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Review

Molecular aspects of membrane fission in the secretory pathway

D. Corda*, C. Hidalgo Carcedo, M. Bonazzi, A. Luini and S. Spanò

Department of Cell Biology and Oncology, Istituto di Ricerche Farmacologiche 'Mario Negri', Consorzio Mario Negri Sud, Via Nazionale, 66030 Santa Maria Imbaro, Chieti (Italy), Fax + 39 0872 570 412,
e-mail: corda@dcbo.negrisud.it

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Abstract. Membrane fission is essential in various intracellular dissociative transport steps. The molecular mechanisms by which endocytic vesicles detach from the plasma membrane are being rapidly elucidated. Much less is known about the fission mechanisms operating at Golgi tubular networks; these include the Golgi transport and sorting stations, the trans-Golgi and cis-Golgi networks, where the geometry and physical properties of the membranes differ from those at the cell surface. Here we discuss the lipid and protein machineries that have so far been related to the fission process, with emphasis on those acting in the Golgi complex.

Key words. CtBP3/BARS; Golgi complex; membrane traffic; acyl-transferase; acyl-CoA; phosphatidic acid; phospholipase D; protein kinase; PITP.

Introduction

Intracellular membrane traffic is a highly dynamic process based on the structural and functional interactions among membrane lipids and proteins. It includes, for example, the secretory pathway starting from the endoplasmic reticulum (ER) towards the Golgi complex, from which transport intermediates pinch off and move to the plasma membrane (or other destinations), where they fuse and release their cargo. Similarly, endocytosis requires the formation and detachment of endocytic vesicles at the plasma membrane; these then move towards, and merge with, intracellular membranes. The ability of biological membranes to give rise to transport intermediates through the pinching-off (fission) and then merging (fusion) mechanisms in a controlled, spatially defined manner is thus essential for cell function, and its understanding is central to cell biology. Indeed, this 'rearrangement' of cellular membranes is not only important in endo- and exocytosis, but is also crucial in processes such as cell division, neurotransmission and fertilization.

In recent years, the work of several laboratories has led to the identification of fusion- and fission-inducing proteins (see below); however, full comprehension of the lipid-protein dynamics in these processes remains far from complete. In the following, we will focus on the fission machineries that have been proposed to act at the Golgi membranes. We refer readers to recent reviews for other aspects of membrane traffic, such as fission in endocytosis [1] and membrane fusion [2–4].

Membrane fission

Membrane fission and fusion share some common features: both processes require close contact between lipid bilayers and the merging of leaflets from the bilayers, an energetically unfavourable process. This is accompanied by an extreme, localized membrane curvature (preceding

* Corresponding author.

fusion or fission) that may be facilitated by specific bilayer lipid compositions [5]. Despite these similarities, the fission process is much less understood than fusion. This is probably due in part to the fact that in viral-induced membrane fusion, the fusogenic proteins have been identified and have served as a model to delineate the general principles of fusion [3]. Moreover, the possibility of inducing the fusion of model membranes has generated theoretical studies of the involved bilayer rearrangements [6, 7]. This does not apply to fission. However, the recent identification of proteins and lipids (see below) with putative roles in membrane fission has called attention to this process and generated experimental and theoretical studies [5, 8, 9]. These studies show that several, apparently independent, protein (and lipid) machineries may play a role in fission (see below). This is at variance with fusion, where only one type of protein machinery (including the SNARE proteins) mediates the process [2, 10], with the possible exception of mitochondria [11]. Another potential important difference is that 'lipid metabolism machines' (see below) have been implicated in membrane fission but not, so far, in fusion.

The Golgi complex

The Golgi complex is composed of flat cisternae piled up into stacks, and of tubular-reticular networks (fig. 1 A). The latter constituents comprise, in turn, the transport and sorting stations, the trans-Golgi network (TGN) and the cis-Golgi network (CGN), and the reticular non-compact zones interconnecting adjacent stacks [12]. Golgi tubules are dynamic structures that are known to play a role in trafficking. They have been observed by green-fluorescence protein (GFP)-based video microscopy to emanate from the Golgi mass, to retract, or to detach and move away rapidly [13, 14]. Trans-Golgi tubules detaching from the TGN act as transport intermediates in constitutive traffic to the plasma membrane [15–17], and cis tubules have been proposed to play a similar role in recycling proteins and lipids from the Golgi to the ER [13, 18]. The function of the inter-stack networks is less clear; they have been proposed to be involved in intra-Golgi transport [19, 20].

Only recently have several studies started to elucidate the molecular mechanisms underlying the dynamics of Golgi tubular networks. Golgi tubules can be schematically viewed as resulting from the interplay between the processes of tubule formation/elongation and fission (fig. 1 B). The elongation of Golgi tubules may involve the activity of a phospholipase A_2 (PLA_2) [21, 22]. In the case of fission, a number of different biochemical pathways have been proposed to play a role: CtBP3/BARS [23], PLD [24, 25], protein kinase C (PKC)/protein kinase D (PKD) [26, 27], and phosphatidylinositol (PI) transfer protein (PITP) [28]. Moreover, recent evidence suggests that specific isoforms of dynamin and endophilin might also be localized in the Golgi, and could therefore have roles in membrane fission [29, 30]. Whether these various proteins might act in different Golgi stations, or operate in a coordinated manner, is still a completely open question. Instead, as will be detailed below, a common feature is that all these proteins require lipids for their activity, which would thus delineate the need for a lipid machinery operating at the Golgi that is possibly driven by specific protein complexes.

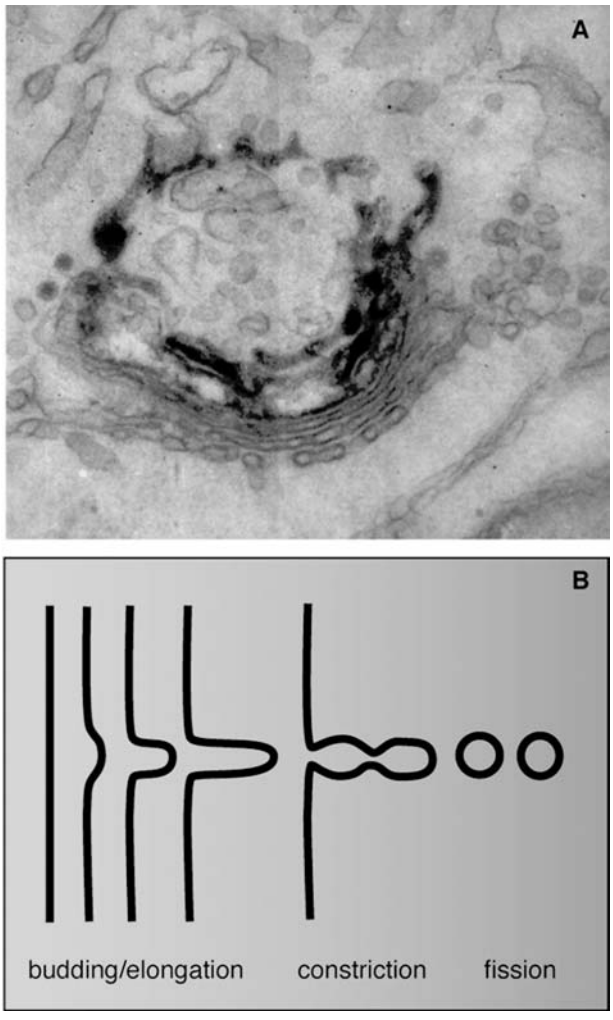

Figure 1. (*A*) Golgi complex structure. Electron micrograph of the Golgi complex from NRK cells stably transfected with a sialyltransferase-horseradish peroxidase fusion protein. The cis side of the Golgi is recognizable by the presence of fenestrated cisternae, whereas the trans side of the Golgi is labelled with horseradish peroxidase. Magnification × 20,000. (*B*) Schematic representation of the steps leading to the formation of transport intermediates from the Golgi complex. Buds are generated from flat membranes, which can then elongate into tubules, and subsequently undergo constriction and fission. See text for details.

The role of lipids in secretory transport

Besides being structural components of the membrane bilayer, lipids are signalling molecules and protein cofactors whose actions are exerted in different cell compartments. In the secretory pathway, phospholipids that are mostly synthesized at the ER are transported to their final destinations via the Golgi complex [31–33]. The lipid composition of cell membranes is not homogeneous, and thus a gradient of bilayer thickness is generated along the secretory pathway due to an increase in the concentrations of sphingomyelin and cholesterol from the ER to the plasma membrane, where microdomains enriched in these two lipids are found [34]. These differences in lipid composition can affect the shape and geometry of the bilayer, important features for protein segregation and sorting, as well as for membrane fusion and fission [5, 35]. Moreover, the bilayer is characterized by an asymmetry in lipid composition between the internal and external leaflets that can also play a role in bending, fusion and fission.

Biological lipids can be classified on the basis of their molecular shapes and structures in an aqueous environment: cylindrical [or bilayer-preferring; phosphatidylcholine (PC) or other phospholipids], conical [or type II; diacylglycerol (DAG), cholesterol] or inverted cones (or type I; lysophospholipids) (see fig. 2A). Membrane bending can be influenced by the relative abundance of cone- or inverted-cone-like lipids, which will tend to confer a negative or positive curvature to the bilayer, depending on their distribution in the inner or outer leaflets of the bilayer (see fig. 2B). Phosphatidic acid (PA), a central lipid in membrane fission as will be discussed below, is a type II lipid with unique physical properties: a very small headgroup characterized by a high charge density that is able to act both as a hydrogen donor and acceptor. When the ionization of PA is reduced (as in the case of divalent-cation binding), PA-rich microdomains are formed through intermolecular hydrogen bonding [36, 37]. Moreover, the shape of PA can vary greatly depending on the presence of free calcium; thus, low calcium ion concentrations favour the cylindrical shape, which becomes conical in the presence of high concentrations (mM) of this ion (see also fig. 3D) [36, 37].

