Lipid homoeostasis and Golgi secretory function

S. Lev

Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel

Abstract

The unique lipid composition of the Golgi membranes is critical for maintaining their structural and functional identity, and is regulated by local lipid metabolism, a variety of lipid-binding, -modifying, -sensing and -transfer proteins, and by selective lipid sorting mechanisms. A growing body of evidence suggests that certain lipids, such as phosphoinositides and diacylglycerol, regulate Golgi-mediated transport events. However, their exact role in this process, and the underlying mechanisms that maintain their critical levels in specific membrane domains of the Golgi apparatus, remain poorly understood. Nevertheless, recent advances have revealed key regulators of lipid homoeostasis in the Golgi complex and have demonstrated their role in Golgi secretory function.

Introduction

The endocytic and secretory organelles possess a unique lipid composition that effectively prevents their homogenization and maintains their distinct structural and functional identities [1]. The distinct segregation of lipids between subcellular organelles requires tight regulation of lipid transport, metabolism and modification [2]. Indeed, many lipid metabolic enzymes are exclusively localized in specific membrane compartments where they generate specific lipid species. These lipids can be transported to different membranous organelles either by vesicular or non-vesicular trafficking. Whereas vesicular trafficking may involve selective lipid sorting mechanisms, non-vesicular transport is mediated by lipid-transport proteins that transport certain lipids from a donor to an acceptor membrane [3,4]. A selective lipid sorting mechanism located at the Golgi complex ensures the unique segregation of certain lipids in other membrane compartments [5]. For example, the mammalian PM (plasma membrane) is rich in cholesterol and sphingolipids, whereas unsaturated PC (phosphatidylcholine) is found mainly in the ER (endoplasmic reticulum). To maintain this distribution, unsaturated PC must be segregated from sphingolipids and cholesterol at the Golgi complex, and selectively included in retrograde carrier vesicles. On the other hand, cholesterol and SM (sphingomyelin) are selectively included in anterograde carrier vesicles or excluded from retrograde ones. This selective lipid sorting is also required for maintaining the unique lipid composition of the Golgi membranes [6].

The Golgi apparatus is not only involved in the processing, sorting and transport of lipids and cellular proteins to private subcellular destinations, but also functions as a membranous scaffold for diverse signalling, metabolic and cytoskeleton proteins. Many lipid-binding, -modifying, -sensing and -regulated proteins are localized in the Golgi complex and affect its lipid composition by integrating multiple signals from their protein partners, from specific lipid species, and from the membrane curvature. The association of these proteins with the Golgi is crucial in maintaining its unique lipid composition and consequently can affect its secretory function [7]. Among the different lipids, phosphoinositides and DAG (diacylglycerol) are generally accepted as key regulators of Golgi-mediated transport. They are actively synthesized in the Golgi complex and are tightly regulated in this organelle [1,8].

Lipid and vesicle biogenesis at the Golgi membranes

The transport of lipids and proteins from the Golgi apparatus is mediated by different transport carriers destined for appropriate subcellular locations. COPI (coatamer protein I) or clathrin-coated vesicles deliver cargo molecules to the ER, Golgi or the endosomal/lysosomal system respectively, whereas transport intermediates that detach from the TGN (trans-Golgi network) are targeted to the PM [9]. The formation of transport intermediates at the Golgi complex is mediated by sequential events that are initiated with bud formation from flat membranes, elongation of the buds into tubules, constriction of the tubules, and finally fission [10]. Motors and cytoskeletal proteins may provide the energy and mechanical forces necessary for deformation of the flat membrane into a bud and its subsequent elongation into a tubule, whereas fission requires local changes in lipid composition to induce membrane curvature. In fact, lipids are actively involved in all stages of vesicle formation, either by recruitment of cytosolic proteins to membranes, allosteric modulation of protein functions, and/or modification of the geometry and fluidity of the membrane bilayer [11]. Biological lipids can be classified according to their molecular
shapes: cylindrical ones that stabilize a flat bilayer such as PC, conical lipids with relatively small polar groups, and inverted cone lipids with large polar groups [12]. Local production of conical and/or inverted cone lipids, even in small amounts, markedly affects the membrane curvature, thereby influencing essentially all stages of vesicle biogenesis. For example, the sites of vesicle budding are often regions of high curvature. Therefore the cytoplasmic leaflet of the neck region of the bud might be accommodated with cone-shaped lipids such as DAG or PA (phosphatidic acid). On the other hand, inverted cone lipids, such as LPA (lyosphatidic acid), might be better accommodated in the luminal leaflet of the neck region. Likewise, fission, the pinching off of transport carriers from the Golgi complex, is achieved by extreme localized membrane curvature. In this process, the two luminal leaflets of the tubules have to come in close proximity and eventually merge. This process requires a strong negative curvature, which is facilitated by a high density of conical lipids in the outer leaflet [13]. The membrane curvature also affects the activity of certain Golgi proteins that regulate vesicle biogenesis. The activity of ArfGAP1 [Arf (ADP-ribosylation factor) GTPase-activating protein 1], which facilitates GTP hydrolysis in Arf1 and controls the dynamics of COPI coats, is dependent on the lipid packing of the bilayer, which is influenced by the shape of the lipid molecules and the curvature of the membrane [14]. Similarly, BAR (Bin-Amphiphysin-Rvs)-domain-containing proteins, which possess the potent amphipathic α-helix that induces spontaneous membrane curvature, might be involved in all stages of vesicle biogenesis, from the initial generation of curvature to vesicle fission [15]. Thus the shape of the lipid molecules and their local distribution within the membrane bilayer and between the bilayer leaflets are crucial for vesicle biogenesis at the Golgi membranes.

