

Trans-Golgi proteins participate in the control of lipid droplet and chylomicron formation

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Synopsis

LDs (lipid droplets) carrying TAG (triacylglycerol) and cholesteryl esters are emerging as dynamic cellular organelles that are generated in nearly every cell. They play a key role in lipid and membrane homeostasis. Abnormal LD dynamics are associated with the pathophysiology of many metabolic diseases, such as obesity, diabetes, atherosclerosis, fatty liver and even cancer. Chylomicrons, stable droplets also consisting of TAG and cholesterol are generated in the intestinal epithelium to transport exogenous (dietary) lipids after meals from the small intestine to tissues for degradation. Defective chylomicron formation is responsible for inherited lipoprotein deficiencies, including abetalipoproteinemia, hypobetalipoproteinemia and chylomicron retention disease. These are disorders sharing characteristics such as fat malabsorption, low levels of circulating lipids and fat-soluble vitamins, failure to thrive in early childhood, ataxic neuropathy and visual impairment. Thus understanding the molecular mechanisms governing the dynamics of LDs and chylomicrons, namely, their biogenesis, growth, maintenance and degradation, will not only clarify their molecular role, but might also provide additional indications to treatment of metabolic diseases. In this review, we highlight the role of two small GTPases [ARFRP1 (ADP-ribosylation factor related protein 1) and ARL1 (ADP-ribosylation factor-like 1)] and their downstream targets acting on the *trans*-Golgi (Golgins and Rab proteins) on LD and chylomicron formation.

Key words: ADP-ribosylation factor related protein 1 (ARFRP1), ADP-ribosylation factor-like 1 (ARL1), Golgin, GTPase, Rab, *trans*-Golgi

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INTRODUCTION

The Golgi apparatus plays a central role on the one hand in the anterograde transport of newly synthesized proteins to the plasma membrane and to intracellular organelles, on the other hand for retrograde traffic from endocytic and recycling pathways [1,2]. A typical mammalian Golgi consists of a pile of stapled cisternae, the Golgi stacks that are interconnected by tubules to form the Golgi ribbon. At the entrance site of the Golgi, the *cis*-Golgi, vesicular tubular clusters form the intermediate between the ER (endoplasmic reticulum) and the Golgi stack. The exit site of the Golgi, the *trans*-Golgi is the major site of sorting proteins to distinct cellular locations [3]. For the correct post-translational modifications of cargos, for efficient sorting, and trafficking of proteins and membranes the Golgi apparatus has to maintain an

ordered structure. Recent work has now established that components of the Golgi membrane trafficking machinery also function in other processes such as cytoskeletal dynamics, organelle biogenesis, receptor signalling, apoptosis and mitosis [4–7]. Here we describe the function of the Golgi, in particular of a cascade of proteins acting on the *trans*-Golgi, for the formation of lipid-carrying organelles such as LDs (lipid droplets) and chylomicrons.

GOLGIN PROTEINS AND THEIR INTERACTION PARTNERS

For the coordination of the form and function of the Golgi, specifically of the *cis*- and *trans*-Golgi, Golgins play an important

Abbreviations used: ABHD5, abhydrolase domain containing 5; ADRP, adipocyte differentiation-related protein; Apo B, apolipoprotein B; ARFRP1, ADP-ribosylation factor related protein 1; ARL1, ADP-ribosylation factor-like 1; ATGL, adipose triacylglycerol lipase; COPII, coating protein II; DAG, diacylglycerol; GPCR, G-protein-coupled receptor; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; FABP, fatty-acid-binding protein; GRIP, Golgin-97, RanBP2 α , Imhp1 and p230/Golgin-245; HSL, hormone-sensitive lipase; LD, lipid droplet; MAG, monoacylglycerol; MGAT, monoacylglycerol acyltransferase; MTP, microsomal triacylglycerol transfer protein; NSF, N-ethylmaleimide-sensitive factor; PAT, perilipin, ADRP and Tip47; pCM, pre-chylomicrons; PCTV, pre-chylomicron transport vesicle; siRNA, small interfering RNA; SNAP-23, synaptosome-associated protein of 23 kDa; SNAP, soluble NSF attachment protein; SNARE, SNAP receptors; TAG, triacylglycerol; Tip47, 47 kDa tail-interacting protein; VAMP, vesicle-associated membrane protein; VLDL, very-low-density lipoprotein.

