
[84] H. Folsch, M. Pypaert, P. Schu, I. Mellman, Distribution and function of AP-1 clathrin adaptor complexes in polarized epithelial cells, J. Cell Biol. 152 (2001) 595–606.
[85] N. Nishimura, H. Plutner, K. Hahn, W.E. Balch, The delta subunit of AP-3 is required for efficient transport of VSV-G from the *trans*-Golgi network to the cell surface, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 6755–6760.
[86] T. Simmen, S. Honing, A. Icking, R. Tikkanen, W. Hunziker, AP-4 binds basolateral signals and participates in basolateral sorting in epithelial MDCK cells, Nat. Cell Biol. 4 (2002) 154–159.
[87] A. Godi, A. Di Campli, G. Di Tullio, M.A. De Matteis, PtdIns4P Controls Membrane Fission at the TGN, Suppl. to Mol. Biol. Cell 12 (2001) 265.
[88] D. Corda, C. Hidalgo Carcedo, M. Bonazzi, A. Luini, S. Spano, Molecular aspects of membrane fission in the secretory pathway, Cell. Mol. Life Sci. 59 (2002) 1819–1832.