**DAG homoeostasis and Golgi secretory function**

**Metabolic pathways that regulate DAG levels at the Golgi complex**

DAG and PA are strongly conical components of the bilayer that can induce membrane bending and the formation of highly curved intermediates, thereby facilitating membrane budding, fusion and fission events [10]. It is generally accepted that PA has a short lifetime in intracellular membranes, and is rapidly converted into DAG by PAP (PA phosphohydrolase). PA is produced by PLD (phospholipase D), which hydrolyses PC, and by CtBP (C-terminal binding protein)/BARS (brefeldin A ADP-ribosylated substrate), an acyl transferase that transfers an acyl moiety from acyl-CoA to LPA. In contrast with PA, DAG has a longer lifetime and exists at constant levels within the Golgi apparatus. It is mainly produced by SM synthase, a Golgi enzyme that converts PC and ceramide into DAG and SM. DAG is also produced by PLC-mediated hydrolysis of PtdIns(4,5)P₂. However, owing to modest quantities of PtdIns(4,5)P₂ in the Golgi membranes, this pathway does not appreciably affect the level of DAG in this organelle. The production of DAG is balanced by metabolic pathways that regulate its consumption [16]. DAG and SM can be converted back into PC and ceramide by SM synthase. In addition, DAG can be phosphorylated by DAG kinase to produce PA, or it can be consumed via the CDP-choline pathway for PC biosynthesis. This pathway is mediated by three enzymes: choline kinase, which phosphorylates choline to produce phosphocholine, CCT (CTP:phosphocholine cytidylyltransferase), which produces CDP-choline from CTP and phosphocholine, and CPT (diphosphate-choline transferase), which produces PC from CDP-choline and DAG [17]. Notably, CPT is localized in the Golgi complex and is mainly regulated by the levels of its substrates, DAG and CDP-choline.

**DAG acts as a scaffold and/or activator of the fission machinery at the TGN**

The conical shape of DAG and its ability to flip-flop across membranes, because of its small and uncharged headgroup, may affect the composition and curvature of the bilayer, and thereby may facilitate membrane budding, fusion and fission [18]. However, DAG is also an important second messenger that recruits to membranes and/or activates specific proteins that function in membrane transport [19]. Previous studies have shown that PKD (protein kinase D) is recruited from the cytosol to the TGN by direct interaction of its N-terminal cysteine-rich domain with DAG [20]. Activation of PKD is required for the fission of transport carriers at the TGN, since overexpression of a catalytically inactive PKD mutant inhibits the detachment of cargo-containing tubules from the TGN and consequently the transport of specific cargo molecules to the cell surface [21]. The ability of PKD to regulate transport in a cargo-specific manner was demonstrated in polarized cells and, accordingly, it was proposed that activated PKD phosphorylates target proteins that drive carrier formation [22]. Activation of PKD at the Golgi complex is mediated by PKCη, which in turn is activated by heterotrimeric G-proteins. According to the proposed model, specific cargos destined for transport to the PM activate a G-protein-coupled receptor(s) at the TGN to release activated Gα and Gβγ subunits. Active Gβ1γ2 or Gβ3γ2 complexes would then lead to the production of DAG, the recruitment of PKCη and PKD to the TGN, and consequently the activation of PKCη [23]. Activated PKCη phosphorylates and activates PKD, which in turn phosphorylates downstream targets to bring about fission of TGN carriers. Although the precise downstream targets of PKD have not yet been identified, several candidates containing the PKD consensus phosphorylation motif, such as DAG kinase or CtBP3/BARS, were proposed to mediate PKD-dependent fission of transport carriers by local production of the fission-promoting lipid PA. More recently, it was shown that activated PKD phosphorylates PI4KIIIβ (phosphoinositide 4-kinase IIIβ) at the Golgi apparatus, and that this phosphorylation enhances the lipid kinase activity of PI4KIIIβ, thereby stimulating the production of PtdIns(4)P [24]. PtdIns(4)P