Fusion of bilayers involves three different steps: membrane adhesion, semifusion and pore formation [5]. Fission can be considered as resulting from a similar series of events proceeding in the opposite order [5, 8]. The semifusion intermediates have a strong net negative (concave) curvature, which would thus be facilitated by a high density of conical lipids in the outer leaflet, whereas inverted-cone-like lipids prevent this formation. In model membranes, conical lipids such as DAG or unsaturated fatty acids have been shown to facilitate fusion, while inverted cones, such as lysophosphatidylcholine, inhibit fusion even at low concentrations [35]. The main difference between fusion and fission relates to the membrane leaflet involved in the process, and thus to the topology of the rearrangements in lipid compositions needed to complete either fusion or fission [5, 8]. Thus, while fusion requires interactions between two cytosolic leaflets of separate bilayers (of an endocytic vesicle and an endosome, for example), in fission, the two lumenal leaflets of a tubule (in the Golgi complex) or the external leaflet of the plasma membrane (in endocytosis) have to come in close

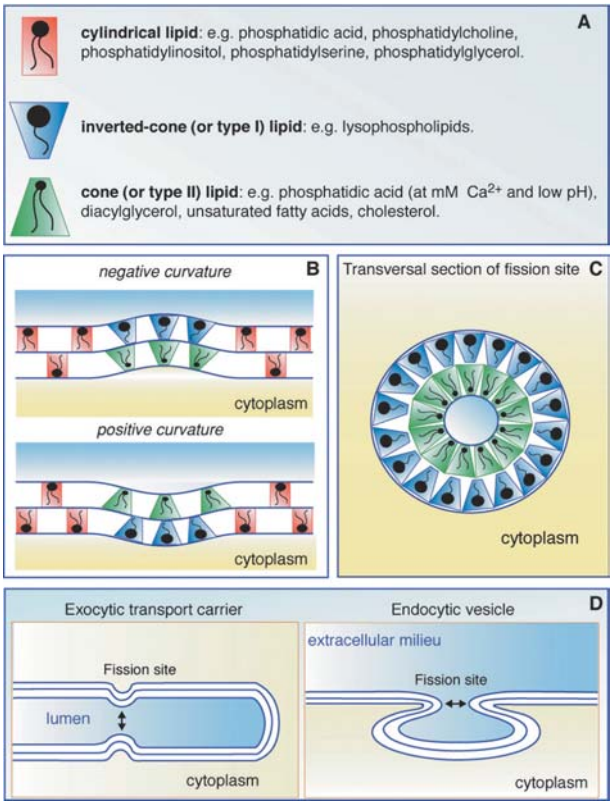

Figure 2. Lipid organization at the fission site. (*A*) Schematic representation of the three classes of lipid. Examples of biologically relevant lipids classified on the basis of their molecular shapes. (*B*) Schematic representation of spontaneous membrane curvature and bending. When the cytoplasmic leaflet is enriched in cone-shaped lipids or the lumenal/external leaflet is enriched in inverted-cone lipids, the bilayer acquires a negative spontaneous curvature and bends towards the organelle lumen or the extracellular space. Conversely, when the cytoplasmic leaflet is enriched in inverted-cone lipids or the lumenal/external leaflet is enriched in cone-shaped lipids, the membrane acquires a positive spontaneous curvature and bends towards the cytoplasm. (*C*) Transversal section of the fission site. In transversal sections, the geometry of the fission site shows a positive curvature that better accommodates cone-shaped lipids in the lumenal/external layer (considering Golgi tubules or endocytic vesicles, as in panel *D*) and inverted-cone lipids in the cytoplasmic layer. (*D*) Membrane fission. This scheme illustrates the contact between bilayers during membrane fission occurring at Golgi tubules (left panel) or at the plasma membrane (right panel), which involves the lumenal and extracellular leaflets of the bilayer, respectively. See text for details.

proximity (fig. 2D). Extreme membrane bending is thus essential for fission in order to bring the lumenal leaflets together (fig. 2D). Moreover, another important difference is that cytosolic factors that act on the relevant leaflet in fusion will act on the 'distant' leaflet in fission. The curvature required for fission can be generated by a difference in lateral pressure between the two membrane leaflets, which can result in membrane deformation. This can be generated by insertion (or modification) of lipids or proteins, or by a difference in spontaneous monolayer curvature between the two membrane leaflets due to selective differences in the types of lipid components of the membrane leaflets [5]. These asymmetries in the bilayer can derive from lipid translocation, lipid metabolism, and/or spontaneous transbilayer movement (flip-flop), all processes that require the action of proteins. Indeed, the activities of several proteins that play roles in fission have been related to their ability to modify the membrane lipid composition or to interact with membrane lipids. Thus, PITP controls the scission of coat protein complex (COP)I-coated vesicles and uncoated vesicles formed at the TGN probably through the delivery of PI to specific sites of the TGN membranes and by increasing the amounts of specific PI-derived metabolites required for fission [ref. 28 and see below]. CtBP3/BARS [23] and endophilin [38] have been proposed to induce fission at the Golgi complex and at the plasma membrane, respectively, through the conversion of lysophosphatidic acid (LPA) into PA. More recently, other enzymes acting on lipids that have been implicated in fission are PLD (which forms PA by hydrolyzing membrane phospholipids) and PKD (which binds DAG) (see below).

CtBP3/BARS

BARS [brefeldin A (BFA)-dependent ADP-ribosylation substrate] was identified as a protein endowed with the ability to antagonize the tubulation-inducing effects of BFA on the Golgi complex [39, 40]. BARS is a 50-kDa cytosolic protein substrate of BFA-induced ADP ribosylation [41, 42]. The ADP-ribosylated form of the protein was purified from rat brain cytosol, cloned (BARS; GenBank accession number (AC) AF067795 [39]), and found to be strongly homologous to the two mammalian members of the C-terminus-binding protein (CtBP) family (CtBP1 and CtBP2, cloned in human and mouse [43–47]). The BARS identity is 97% with human and mouse CtBP1, and 79% with human and mouse CtBP2. The significant region of diversity between CtBP1 and BARS is the N-terminal stretch, where these proteins differ in sequence and length. Although this region is small, it might have significant regulatory or targeting functions. Thus BARS is a third isoform of the CtBP protein group and is now referred to as CtBP3/BARS [39].

Characterization of the role of CtBP3/BARS in the organization of the tubular component of the Golgi complex originated from the demonstration that an ADP-ribosylation reaction mediates some of the effects of BFA on the structure and function of the Golgi complex [40, 48, 49]. Thus, a series of inhibitors of the BFA-dependent ADP-ribosylation reaction also inhibits the effects of BFA on Golgi morphology and, with similar potency, the ADP-ribosylation of CtBP3/BARS and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the two specific substrates of BFA [41, 42, 49]. In addition, in permeabilized RBL cells, the BFA-dependent disassembly of the Golgi complex requires NAD^+ and cytosol [40]. Cytosol that has been previously ADP-ribosylated (hence containing ADP-ribosylated GAPDH and CtBP3/BARS) is sufficient to sustain the Golgi disassembly induced by BFA, also in the absence of NAD^+ [40, 48], indicating that an ADP-ribosylation reaction is indeed part of the mechanism of action of BFA, and that brain cytosol contains factors that prevent the Golgi disassembly induced by BFA [40, 48]. CtBP3/BARS was shown to be one of these factors, based on the observations that: (i) the ADP-ribosylation of CtBP3/BARS by BFA correlates with the loss of inhibitory activity of the cytosol on Golgi disassembly [40]; (ii) anti-BARS antibodies mimic the BFA effect [39]; and (iii) purified CtBP3/BARS prevents the effect of BFA in permeabilized RBL cells [39]. This thus demonstrates that the previously reported ability of brain cytosol to inhibit the effects of BFA on Golgi morphology is mediated largely, albeit not completely, by this protein [39]. These studies also clearly indicate that CtBP3/BARS is inactivated by BFA-dependent ADP-ribosylation [39, 48].

Immunofluorescence microscopy of COS7 cells overexpressing CtBP3/BARS has shown a perinuclear/Golgi area localization as well as a partial/minor nuclear localization, both of which have been confirmed studying the distribution of the endogenous protein in human fibroblasts [unpublished data]. This is compatible with a role of CtBP3/BARS not only in the Golgi, but also in the nucleus, similar to that seen for the other CtBP family members [39, 46, 47]. In permeabilized RBL cells, exposure to CtBP3/BARS-enriched cytosol causes a reduction in the tubular-reticular zones of the Golgi and their partial replacement with fragments of variable sizes [unpublished data]. Cisternae are partially preserved, while other cellular compartments seem not to be affected [unpublished data].