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role. They are peripheral membrane proteins associating with the cytoplasmic side of Golgi membranes to which they are recruited in a tightly regulated manner [8]. Golgins form a family of coiled-coil proteins defined by their localization at the Golgi complex. They exhibit the ability to recruit diverse effector molecules thereby coordinating various membrane-mediated processes. Golgins associating with the *trans*-Golgi comprise among others (e.g. BICD1 and BICD2) well-characterized GRIP (glutamate receptor-interacting protein) domain proteins (Golgin-245, Golgin-97, GCC88 and GCC185) that share aside from the abundance of predicted coiled-coil structure sequence homology within the GRIP domain. The GTPase ARL1 (ADP-ribosylation factor-like 1) coordinates the recruitment of Golgins from the cytosol to Golgi membranes via binding to the C-terminal GRIP domain in a GTP-dependent manner. Golgin-245 forms a dimer, and each GRIP domain interacts with one ARL1 that associates with the membranes. This membrane association requires the presence of another GTPase, ARFRP1 (ADP-ribosylation factor-related protein 1) because inhibition or deletion of ARFRP1 dissociates ARL1 from Golgi membranes even in its GTP-activated state [9–11], resulting in a cytosolic localization of Golgin-245 [11].

In addition to their binding to ARL1 Golgins interact with several other GTPases of the Rab family that are regulators of membrane trafficking via their coiled-coil domain [12]. *Drosophila* GRIP domain containing Golgins were shown to bind four Rab proteins: Golgin-245 binds Rab2 and Rab30, Golgin-97 binds Rab6 and Rab19, GCC88 binds Rab6 and Rab30, GCC185 binds Rab2 and Rab30 [12] (Figure 1). The interaction sites of Rab proteins are located at different positions in the coiled-coil region and do not appear to be essential for the recruitment of GRIP proteins to the Golgi membranes. Thus, it is believed that *trans*-Golgi Golgins project into the cytoplasm to capture membranes bearing specific Rab proteins, and thereby regulating trafficking events [12] and facilitating tethering of transport carriers to the *trans*-Golgi. It is also possible that this complex interaction defines the spatial relationship between *trans*-Golgi membranes and membranes of other compartments, e.g. Golgi stacks, perinuclear recycling endosomes or pre-Golgi compartments. It is quite possible that Golgins act as linkers between pathways and/or compartments [7].

SIMILAR STRUCTURES OF LDS AND CHYLOMICRONS, TWO DYNAMIC ORGANELLES

LDS that can be generated in every cell, share structural features with chylomicrons that are formed in intestinal epithelium and with lipoproteins that are generated in the liver and released into the plasma. They consist of a lipid core that is surrounded by proteins. LDS differ from chylomicrons and lipoproteins in their size and protein composition (Figure 2).

Lipid droplets

Lipids are stored in the cytosol in so-called LDS, dynamic organelles in which neutral lipids [consisting of TAG (triacylglycerol)

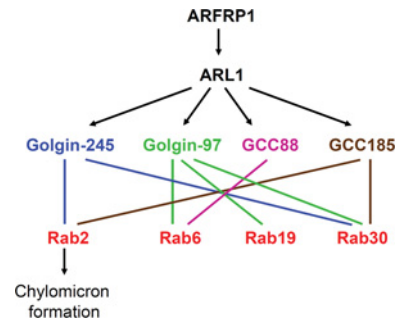
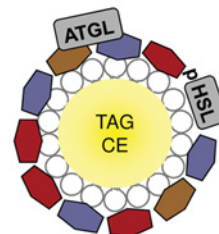


Figure 1 Overview of the ARFRP1-ARL1-Golgin-Rab cascade

Summary of interactions that have been identified in different systems. ARFRP1 recruits ARL1 to the Golgi. ARL1 binds to the GRIP domain of several Golgins (Golgin-245, Golgin-97, GCC88 and GCC185). In their coiled-coil domain Golgins possess binding sites for different Rab GTPases that can regulate trafficking.

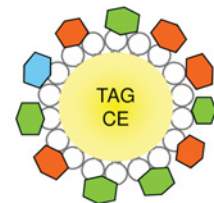
Lipid droplet in adipocytes



Size: 0.1–100 μm

- Perilipin/PLIN1
- ADRP/PLIN2
- Lipid droplet coating proteins attached to the LD in the ER
- Lipases (e.g. ATGL, HSL)

Chylomicron in enterocytes



Size: 75–450 nm

- ApoB48
- ApoA-I
- ApoA-IV

Figure 2 Similar organization of LDS and chylomicrons

LDS and chylomicrons consist of a core-containing TAG and CE (cholesterol esters) encircled by a lipid monolayer, which is surrounded by a protein layer (LD-coating proteins and lipases in LDS; apolipoproteins in chylomicrons).

and cholesterol esters] are surrounded by a monolayer of amphipathic lipids (phospholipids and cholesterol) [13]. Several proteins associate with the monolayer and participate in the turnover of lipids and in the formation and trafficking of the droplets. LDS are formed in microsomal membranes as primordial droplets with a diameter of 0.1–0.4 μm and increase in size by fusion [14]. The size and number of LDS in diverse cell types differ considerably. White adipocytes for instance store only one LD with a diameter of approximately 100 μm that fills nearly the entire cell. Many other cells are also able to generate and store small LDS with variable sizes between 0.1 and 0.2 μm. The LD size can change rapidly according to the metabolic state of the cell.

cultivation in the presence of fatty acids or induction of adipogenesis results in a rapid growth, whereas LDs shrink during starvation of cells [15].