©2006 Biochemical Society
plays a crucial role in maintaining the Golgi structure and secretory function, which has been demonstrated both in mammals and in yeast [1]. Moreover, it was proposed that PtdIns(4)P is required for the recruitment and/or activation of certain proteins that govern transport carrier formation. Indeed, PtdIns(4)P mediates the recruitment of the clathrin adaptor complex AP-1 (adaptor protein 1) and FAPPs (four-phosphate-adaptor proteins) to the TGN and thereby may regulate protein transport from the TGN to endosomes and PM respectively [25,26].

The production of PtdIns(4)P at the Golgi complex of mammalian cells is mediated by three lipid kinases: PI4KIIα, PI4KIIβ and PI4KIIIβ. These kinases phosphorylate PI (phosphatidylinositol) at position 4 of the inositol head group to generate PtdIns(4)P. PI is probably presented to these lipid kinases by PITPs (PI-transfer proteins) [27]. Indeed, a direct interaction between PI4KIII and the Nir2 protein (also known as PITPnm), a peripheral Golgi protein containing a PI-transfer domain, was detected in mammalian cells. It was proposed that the PI4KIII–Nir2 interaction enhances the production of PtdIns(4)P at the Golgi complex [28]. Thus PITPs may link signalling pathways controlling DAG and phosphoinositide homeostasis at the Golgi complex.

The role of PITPs in regulating DAG levels in the Golgi complex

The involvement of PITPs in Golgi-mediated transport was first documented in yeast by demonstrating that Sec14p is essential for protein trafficking from the yeast–Golgi complex [29]. Sec14p is the major PITP in Saccharomyces cerevisiae, and, like other PITPs, catalyses the monomeric exchange of PI or PC between membrane bilayers in vitro [30]. It was previously shown that Sec14p regulates protein transport from the TGN by maintaining a critical DAG pool in this organelle [31]. In its PC-bound form, Sec14p negatively regulates PC biosynthesis by inhibiting the activity of CCT, the rate-limiting enzyme of the CDP-choline pathway, thereby preventing DAG consumption in the yeast–Golgi complex [32]. On the other hand, the PI-bound form promotes the synthesis of phosphoinositides, presumably to increase DAG production. Thus Sec14p maintains the DAG level in the Golgi by regulating its consumption and production pathways [33]. More recently, we have found that the human Nir2, a protein containing a PI-transfer domain, regulates the Golgi structure and secretory function by maintaining a critical pool of DAG in this organelle [34]. Nir2 belongs to a highly conserved family of proteins, and it is mainly localized in the Golgi complex in interphase cells [35,36]. Down-regulation of Nir2 by RNAi (RNA interference) markedly affects the structural integrity of the Golgi apparatus, and substantially inhibits protein transport from the TGN to the PM due to impaired fission of transport carriers at the TGN. We also found that depletion of Nir2 reduces the DAG level and concomitantly elevates the PC level in the Golgi complex. Strikingly, we found that Nir2 has no effect on the production of DAG from ceramide and PC, but instead regulates its consumption via the CDP-choline pathway for PC biosynthesis. Furthermore, we showed that the expression of wild-type Nir2 containing silent mutations within the RNAi-targeting sequence restores the DAG level in the Golgi as well as protein transport from the TGN of Nir2-depleted cells. However, mutants of Nir2 lacking an intact PI-transfer domain failed to restore the Golgi-secretory function, indicating that this domain is crucial for Nir2 function in the Golgi complex. Accordingly, we propose that Nir2 uses its PI-transfer domain to sense the PI/PC ratio at the TGN, and also to regulate local levels of DAG and to interact with PI4K at the Golgi, thus regulating the production of PC, thereby regulating the level of DAG. The reduced level of DAG in the Golgi of Nir2-depleted cells induces the formation of cargo-containing tubules that fail to detach from the TGN, consequently attenuating protein transport to the cell surface [34]. These results are consistent with the proposed function of DAG in membrane fission. Thus the ability of Nir2 to bind PI or PC via its PI-transfer domain, and also to regulate local levels of DAG and to interact with PI4K at the Golgi, may provide a point of convergence between phosphoinositide and DAG homeostasis at the Golgi complex (Scheme 1). Collectively, these results suggest a novel mechanism for maintaining a critical pool of DAG.
in the Golgi apparatus of mammalian cells, and demonstrate the interface between lipid homoeostasis and Golgi secretory function.

References


Received 21 November 2005