In isolated Golgi membranes, CtBP3/BARS has been shown to be a direct, potent activator of the fission of Golgi tubular-reticular domains into fragments of variable sizes and to be an essential component of a fission machinery normally present in the cytosol (fig. 3A–C) [23]. The fission events induced by CtBP3/BARS are preceded by the formation of structures where tubules are

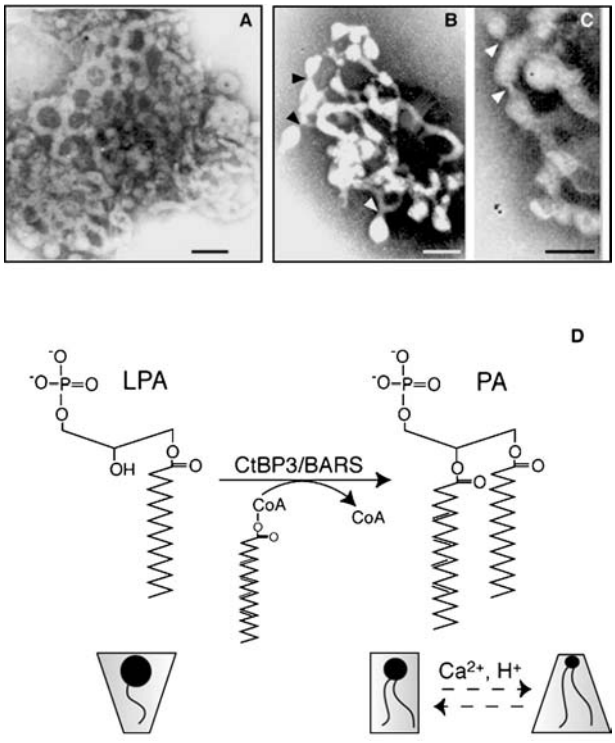

Figure 3. Fissioning of Golgi membranes. (*A–C*) Negative staining [following the method and EM analysis described in ref. 23] of isolated Golgi membranes incubated with cytosol alone (*A*) or with cytosol and CtBP3/BARS (*B, C*). In *C*, the Golgi membranes are shown at higher magnification to show constriction sites (arrowheads). Bars, 100 nm. (*D*) Scheme of the reaction catalyzed by CtBP3/BARS. LPA, an inverted-cone lipid, is converted to PA, a cylindrical lipid, through the addition of an acyl moiety from acyl-CoA. The newly formed PA can acquire a conical shape in the presence of millimolar concentrations of free calcium and at low pH.

extremely constricted, and which most likely represent intermediates in the fission process (see below). These effects of CtBP3/BARS are structurally different from the in vitro effects on isolated membranes induced by dynamin, a GTPase involved in the formation of clathrin-coated vesicles from the cell surface [50–53], suggesting that the events involving the two proteins are mechanistically distinct.

Clues concerning the mechanism of CtBP3/BARS-dependent fission have been derived from the morphology of the sites where this protein induces constriction of Golgi tubules (fig. 3B, C). As noted above, based on their unique shape, the time course of their appearance, and the observation that the distance between them reflects the size of the fragments generated by fission, these sites are most likely placed where fission later procedes to completion [23]. Some of them might also represent incomplete fission events which will later revert to a normal tubule calibre. Their most interesting feature is their high level of membrane curvature, which implies that their structure is almost certainly incompatible with the lipid composition of a normal membrane bilayer. The minimum vesicular or tubular diameter that will accommodate 'cylindrical' lipids is 25 nm [54]. Thus, normally abundant lipid species such as PC, PI and phosphatidylserine, which are all cylindrical, must be scarce in the constricted zones. Instead, assuming that the predominant curvature is the one visible in transversal sections, type I (inverted-cone-like) lipids, such as lysolipids or polyphosphoinositides, are likely to predominate in the external leaflet, and type II (conical) lipids, such as phosphatidylethanolamine, DAG and arachidonic acid, in the inner leaflet (fig. 2C) [54]. Of interest here is to note that the distance found between facing inner leaflets of the constricted Golgi tubes (about 4 nm) is comparable to that found in typical hexagonal-II tubes (formed by pure type II lipids [55, 56]). These considerations suggest that local changes in lipid composition play a role in the generation of the fission intermediates. Indeed, CtBP3/BARS can induce modifications in the lipid bilayer of a kind that would suit this model [23]. Thus, CtBP3/BARS is endowed with an intrinsic acyl transferase activity by which it transfers an acyl moiety from acyl-CoA specifically to LPA, forming PA (fig. 3D) [23]. The synthesis of PA from LPA is sufficient for membrane fission, and the addition of LPA greatly facilitates this, demonstrating a role for this specific lipid metabolic pathway in the fission of cellular membranes [23]. Interestingly, the percentage of PA required to induce Golgi membrane fission is significantly lowered by CtBP3/BARS, suggesting that this protein acts by 'concentrating' the newly formed PA in specific membrane sites, which then undergo fission [23]. BFA-induced ADP-ribosylation of CtBP3/BARS abolishes its transferase activity and the subsequent fission of Golgi tubules [23]. A conclusion from this study is that acyl-CoAs are absolutely required for CtBP3/BARS-induced fission. The requirement for acyl-CoA first emerged from studies on the fission of COPI-coated vesicles [57]; in that report, however, acyl-CoA was proposed to be related to protein (rather than lipid) modifications.

The concept that changes in lipid composition play a role in fission does not exclude facilitation of the formation of fission intermediates by mechanical stress imposed on the membrane, such as that proposed to be induced by dynamin [58, 59], and that could be brought about by the cytoskeletal elements relevant in Golgi organization [60, 61]. So far though, ring- or coat-like structures reminiscent of dynamin spirals or protein coats have not been reported at fission sites in the Golgi. Moreover, the CtBP3/BARS-dependent fission sites are very different from the uniform tubules generated by dynamin in vitro [59, 62, 63].

An alternative model to explain the role of the acyl transferases in membrane fission proposes that rather than being related to the enzymatic conversion of LPA to

PA (which is very slow for both endophilin and CtBP3/BARS [23, 38]), the changes in bilayer geometry are induced by a conformational switch of the acyl transferase, determined by the lipid (LPA, PA or acyl-CoA) bound to it [1]. Thus, the conformation and orientation of the protein in the bilayer would be affected by the conversion of the LPA- (or acyl-CoA-) to the PA-bound state, and this could lead to fission by a mechanism that still remains hypothetical [1].

Phospholipase D

Due to the proposed involvement of PA in membrane fission (see above), all enzymatic pathways leading to its production could potentially be involved in the induction/regulation of this process. Among these, PLD, that catalyses the hydrolysis of PC (or other phospholipids) to yield PA and choline, has already been proposed to play a role in secretion [24, 25, 64–66; also reviewed in ref. 67]. Two PLDs, PLD1 and PLD2, have been identified in mammals [68, 69], with PLD1a and PLD1b being two alternative splicing forms of PLD1 [70, 71]. PLD1 can be activated in vitro by PKCα, ADP-ribosylating factor (ARF) and Rho in the presence of phosphatidylinositol 4,5-bisphosphate (PIP_2) [72]. PLD2 is 50% identical to PLD1, exists as three splicing variants [73], and is constitutively active in vitro and in vivo [69]. Among the interactors having an inhibitory effect on its enzymatic activity, AP180 and the amphiphysins have been shown to inhibit PLDs through direct interactions, while the 5′-phosphatase synaptojanin does so by reducing the levels of PIP_2 [74–76]. Recently, PLD2 has also been found to directly interact with the glycolytic enzyme aldolase through its pleckstrin homology (PH) domain [77].

After the initial studies on ARF-dependent involvement of PLD in secretion, PLD activity and PA synthesis were proposed as being involved in the budding of intermediates from the Golgi complex [24, 25]. The activation of PLD by ARF was proposed to regulate the recruitment of coatomer, and thus control the formation of COPI-coated vesicles, suggesting a role for PA, and potentially PA-derived lipids, in membrane budding [25]. In line with these data, stimulation of PA synthesis through addition of exogenous PLD specifically enhances the formation of hormone-containing secretory vesicles in permeabilized rat pituitary GH_3 cells, whereas addition of an inactive form of PLD does not stimulate this process [24]. Accordingly, inhibition of PA synthesis using primary alcohols also inhibits transport-intermediate formation [24]. This treatment also induces the complete fragmentation of the Golgi complex [78]. Whether these effects of PA are direct or are mediated by the PA-dependent activation of lipid kinases leading to PIP_2 synthesis has yet to be elucidated [78].

PA and PIP_2 have also been proposed to be part of a positive feedback loop, which would create membrane microdomains enriched in these two acidic phospholipids that could then promote membrane fusion; these domains also have the potential to be involved in membrane fission [26, 79]. This positive feedback would result in the PLD-dependent formation of PA, which can stimulate a phosphatidylinositol 4-phosphate (PI4P) 5-kinase to generate PIP_2 [80], an activator of PLD [81], hence further stimulating PA formation.

The above conclusions are somewhat controversial. Other data have indicated that PLD activity is not present in the Golgi [82, 83] and have not confirmed the ARF-dependent activation of PLD and PLD-mediated control of coatomer assembly [84, 85]. A similar controversy also surrounds the definition of the cellular localization of the PLDs [reviewed in ref. 67]. Most studies have been performed by analysing the localization of transfected tagged PLDs. Thus, PLD1 has been reported to be localized in the ER and Golgi [69], and on secretory granules, late endosomes and lysosomes [86, 87], while PLD2 has been localized mainly in the plasma membrane and in endocytic vesicles [69]. Recently, divergent findings about the localization of endogenous PLDs in different cell lines have been reported. In epithelial cells (HT29-cl19), PLD1 appears to be in cytoplasmic spots and to be translocated to the plasma membrane after phorbol ester treatment, whereas PLD2 appears to be localized in the Golgi complex [88]. In contrast, in NRK and GH_3 cells, Shields and collaborators found that an antiserum raised against PLD1 detects this protein mostly at the level of the Golgi complex, both by immunofluorescence and by electron microscopy (EM) analysis [89]. They have also reported that PLD1 overexpression leads to mislocalization of the endogenous protein [89].

Thus, while PLD activity in the Golgi would fit with the PA requirement in fission, the localization of either isozyme in this organelle remains to be fully demonstrated. Whether other proteins with a PLD activity, but not homologous to the known family members, act at the Golgi is an interesting possibility that has not, however, the support of experimental data.

Dynamin-driven machinery

Dynamin, the best characterized fission-inducing protein so far, was initially implicated in the formation of clathrin-coated vesicles at the plasma membrane [90–92], and shown to localize at tubular invaginations of clathrin-coated pits [93]. Recently, a number of comprehensive reviews covering all aspects of dynamin cell biology have appeared [50–53].

Briefly, mammalian dynamins are encoded by three distinct genes with different tissue expression: dynamin I,

originally isolated from rat brain as a microtubule-binding protein, is a neuron-specific isoform; dynamin II is a ubiquitous protein, and dynamin III is expressed in brain, testis and lung [reviewed in ref. 50]. They are 100-kDa GTPases with a multidomain structure, comprising an N-terminal tripartite, highly conserved GTP-hydrolysis domain, an intermediate domain whose function is still not clear, a PH domain responsible for the interaction with PIP_2 and membrane targeting, a coiled-coil region that acts as a GTPase-effector domain (GED) and as a GTPase-activating protein (GAP) domain, and a C-terminal proline-arginine-rich domain (PRD) containing several Src homology (SH3)-domain-binding sites. Each domain is involved in interactions with several other proteins or with lipid components of the membranes, indicating that dynamin is a likely target of complex intracellular regulatory processes [50].

Several studies have supported the role of dynamins in the formation of transport intermediates in different steps of the endocytic pathway [90, 91, 94–97]. In contrast, the localization and involvement of dynamins in fission events at the TGN is still being debated. Dynamin II has been reported as localized at the Golgi complex [98, 99]. McNiven and collaborators have also shown that dynamin II inhibitory antibodies [99] and dynamin II mutants [30] are able to interfere with the transport of membrane proteins from the Golgi complex to the plasma membrane and to affect Golgi morphology. A similar conclusion was reached by Rodriguez-Boulan and collaborators, who showed that dynamin II mutants block the formation of transport intermediates directed from the TGN to the plasma membrane [100]. In contrast, other authors have shown that both dynamin I and dynamin II mutants only affect endocytic transport, without interfering with the biosynthetic transport from the Golgi [101]. This discrepancy may originate from the fact that different dynamin II splicing variants were employed in these studies, and leads to the hypothesis that only specific dynamin II isoforms are localized at the Golgi and act in the formation of transport intermediates from the TGN.

The exact mechanism by which dynamin controls membrane dynamics during the formation of membranous carriers has been investigated in different experimental systems and has given rise to several models which have tried to accommodate the experimental evidence [reviewed in refs 52 and 53]. Most of these models take into account data showing that dynamin is a mechanoenzyme able to generate a net motive force from the hydrolysis of GTP. When applied to lipid membranes, this force would result in the generation of tubules from flat membranes and in the constriction and severing of these tubules, leading to the formation of transport intermediates [59, 62, 63]. Mention needs to be made, however, that other mechanisms of action of dynamin have also been proposed which relate either to its GTPase activity, which could play a regulatory role in the fissioning process [53, 102, 103], or to its ability to interact and thus regulate the actin cytoskeleton, which has also been proposed to play a role in fission [104, 105].

The putative role of dynamin at the Golgi raises the question as to whether CtBP3/BARS and dynamin participate in the same, or in distinct, membrane fission events. There are marked dissimilarities between the cellular structures on which the two proteins exert their known effects at the plasma membrane and at the Golgi tubules [23, 51]. Thus, the fission of a clathrin-coated vesicle from the plasma membrane, an organelle with a unique cholesterol-rich lipid composition, flat geometry, and complex cytoskeletal scaffold, might well differ profoundly in terms of kinetics and machinery from the process involved in the clipping of an elongated, flexible structure such as a Golgi tubule. However, if dynamin can also operate at the Golgi, the possibility should be considered that dynamin operates by a different mechanism (possibly similar to that mediated by CtBP3/BARS). The information available so far on Golgi dynamin is too scarce to define a more refined model. Better understanding of the differences and commonalities between fission at the plasma membrane and at the Golgi will be instrumental in elucidating the general mechanisms of membrane fission.

Endophilins

The endophilins (A1, A2 and A3) were identified as SH3-domain-containing interactors of synaptojanin and dynamin I, two proteins involved in synaptic vesicle traffic [106, 107]. They show different distributions: endophilin A1 was detected only in brain, endophilin A2 in multiple tissues, and endophilin A3 in brain and testis [reviewed in ref. 1]. They localize at the level of nerve terminals, where they colocalize with dynamin I, synaptojanin and amphiphysin I [106, 108].

Endophilins are players in clathrin-mediated endocytosis, and have been implicated in different stages of this process: in generating membrane curvature, in fission and in the release of coat [1, 38, 109, 110]. Moreover, endophilin has also recently been shown to induce tubule formation in liposomes made from a brain lipid extract [29]. With regard to the regulation of membrane curvature and fission, endophilin A1 has been shown to be required for synaptic vesicle endocytosis in an in vitro assay on perforated PC12 cells [38]. Interestingly, and similar to CtBP3/BARS, endophilin A1 is an LPA-specific acyltransferase that converts LPA to PA by transferring an acyl chain from an acyl-CoA molecule to the sn-2 carbon of LPA [38]. Endophilin A1 is able to use both saturated and unsaturated acyl chains as in vitro substrates, but the formation of synaptic vesicles is strictly dependent on the

presence of arachidonoyl-CoA, while it is inhibited by palmitoyl-CoA [38]. This result suggests that at least in the case of generation of curvature in endocytic events occurring at the plasma membrane, the length and shape of the acyl chains of phospholipids is crucial.

Recently, a newly discovered member of the family, endophilin B1 (25% identical and 39% similar to endophilin A1), has been found to localize at the Golgi complex in CHO cells [29]. Endophilin B1 also binds to lipids, tubulates liposomes and has LPA-specific acyl transferase activity [29, 111]. This recent finding opens the possibility that endophilins can also control tubulation/fission processes at the TGN, and is in line with the proposal of a dynamin-codriven machinery operating at the Golgi [30].

PKC and PKD

A PKC-like activity has been proposed to be involved in Golgi membrane fission, as characterized by Sabatini and colleagues in Golgi membranes isolated from MDCK cells [26, 112]. This process consists of two sequential steps, coat assembly and bud formation, which are triggered by the GTPγS-dependent activation of ARF; this is followed by the scission of coated buds, a process that requires the presence of cytosolic proteins [112]. One of the proposed relevant cytosolic elements is a PKC-like activity, since fragmentation is suppressed by PKC inhibitors and enhanced by the PKC stimulator tetradecanoyl phorbol acetate (TPA) [26]. Indeed, staurosporine (an inhibitor of PKC, but active also on PKA and tyrosine kinases), calphostin C (a specific PKC inhibitor) and a monoclonal antibody that recognizes all isozymes of PKC all have an inhibitory effect on Golgi fragmentation in vitro; conversely, TPA stimulates this process. Interestingly, these effects do not require ATP, suggesting that the PKC action is independent of its kinase activity [26]. Another known ATP-independent PKC action is the activation of PLD; the regulatory domain of PKCα is sufficient to cause stimulation of PLD, suggesting that a DAG/TPA-activated PKC interacts directly with PLD, activating it by an allosteric mechanism [113, 114]. A hypothesis proposed by Sabatini and colleagues is that during coat assembly, PKC activates PLD, which then promotes Golgi fragmentation by remodelling the phospholipid bilayer and severing connections between the transport intermediates and the donor membranes [26]. So far, however, there is no direct evidence for an involvement of PKC. Also, as a note of caution in this context, one should note that other proteins, such as the chimaerins, are sensitive to activators and inhibitors of PKC [115–117].

PKC is also an activator of PKD [reviewed in ref. 118], itself a member of the PKC family that is also known as PKCμ, which has been localized in the Golgi and proposed to play a role in the formation of post-Golgi transport intermediates [119, 120]. Thus, PKD can be activated by receptor-induced activation of PLC, followed by the formation of DAG and activation of PKCε or PKCη, which can directly (or indirectly) phosphorylate the activation loop of PKD1 [118, 121]. Alternatively, PKD activation at the Golgi can be mediated by the GTP-binding (G) protein $\beta\gamma$ dimer, which has previously been shown to induce the fragmentation of the Golgi complex [122]. Thus, in permeabilized NRK cells, the addition of free G$\beta\gamma$ is sufficient to increase Golgi fragmentation, an effect that is inhibited by the addition of the inactive GDP-bound Gα, which causes reassociation of the heterotrimer. Addition of active, GTPγS-bound Gα also has no effect on the Golgi, supporting the idea that fragmentation is due to the $\beta\gamma$ subunit [122]. G proteins are also targets of the Golgi-fragmenting toxin ilimaquinone, which has been proposed to act via the release of the free $\beta\gamma$ subunit which, in turn, interacts directly with the PKD PH domain, thus activating a downstream pathway [27].

Expression of a kinase-inactive form of PKD (PKD-K618N) causes tubulation of the TGN. These tubules contain cargo, but do not detach from the TGN, confirming the hampered Golgi tubule fission and transport to the plasma membrane following PKD inactivation [120]. Wild-type PKD competes with the tubulation activity of the PKD-K618N and restores fragmentation, showing that TGN tubulation/fragmentation correlates with the state of activity of PKD. Thus, unlike other intracellular transport events, protein transport from the TGN to the plasma membrane requires PKD activity [120]. PKD might intervene in the regulation of the fission of Golgi membranes; overactivation of PKD (by ilimaquinone and $\beta\gamma$ subunits) converts Golgi stacks into fragments via the activation of the fission machinery, while inactivation of PKD causes tubulation due to a defect in membrane fission [120].

PKD has also been shown to bind DAG via its first cysteine-rich domain (C1a); this binding is necessary for its recruitment to the TGN [123, 124], where PKD-K618N predominantly localizes [118, 123]. The downstream interactors of PKD are still unknown. Good candidates could be the lipid kinases, since PKD has been shown to associate with both PI 4-kinase and PI4P 5-kinase activities; however, the specific interacting enzyme isoforms remain to be defined [125].

PI transfer proteins

There are two classes of PITPs, class I (which includes mammalian PITPα and β) and class II (which includes the yeast Sec14p); these bind PI with higher affinity than PC [126, 127]. The PITPs bring PI to the PI pools participating in the synthesis of PIP_2, which then serves as the

PLC substrate to form inositol 1,4,5-trisphosphate and DAG [128]. DAG acts as signalling lipid and is capable of rapid flip-flop between the leaflets of the bilayer, thus potentially affecting membrane curvature (see below).

Sec14p, the yeast analogue of mammalian PITP, is essential for protein transport from the TGN [129, 130]. In yeast, this protein is known to regulate both PI and PC synthesis by promoting PI4P and PIP_2 generation, and by inhibiting PC synthesis via the CDP-choline pathway (a potent consumer of DAG), respectively [127]. Yeast strains expressing a temperature-sensitive mutant of Sec14p are not able to export cargo from the late Golgi compartment at the non-permissive temperature [126]. The mechanisms leading to the secretion block have been reviewed recently and are beyond the scope of this review [126, 127, 131]. Although not yet fully elucidated, they point to a role of several lipids, including DAG, PA and PIP_2, as critical regulators of Sec14p-dependent Golgi secretory function [127]. However, these studies have not clarified whether the secretion block in yeast is due to inhibition of carrier budding or fission.

PITP has also been implicated in fission in animal cells in more than one independent line of research. In a cell-free system derived from a neuroendocrine cell line, PITP is a cytosolic factor that induces the formation of constitutive secretory vesicles and immature secretory granules from the TGN [132]. Sec14p is able to substitute for mammalian PITP in secretory vesicle formation; this, together with the use of PIP_2 inhibitors, has led to the proposal that this PITP effect could be related to PIP_2 formation [132, 133].

Sabatini and coworkers have shown that PITP is involved in the scission of COPI-coated buds from the trans-Golgi by a mechanism that is nucleotide independent and regulated by cytosolic components [28]. In the PI-bound form, Sec14p is capable of sustaining the scission reaction, which does not occur with PC-bound Sec14p [28]. The vesiculation caused by PI-Sec14p is not affected by PI alone since PI-containing liposomes do not cause vesiculation. Furthermore, vesicle scission does not require the conversion of PI into its phosphorylated derivatives since vesicle generation can be supported by non-hydrolysable GTP analogues in the nearly complete absence of ATP in this system [28]. Thus, the role of PITP could be in the remodelling of the phospholipid bilayer in the vicinity of a coated bud that results from replacing a resident phospholipid with PI. This PI could serve as a substrate for local phospholipases to form PA or DAG.

In an independent line of research, Howell and collaborators have also indicated a role for PITPs on Golgi membranes, demonstrating a synergistic activity of PITPs and the p62-complex-associated PI 3-kinase [134]. In a cell-free system, the formation of vesicles from the trans-Golgi compartment depends on the presence of cytosol and, more specifically, of active PITP. Moreover, antibodies against both PITPα and β inhibit vesicle formation in a concentration-dependent manner. In the presence of limiting cytosol, the addition of PITP allows a partial reconstitution of the vesiculation [134]. The p62-complex-associated PI 3-kinase was identified as the cofactor needed for the complete reconstitution of the machinery that leads to vesicle formation; in line with this, increased PI 3-phosphate production was also detected [134]. The model that originates from these data proposes that PITP presents PI (the substrate) to a PI-specific kinase that, when stimulated, generates a localized pool of PI 3-phosphate, which in turn participates in the regulation of cargo sorting and vesicle formation [134].

In summary, a complex body of evidence implicates PITP in membrane fission at the TGN. The precise mechanism(s) of action of this protein, however, remain to be clarified.

Conclusions

An interpretation of the pathways reported above is that membrane fission requires protein and lipid machineries; these work in a coordinated fashion to induce changes in the bilayer organization that favours fission. Different fissioning molecules appear to converge on key lipid species, such as PA and DAG (see above and fig. 4). We have already discussed how the shape of PA and thus the curvature of PA-containing bilayers depend on their environmental conditions, such as pH and calcium concentration (see above). In particular, in the Golgi complex, which contains millimolar calcium [135], PA should be present in the inner leaflet of the Golgi bilayer mostly as a cone. In contrast, like all lysolipids, LPA has a stable inverted-cone shape [54, 136]. An additional relevant characteristic of PA is its rapid dephosphorylation to DAG, a strongly conical component of the bilayer [54, 136, 137] which, due to its small and uncharged headgroup, can spontaneously flip-flop across membranes. This mechanism can affect the composition and curvature of both the leaflets in the bilayer no matter where the conversion of PA to DAG actually occurs. In the case of CtBP3/BARS, the DAG formed at the cytosolic leaflet could thus change the curvature in the lumen of the Golgi tubules [23].

In line with these types of modification, we hypothesize that an acyl-transferase activity involved in fission could be correlated with the potential build-up of a lipid microdomain enriched in LPA, PA and DAG, which, via the rapid interconversions among these three species, can facilitate the formation of highly curved fission intermediates, and complete fission through coordinated changes in local membrane curvature (fig. 4). These lipid modifications can be thought of as lipid-driven machines that, together with specific protein complexes, take part in membrane fission.

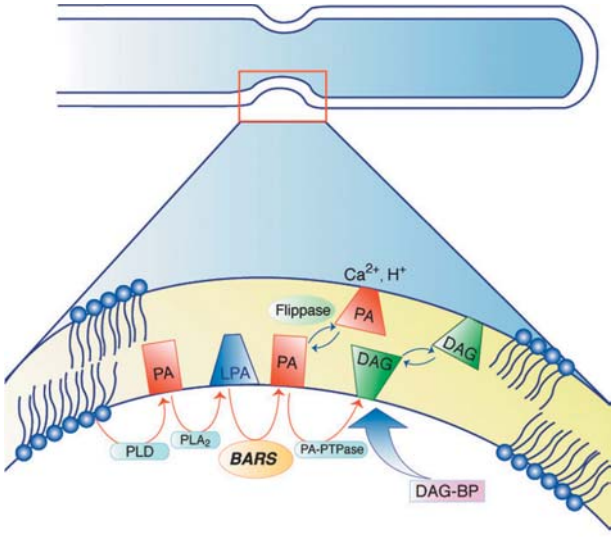

Figure 4. Schematic representation of the lipid metabolism proposed to be implicated in the modification of membrane curvature at the fission site. PA is generated on the cytosolic leaflet from LPA through the action of CtBP3/BARS. LPA can derive from the sequential action of PLD and PLA_2 acting on the phospholipids. PA can also be generated from phospholipids by the action of PLD. PA can then be brought to the lumenal leaflet by a flippase activity. Once on the lumenal leaflet of the bilayer, PA can acquire a conical shape at the millimolar free calcium concentration (characteristic of the Golgi complex [135]) and acidic pH. Alternatively, PA can be converted into DAG by a PA-phosphatase (PA-PTPase) activity. DAG is able to flip spontaneously to the lumenal side of the bilayer. Membrane domains enriched in DAG could also recruit DAG-binding proteins (DAG-BP), such as PKC and PKD. Other pathways leading to the formation of these lipids (such as the PLC-dependent formation of DAG [128]) are not indicated for simplicity. See text for details.

An obvious question that arises relates to the specific lipid fission machinery at the Golgi and in other membrane compartments. First, information on the biological significance of the different fission machineries is scarce. We do not know whether these molecules cooperate to affect fission, or whether they represent functionally similar machineries that act independently, in different cell compartments; whether different machineries act to release specific transport intermediates is also not known. We believe that the proteins with fissioning activity identified so far just begin to delineate a molecular mechanism that is certainly more complex, and should involve additional interacting proteins/enzymes. For example, phospholipases could be crucial in generating the local enrichment in LPA on which CtBP3/BARS or other acyl transferases can act, whereas PA phosphatases would facilitate the rapid interconversion of PA and DAG. Other lipid-binding proteins, as discussed above for PITP, could also be crucial in creating discrete lipid domains in which fission would be more likely to occur.

A simplified model could be that in different membrane compartments, proteins able to bring about the relevant changes in lipid composition (DAG formation, for example) and curvature (as induced by flippase activities) lead to the same membrane transformation, i.e. fission. CtBP3/BARS in the Golgi and endophilins in endocytosis are examples of totally unrelated proteins that are able to produce identical membrane transformation [23, 38]. Thus, if we consider DAG as the crucial molecule, we would expect protein complexes producing the PLC-dependent hydrolysis of PIP_2 to have the same effect at the membrane level as proteins characterized by the PLD-dependent hydrolysis of PC (plus a phosphatase activity). A still missing element in this scheme is the recognition module that would determine where the proteinaceous-fissioning complex would localize to activate the lipid machineries. Potentially, the initial recognition of an LPA molecule by a specific protein of the fissioning complex would then catalyze the formation of additional LPA by interacting enzymes (in the complex), and thus constitute the fissioning lipid domain, which would rapidly become the 'lipid-fissioning machine'. This would be in line with the observations that CtBP3/BARS [unpublished data] and endophilin [38] are both able to bind LPA.

These active and coordinated interactions among and between proteins and lipids at specific membrane sites remain to be demonstrated; however, we like to speculate that rather than requiring specific protein structures, membrane fission requires specific enzymatic activities affecting membrane lipid dynamics.

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1 Huttner W. B. and Schmidt A. (2000) Lipids, lipid modification and lipid-protein interaction in membrane budding and fission – insights from the roles of endophilin A1 and synaptophysin in synaptic vesicle endocytosis. Curr. Opin. Neurobiol. **10:** 543–551
2 Pelham H. R. (2001) SNAREs and the specificity of membrane fusion. Trends Cell Biol. **11:** 99–101
3 Stegmann T. (2000) Membrane fusion mechanisms: the influenza hemagglutinin paradigm and its implications for intracellular fusion. Traffic **1:** 598–604
4 Burger K. N. J. (1998) Morphology of membrane fusion. Curr. Top. Membr. **44:** 403–445
5 Burger K. N. (2000) Greasing membrane fusion and fission machineries. Traffic **1:** 605–613
6 Chernomordik L., Kozlov M. M., Melikyan G. B., Abidor I. G., Markin V. S. and Chizmadzhev Y. A. (1985) The shape of lipid molecules and monolayer membrane fusion. Biochim. Biophys. Acta **812:** 643–655
7 Kozlov M. M., Leikin S. L., Chernomordik L. V., Markin V. S. and Chizmadzhev Y. A. (1989) Stalk mechanism of vesicle fu-

sion: intermixing of aqueous contents. Eur. Biophys. J. **17:** 121–129
8 Kozlov M. M. and Chernomordik L. V. (2002) The protein coat in membrane fusion: lessons from fission. Traffic **3:** 256–267
9 Huttner W. B. and Zimmerberg J. (2001) Implications of lipid microdomains for membrane curvature, budding and fission. Curr. Opin. Cell Biol. **13:** 478–484
10 Chen Y. A. and Scheller R. H. (2001) SNARE-mediated membrane fusion. Nat. Rev. Mol. Cell Biol. **2:** 98–106
11 Yoon Y. and McNiven M. A. (2001) Mitochondrial division: new partners in membrane pinching. Curr. Biol. **11:** R67–R70
12 Rambourg A. and Clermont Y. (1997) Three-dimensional structure of the Golgi apparatus in mammalian cells. In: The Golgi Apparatus, pp. 37–60, Berger E. G. and Roth J. (eds), Birkhäuser, Basel
13 Sciaky N., Presley J., Smith C., Zaal K. J., Cole N., Moreira J. E. et al. (1997) Golgi tubule traffic and the effects of brefeldin A visualized in living cells. J. Cell Biol. **139:** 1137–1155
14 Presley J. F., Smith C., Hirschberg K., Miller C., Cole N. B., Zaal K. J. et al. (1998) Golgi membrane dynamics. Mol. Biol. Cell **9:** 1617–1626
15 Hirschberg K., Miller C. M., Ellenberg J., Presley J. F., Siggia E. D., Phair R. D. et al. (1998) Kinetic analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells. J. Cell Biol. **143:** 1485–1503
16 Nakata T., Terada S. and Hirokawa N. (1998) Visualization of the dynamics of synaptic vesicle and plasma membrane proteins in living axons. J. Cell Biol. **140:** 659–674
17 Toomre D., Keller P., White J., Olivo J. C. and Simons K. (1999) Dual-color visualization of trans-Golgi network to plasma membrane traffic along microtubules in living cells. J. Cell Sci. **112:** 21–33
18 Hsu V. W., Yuan L. C., Nuchtern J. G., Lippincott-Schwartz J., Hammerling G. J. and Klausner R. D. (1991) A recycling pathway between the endoplasmic reticulum and the Golgi apparatus for retention of unassembled MHC class I molecules. Nature **352:** 441–444
19 Mironov A. A., Weidman P. and Luini A. (1997) Variations on the intracellular transport theme: maturing cisternae and trafficking tubules. J. Cell Biol. **138:** 481–484
20 Weidman P. J. (1995) Anterograde transport through the Golgi complex: do Golgi tubules hold the key? Trends Cell Biol. **5:** 302–305
21 Figueiredo P. de, Drecktrah D., Katzenellenbogen J. A., Strang M. and Brown W. J. (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
22 Figueiredo P. de, Polizotto R. S., Drecktrah D. and Brown W. J. (1999) Membrane tubule-mediated reassembly and maintenance of the Golgi complex is disrupted by phospholipase A2 antagonists. Mol. Biol. Cell **10:** 1763–1782
23 Weigert R., Silletta M. G., Spano S., Turacchio G., Cericola C., Colanzi A. et al. (1999) CtBP/BARS induces fission of Golgi membranes by acylating lysophosphatidic acid. Nature **402:** 429–433
24 Chen Y. G., Siddhanta A., Austin C. D., Hammond S. M., Sung T. C., Frohman M. A. et al. (1997) Phospholipase D stimulates release of nascent secretory vesicles from the trans-Golgi network. J. Cell Biol. **138:** 495–504
25 Ktistakis N. T., Brown H. A., Waters M. G., Sternweis P. C. and Roth M. G. (1996) Evidence that phospholipase D mediates ADP ribosylation factor-dependent formation of Golgi coated vesicles. J. Cell Biol. **134:** 295–306
26 Simon J. P., Ivanov I. E., Adesnik M. and Sabatini D. D. (1996) The production of post-Golgi vesicles requires a protein kinase C-like molecule, but not its phosphorylating activity. J. Cell Biol. **135:** 355–370
27 Jamora C., Yamanouye N., Van Lint J., Laudenslager J., Vandenheede J. R., Faulkner D. J. et al. (1999) G$\beta\gamma$-mediated regulation of Golgi organization is through the direct activation of protein kinase D. Cell **98:** 59–68
28 Simon J. P., Morimoto T., Bankaitis V. A., Gottlieb T. A., Ivanov I. E., Adesnik M. et al. (1998) An essential role for the phosphatidylinositol transfer protein in the scission of coatomer-coated vesicles from the trans-Golgi network. Proc. Natl. Acad. Sci. USA **95:** 11181–11186
29 Farsad K., Ringstad N., Takei K., Floyd S. R., Rose K. and De Camilli P. (2001) Generation of high curvature membranes mediated by direct endophilin bilayer interactions. J. Cell Biol. **155:** 193–200
30 Cao H., Thompson H. M., Krueger E. W. and McNiven M. A. (2000) Disruption of Golgi structure and function in mammalian cells expressing a mutant dynamin. J. Cell Sci. **113:** 1993–2002
31 De Matteis M. A., Godi A. and Corda D. (2002) The phosphoinositides and the Golgi Complex. Curr. Opin. Cell Biol. **14:** 434–447
32 Kent C. and Carman G. M. (1999) Interactions among pathways for phosphatidylcholine metabolism, CTP synthesis and secretion through the Golgi apparatus. Trends Biochem. Sci. **24:** 146–150
33 Monaco M. E. and Gershengorn M. C. (1992) Subcellular organization of receptor-mediated phosphoinositide turnover. Endocr. Rev. **13:** 707–718
34 Meer G. van (1998) Lipids of the Golgi membrane. Trends Cell Biol. **8:** 29–33
35 Chernomordik L., Kozlov M. M. and Zimmerberg J. (1995) Lipids in biological membrane fusion. J. Membr. Biol. **146:** 1–14
36 Boggs J. M. (1987) Lipid intermolecular hydrogen bonding: influence on structural organization and membrane function. Biochim. Biophys. Acta **906:** 353–404
37 Demel R. A., Yin C. C., Lin B. Z. and Hauser H. (1992) Monolayer characteristics and thermal behaviour of phosphatidic acids. Chem. Phys. Lipids **60:** 209–223
38 Schmidt A., Wolde M., Thiele C., Fest W., Kratzin H., Podtelejnikov A. V. et al. (1999) Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. Nature **401:** 133–141
39 Spano S., Silletta M. G., Colanzi A., Alberti S., Fiucci G., Valente C. et al. (1999) Molecular cloning and functional characterization of brefeldin A-ADP-ribosylated substrate: a novel protein involved in the maintenance of the Golgi structure. J. Biol. Chem. **274:** 17705–17710
40 Mironov A., Colanzi A., Silletta M. G., Fiucci G., Flati S., Fusella A. et al. (1997) Role of NAD^+ and ADP-ribosylation in the maintenance of the Golgi structure. J. Cell Biol. **139:** 1109–1118
41 De Matteis M. A., Di Girolamo M., Colanzi A., Pallas M., Di Tullio G., McDonald L. J. et al. (1994) Stimulation of endogenous ADP-ribosylation by brefeldin A. Proc. Natl. Acad. Sci. USA **91:** 1114–1118
42 Di Girolamo M., Silletta M. G., De Matteis M. A., Braca A., Colanzi A., Pawlak D. et al. (1995) Evidence that the 50-kDa substrate of brefeldin A-dependent ADP-ribosylation binds GTP and is modulated by the G-protein beta gamma subunit complex. Proc. Natl. Acad. Sci. USA **92:** 7065–7069
43 Schaeper U., Boyd J. M., Verma S., Uhlmann E., Subramanian T. and Chinnadurai G. (1995) Molecular cloning and characterization of a cellular phosphoprotein that interacts with a conserved C-terminal domain of adenovirus E1A involved in negative modulation of oncogenic transformation. Proc. Natl. Acad. Sci. USA **92:** 10467–10471

44 Katsanis N. and Fisher E. M. (1998) A novel C-terminal binding protein (CtBP2) is closely related to CtBP1, an adenovirus E1A-binding protein, and maps to human chromosome 21q21.3. Genomics **47:** 294–299

45 Turner J. and Crossley M. (1998) Cloning and characterization of mCtBP2, a co-repressor that associates with basic Kruppel-like factor and other mammalian transcriptional regulators. EMBO J. **17:** 5129–5140

46 Chinnadurai G. (2002) CtBP, an unconventional transcriptional corepressor in development and oncogenesis. Mol. Cell **9:** 213–224

47 Turner J. and Crossley M. (2001) The CtBP family: enigmatic and enzymatic transcriptional co-repressors. Bioessays **23:** 683–690

48 Silletta M. G., Colanzi A., Weigert R., Di Girolamo M., Santone I., Fiucci G. et al. (1999) Role of brefeldin A-dependent ADP-ribosylation in the control of intracellular membrane transport. Mol. Cell. Biochem. **193:** 43–51

49 Weigert R., Colanzi A., Mironov A., Buccione R., Cericola C., Sciulli M. G. et al. (1997) Characterization of chemical inhibitors of brefeldin A-activated mono-ADP-ribosylation. J. Biol. Chem. **272:** 14200–14207

50 Hinshaw J. E. (2000) Dynamin and its role in membrane fission. Annu. Rev. Cell Dev. Biol. **16:** 483–519

51 McNiven M. A. (1998) Dynamin: a molecular motor with pinchase action. Cell **94:** 151–154

52 McNiven M. A., Cao H., Pitts K. R. and Yoon Y. (2000) The dynamin family of mechanoenzymes: pinching in new places. Trends Biochem. Sci. **25:** 115–120

53 Sever S., Damke H. and Schmid S. L. (2000) Garrotes, springs, ratchets, and whips: putting dynamin models to the test. Traffic **1:** 385–392

54 Cullis P. P., Fenske D. B. and Hope M. (1996) Physical properties and functional roles of lipids in membranes. In: Biochemistry of Lipids, Lipoproteins and Membranes, pp. 1–33, Vance D. E. and Vance J. E. (eds), Elsevier, Amsterdam

55 Epand R. M. (1988) Lipid Polymorphism and Membrane Properties, vol. 44, Academic Press, San Diego

56 Kruijff B. de, Cullis P. R., Verkleij A. J., Hope M. J., Van Echteld C. J. A., Taraschi T. F. et al. (1985) Modulation of lipid polymorphism by lipid-protein interactions. In: Progress in Protein-Lipid interactions, pp. 89–142, Watts A. and De Pont J. J. H. M. (eds), Elsevier, Amsterdam

57 Pfanner N., Orci L., Glick B. S., Amherdt M., Arden S. R., Malhotra V. et al. (1989) Fatty acyl-coenzyme A is required for budding of transport vesicles from Golgi cisternae. Cell **59:** 95–102

58 Kelly R. B. (1999) New twists for dynamin. Nat. Cell Biol. **1:** E8–E9

59 Stowell M. H., Marks B., Wigge P. and McMahon H. T. (1999) Nucleotide-dependent conformational changes in dynamin: evidence for a mechanochemical molecular spring. Nat. Cell Biol. **1:** 27–32

60 De Matteis M. A. and Morrow J. S. (2000) Spectrin tethers and mesh in the biosynthetic pathway. J. Cell Sci. **113:** 2331–2343

61 Fucini R. V., Chen J.-L., Sharma C., Kessels M. M. and Stamnes M. (2002) Golgi vesicle proteins are linked to the assembly of an actin complex defined by mAbp1. Mol. Biol. Cell **13:** 621–631

62 Sweitzer S. M. and Hinshaw J. E. (1998) Dynamin undergoes a GTP-dependent conformational change causing vesiculation. Cell **93:** 1021–1029

63 Takei K., Haucke V., Slepnev V., Farsad K., Salazar M., Chen H. et al. (1998) Generation of coated intermediates of clathrin-mediated endocytosis on protein-free liposomes. Cell **94:** 131–141

64 Brown H. A., Gutowski S., Moomaw C. R., Slaughter C. and Sternweis P. C. (1993) ADP-ribosylation factor, a small GTP-dependent regulatory protein, stimulates phospholipase D activity. Cell **75:** 1137–1144

65 Cockcroft S., Thomas G. M., Fensome A., Geny B., Cunningham E., Gout I. et al. (1994) Phospholipase D: a downstream effector of ARF in granulocytes. Science **263:** 523–526

66 Stutchfield J. and Cockcroft S. (1993) Correlation between secretion and phospholipase D activation in differentiated HL60 cells. Biochem. J. **293:** 649–655

67 Cockcroft S. (2001) Signalling roles of mammalian phospholipase D1 and D2. Cell. Mol. Life Sci. **58:** 1674–1687

68 Hammond S. M., Altshuller Y. M., Sung T. C., Rudge S. A., Rose K., Engebrecht J. et al. (1995) Human ADP-ribosylation factor-activated phosphatidylcholine-specific phospholipase D defines a new and highly conserved gene family. J. Biol. Chem. **270:** 29640–29643

69 Colley W. C., Sung T. C., Roll R., Jenco J., Hammond S. M., Altshuller Y. et al. (1997) Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization. Curr. Biol. **7:** 191–201

70 Park S. K., Provost J. J., Bae C. D., Ho W. T. and Exton J. H. (1997) Cloning and characterization of phospholipase D from rat brain. J. Biol. Chem. **272:** 29263–29271

71 Hammond S. M., Jenco J. M., Nakashima S., Cadwallader K., Gu Q., Cook S. et al. (1997) Characterization of two alternately spliced forms of phospholipase D1: activation of the purified enzymes by phosphatidylinositol 4,5-bisphosphate, ADP-ribosylation factor, and Rho family monomeric GTP-binding proteins and protein kinase C-alpha. J. Biol. Chem. **272:** 3860–3868

72 Exton J. H. (1999) Regulation of phospholipase D. Biochim. Biophys. Acta **1439:** 121–133

73 Sung T. C., Roper R. L., Zhang Y., Rudge S. A., Temel R., Hammond S. M. et al. (1997) Mutagenesis of phospholipase D defines a superfamily including a trans-Golgi viral protein required for poxvirus pathogenicity. EMBO J. **16:** 4519–4530

74 Chung J. K., Sekiya F., Kang H. S., Lee C., Han J. S., Kim S. R. et al. (1997) Synaptojanin inhibition of phospholipase D activity by hydrolysis of phosphatidylinositol 4,5-bisphosphate. J. Biol. Chem. **272:** 15980–15985

75 Lee C., Kang H. S., Chung J. K., Sekiya F., Kim J. R., Han J. S. et al. (1997) Inhibition of phospholipase D by clathrin assembly protein 3 (AP3). J. Biol. Chem. **272:** 15986–15992

76 Lee C., Kim S. R., Chung J. K., Frohman M. A., Kilimann M. W. and Rhee S. G. (2000) Inhibition of phospholipase D by amphiphysins. J. Biol. Chem. **275:** 18751–18758

77 Kim J. H., Lee S., Lee T. G., Hirata M., Suh P. G. and Ryu S. H. (2002) Phospholipase D2 directly interacts with aldolase via its PH domain. Biochemistry **41:** 3414–3421

78 Siddhanta A., Backer J. M. and 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

79 Liscovitch M. and Cantley L. C. (1995) Signal transduction and membrane traffic: the PITP/phosphoinositide connection. Cell **81:** 659–662

80 Jenkins G. H., Fisette P. L. and Anderson R. A. (1994) Type I phosphatidylinositol 4-phosphate 5-kinase isoforms are specifically stimulated by phosphatidic acid. J. Biol. Chem. **269:** 11547–11554

81 Liscovitch M., Chalifa V., Pertile P., Chen C. S. and Cantley L. C. (1994) Novel function of phosphatidylinositol 4,5-bisphosphate as a cofactor for brain membrane phospholipase D. J. Biol. Chem. **269:** 21403–21406

82 Jones D. R., Sanjuan M. A. and Merida I. (2000) Type Ialpha phosphatidylinositol 4-phosphate 5-kinase is a putative target for increased intracellular phosphatidic acid. FEBS Lett. **476:** 160–165

83 Arneson L. S., Kunz J., Anderson R. A. and Traub L. M. (1999) Coupled inositide phosphorylation and phospholipase

D activation initiates clathrin-coat assembly on lysosomes. J. Biol. Chem. **274:** 17794–17805
84 Jones D., Morgan C. and Cockcroft S. (1999) Phospholipase D and membrane traffic: potential roles in regulated exocytosis, membrane delivery and vesicle budding. Biochim. Biophys. Acta **1439:** 229–244
85 Kuai J., Boman A. L., Arnold R. S., Zhu X. and Kahn R. A. (2000) Effects of activated ADP-ribosylation factors on Golgi morphology require neither activation of phospholipase D1 nor recruitment of coatomer. J. Biol. Chem. **275:** 4022–4032
86 Brown F. D., Thompson N., Saqib K. M., Clark J. M., Powner D., Thompson N. T. et al. (1998) Phospholipase D1 localises to secretory granules and lysosomes and is plasma-membrane translocated on cellular stimulation. Curr. Biol. **8:** 835–838
87 Toda K., Nogami M., Murakami K., Kanaho Y. and Nakayama K. (1999) Colocalization of phospholipase D1 and GTP-binding-defective mutant of ADP-ribosylation factor 6 to endosomes and lysosomes. FEBS Lett. **442:** 221–225
88 Denmat-Ouisse L. A., Phebidias C., Honkavaara P., Robin P., Geny B., Min do S. et al. (2001) Regulation of constitutive protein transit by phospholipase D in HT29-cl19A cells. J. Biol. Chem. **276:** 48840–48846
89 Freyberg Z., Sweeney D., Siddhanta A., Bourgoin S., Frohman M. and Shields D. (2001) Intracellular localization of phospholipase D1 in mammalian cells. Mol. Biol. Cell **12:** 943–955
90 Damke H., Baba T., Warnock D. E. and Schmid S. L. (1994) Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. J. Cell Biol. **127:** 915–934
91 Herskovits J. S., Burgess C. C., Obar R. A. and Vallee R. B. (1993) Effects of mutant rat dynamin on endocytosis. J. Cell Biol. **122:** 565–578
92 Bliek A. M. van der and Meyerowitz E. M. (1991) Dynamin-like protein encoded by the *Drosophila* shibire gene associated with vesicular traffic. Nature **351:** 411–414
93 Takei K., McPherson P. S., Schmid S. L. and De Camilli P. (1995) Tubular membrane invaginations coated by dynamin rings are induced by GTP-gamma S in nerve terminals. Nature **374:** 186–190
94 Kosaka T. and Ikeda K. (1983) Reversible blockage of membrane retrieval and endocytosis in the garland cell of the temperature-sensitive mutant of *Drosophila melanogaster*, shibirets1. J. Cell Biol. **97:** 499–507
95 Henley J. R., Krueger E. W., Oswald B. J. and McNiven M. A. (1998) Dynamin-mediated internalization of caveolae. J. Cell Biol. **141:** 85–99
96 Henley J. R., Cao H. and McNiven M. A. (1999) Participation of dynamin in the biogenesis of cytoplasmic vesicles. FASEB J. **13 Suppl 2:** S243–S247
97 Gold E. S., Underhill D. M., Morrissette N. S., Guo J., McNiven M. A. and Aderem A. (1999) Dynamin 2 is required for phagocytosis in macrophages. J. Exp. Med. **190:** 1849–1856
98 Mayer A., Ivanov I. E., Gravotta D., Adesnik M. and Sabatini D. D. (1996) Cell-free reconstitution of the transport of viral glycoproteins from the TGN to the basolateral plasma membrane of MDCK cells. J. Cell Sci. **109:** 1667–1676
99 Jones S. M., Howell K. E., Henley J. R., Cao H. and McNiven M. A. (1998) Role of dynamin in the formation of transport vesicles from the trans-Golgi network. Science **279:** 573–577
100 Kreitzer G., Marmorstein A., Okamoto P., Vallee R. and Rodriguez-Boulan E. (2000) Kinesin and dynamin are required for post-Golgi transport of a plasma-membrane protein. Nat. Cell Biol. **2:** 125–127
101 Altschuler Y., Barbas S. M., Terlecky L. J., Tang K., Hardy S., Mostov K. E. et al. (1998) Redundant and distinct functions for dynamin-1 and dynamin-2 isoforms. J. Cell Biol. **143:** 1871–1881
102 Marks B., Stowell M. H., Vallis Y., Mills I. G., Gibson A., Hopkins C. R. et al. (2001) GTPase activity of dynamin and resulting conformation change are essential for endocytosis. Nature **410:** 231–235
103 Sever S., Damke H. and Schmid S. L. (2000) Dynamin:GTP controls the formation of constricted coated pits, the rate limiting step in clathrin-mediated endocytosis. J. Cell Biol. **150:** 1137–1148
104 Lee E. and De Camilli P. (2002) Dynamin at actin tails. Proc. Natl. Acad. Sci. USA **99:** 161–166
105 Orth J. D., Krueger E. W., Cao H. and McNiven M. A. (2002) The large GTPase dynamin regulates actin comet formation and movement in living cells. Proc. Natl. Acad. Sci. USA **99:** 167–172
106 Ringstad N., Nemoto Y. and De Camilli P. (1997) The SH3p4/Sh3p8/SH3p13 protein family: binding partners for synaptojanin and dynamin via a Grb2-like Src homology 3 domain. Proc. Natl. Acad. Sci. USA **94:** 8569–8574
107 Heuvel E. de, Bell A. W., Ramjaun A. R., Wong K., Sossin W. S. and McPherson P. S. (1997) Identification of the major synaptojanin-binding proteins in brain. J. Biol. Chem. **272:** 8710–8716
108 Micheva K. D., Kay B. K. and McPherson P. S. (1997) Synaptojanin forms two separate complexes in the nerve terminal: interactions with endophilin and amphiphysin. J. Biol. Chem. **272:** 27239–27245
109 Ringstad N., Gad H., Low P., Di Paolo G., Brodin L., Shupliakov O. et al. (1999) Endophilin/SH3p4 is required for the transition from early to late stages in clathrin-mediated synaptic vesicle endocytosis. Neuron **24:** 143–154
110 Gad H., Ringstad N., Low P., Kjaerulff O., Gustafsson J., Wenk M. et al. (2000) Fission and uncoating of synaptic clathrin-coated vesicles are perturbed by disruption of interactions with the SH3 domain of endophilin. Neuron **27:** 301–312
111 Huttner W. B. and Schmidt A. A. (2002) Membrane curvature: a case of endofeelin' em leader. Trends Cell Biol. **12:** 155–158
112 Simon J. P., Ivanov I. E., Shopsin B., Hersh D., Adesnik M. and Sabatini D. D. (1996) 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:** 16952–16961
113 Conricode K. M., Brewer K. A. and Exton J. H. (1992) Activation of phospholipase D by protein kinase C: evidence for a phosphorylation-independent mechanism. J. Biol. Chem. **267:** 7199–7202
114 Singer W. D., Brown H. A., Jiang X. and Sternweis P. C. (1996) Regulation of phospholipase D by protein kinase C is synergistic with ADP-ribosylation factor and independent of protein kinase activity. J. Biol. Chem. **271:** 4504–4510
115 Ahmed S., Lee J., Kozma R., Best A., Monfries C. and Lim L. (1993) A novel functional target for tumor-promoting phorbol esters and lysophosphatidic acid: the p21rac-GTPase activating protein n-chimaerin. J. Biol. Chem. **268:** 10709–10712
116 Ahmed S., Kozma R., Monfries C., Hall C., Lim H. H., Smith P. et al. (1990) Human brain n-chimaerin cDNA encodes a novel phorbol ester receptor. Biochem. J. **272:** 767–773
117 Kazanietz M. G. (2002) Novel 'nonkinase' phorbol ester receptors: the C1 domain connection. Mol. Pharmacol. **61:** 759–767
118 Van Lint J., Rykx A., Maeda Y., Vantus T., Sturany S., Malhotra V. et al. (2002) Protein kinase D: an intracellular traffic regulator on the move. Trends Cell Biol. **12:** 193–200
119 Prestle J., Pfizenmaier K., Brenner J. and Johannes F. J. (1996) Protein kinase C mu is located at the Golgi compartment. J. Cell Biol. **134:** 1401–1410
120 Liljedahl M., Maeda Y., Colanzi A., Ayala I., Van Lint J. and Malhotra V. (2001) Protein kinase D regulates the fission of

cell surface destined transport carriers from the trans-Golgi network. Cell **104:** 409–420
121 Van Lint J. V., Sinnett-Smith J. and Rozengurt E. (1995) Expression and characterization of PKD, a phorbol ester and diacylglycerol-stimulated serine protein kinase. J. Biol. Chem. **270:** 1455–1461
122 Jamora C., Takizawa P. A., Zaarour R. F., Denesvre C., Faulkner D. J. and Malhotra V. (1997) Regulation of Golgi structure through heterotrimeric G proteins. Cell **91:** 617–626
123 Maeda Y., Beznoussenko G. V., Van Lint J., Mironov A. A. and Malhotra V. (2001) Recruitment of protein kinase D to the trans-Golgi network via the first cysteine-rich domain. EMBO J. **20:** 5982–5990
124 Baron C. L. and Malhotra V. (2002) Role of diacylglycerol in PKD recruitment to the TGN and protein transport to the plasma membrane. Science **295:** 325–328
125 Nishikawa K., Toker A., Wong K., Marignani P. A., Johannes F. J. and Cantley L. C. (1998) Association of protein kinase Cmu with type II phosphatidylinositol 4-kinase and type I phosphatidylinositol-4-phosphate 5-kinase. J. Biol. Chem. **273:** 23126–23133
126 Kearns B. G., Alb J. G. Jr and Bankaitis V. (1998) Phosphatidylinositol transfer proteins: the long and winding road to physiological function. Trends Cell Biol. **8:** 276–282
127 Li X., Xie Z. and Bankaitis V. A. (2000) Phosphatidylinositol/phosphatidylcholine transfer proteins in yeast. Biochim. Biophys. Acta **1486:** 55–71
128 Wiedemann C. and Cockcroft S. (1998) The role of phosphatidylinositol transfer proteins (PITPs) in intracellular signalling. Trends Endocrinol. Metab. **9:** 324–328
129 Bankaitis V. A., Malehorn D. E., Emr S. D. and Greene R. (1989) The *Saccharomyces cerevisiae* SEC14 gene encodes a cytosolic factor that is required for transport of secretory proteins from the yeast Golgi complex. J. Cell Biol. **108:** 1271–1281
130 Bankaitis V. A., Aitken J. R., Cleves A. E. and Dowhan W. (1990) An essential role for a phospholipid transfer protein in yeast Golgi function. Nature **347:** 561–562
131 Fang M., Rivas M. P. and Bankaitis V. A. (1998) The contribution of lipids and lipid metabolism to cellular functions of the Golgi complex. Biochim. Biophys. Acta **1404:** 85–100
132 Ohashi M., Jan de Vries K., Frank R., Snoek G., Bankaitis V., Wirtz K. et al. (1995) A role for phosphatidylinositol transfer protein in secretory vesicle formation. Nature **377:** 544–547
133 Tuscher O., Lorra C., Bouma B., Wirtz K. W. and Huttner W. B. (1997) Cooperativity of phosphatidylinositol transfer protein and phospholipase D in secretory vesicle formation from the TGN – phosphoinositides as a common denominator? FEBS Lett. **419:** 271–275
134 Jones S. M., Alb J. G. Jr, Phillips S. E., Bankaitis V. A. and Howell K. E. (1998) A phosphatidylinositol 3-kinase and phosphatidylinositol transfer protein act synergistically in formation of constitutive transport vesicles from the trans-Golgi network. J. Biol. Chem. **273:** 10349–10354
135 Pinton P., Pozzan T. and Rizzuto R. (1998) The Golgi apparatus is an inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store, with functional properties distinct from those of the endoplasmic reticulum. EMBO J. **17:** 5298–5308
136 Cullis P. R. and de Kruijff B. (1979) Lipid polymorphism and the functional roles of lipids in biological membranes. Biochim. Biophys. Acta **559:** 399–420
137 Kent C. (1995) Eukaryotic phospholipid biosynthesis. Annu. Rev. Biochem. **64:** 315–343