A large number of studies indicate that the formation of LDs is initiated in the ER [16] where enzymes such as MGAT (monoacylglycerol acyltransferase) and DGAT (diacylglycerol acyltransferase) catalyse the synthesis of TAG in the interspace between the bilayer leaflets of the ER membrane. The synthesis of TAG starts by acylation of glycerol-3-phosphate with two fatty acids to give PA (phosphatidic acid). Subsequent dephosphorylation yields DAG (diacylglycerol), the precursor for synthesis of both phospholipids and, by a third acylation, TAG (Figure 3). Two enzymes DGAT1 [17] and DGAT2 [18] catalyse the last step of TAG synthesis. This is followed by a budding-out of the cytoplasm-oriented hemimembrane to form the fat-bearing LD. LDs are coated by a large number of proteins, e.g. the PAT proteins [perilipin/PLIN1, ADRP (adipocyte differentiation-related protein)/PLIN2 and Tip47 (47 kDa tail-interacting protein)/PLIN3] and lipases [ATGL (adipose triacylglycerol lipase) and HSL (hormone-sensitive lipase)]. The origin of the protein coat of LDs is not yet fully understood. Some LD-coating proteins were recently shown to be inserted into the ER membrane before localization to the LD occurs [19,20], whereas other proteins, such as the PAT-domain protein perilipin/PLIN1, are never found in association with the ER [21]. The details of how these proteins attach to or insert into the LD membrane have not yet been explored in any detail.

When primordial droplets are released from ER, they increase their volume either by lipid synthesis in close proximity to LDs mediated by DGAT2 [22] or by fusion of LDs [23]. By fluorescence imaging in living 3T3-L1 adipocytes and COS-7 cells Kuerschner et al. [22] demonstrated that not only TAG but also its direct metabolic precursor DAG accumulates on LDs. They found the essential enzyme DGAT2 to associate specifically with LDs where it catalyses the conversion of DAG to TAG. These data indicate that at least part of TAG biosynthesis occurs in the immediate vicinity of LDs and that these newly synthesized TAG molecules are directly inserted into the growing droplet. Furthermore, data of Boström et al. [23] indicated that LDs grow by fusion and that LDs are associated with proteins involved in fusion processes in the cell: NSF (*N*-ethylmaleimide-sensitive factor), α -SNAP (soluble NSF attachment protein) and the SNAREs (SNAP receptors) SNAP-23 (synaptosome-associated protein of 23 kDa), syntaxin-5 and VAMP4 (vesicle-associated membrane protein 4).

For their dynamics, growth and shrinkage, it is essential that LDs travel through the cell via cytoskeletal tracks. As stated above they have their origin at the ER when TAG synthesis is initiated. In the state of starvation, they are found in close proximity to mitochondria where fatty acids are metabolized via β -oxidation for energy production. A first step of TAG breakdown for instance induced either by cAMP-dependent signalling via GPCRs (G-protein-coupled receptors) of the Gs/Gi family or via GPCRs of the Gq family, through PLC (phospholipase C) calmodulin and PKC (protein kinase C) signals is the recruitment and activation of the lipases ATGL, HSL and MAG (monoacyl-

glycerol) lipase [24]. ATGL initiates TAG breakdown producing DAG, which is subsequently hydrolysed by HSL. In the basal state, HSL is unphosphorylated and remains in the cytosol, whereas ATGL, perilipin/PLIN1 and the ATGL coactivator ABHD5 (abhydrolase domain containing 5; also known as CGI-58) are located at the LD surface; perilipin/PLIN1 and ABHD5 form a complex [25]. Upon stimulation perilipin/PLIN1 and HSL are phosphorylated, resulting in the translocation of HSL to the LD and in the release of perilipin/PLIN1 from ABHD5, which then interacts with ATGL to induce its activity and TAG cleavage [26–29; for review see 30].

Genome-wide screening approaches that were performed to identify genes that participate in the regulation of fat storage and LD formation indicate that besides ER and mitochondria other organelles such as the Golgi or the proteasome also regulate LD morphology. By siRNA (small interfering RNA) screens performed in *Drosophila* cells [31–33] genes of the vesicular trafficking machinery, e.g. ARF/COPI (coating protein 1) that act in the retrograde transport of proteins and vesicles from Golgi to ER were identified. Their knockdown resulted in enlarged LDs presumably due to a defective targeting of the lipase ATGL (*brummer* in *Drosophila*) to the LD [33]. By characterizing the phenotype of the adipocyte-specific *Arfrp1* null mutant, we have recently shown that the *trans*-Golgi GTPase ARFRP1 plays a pivotal role in LD fusion and lipolysis (see below) [34].

Chylomicrons

Intestinal cells synthesize and secrete chylomicrons in the postprandial state after nutrient uptake. Synthesis of these particles is defective in abetalipoproteinaemia and chylomicron retention disease [35,36]. Chylomicrons are heterogeneous, lipid-rich particles ranging in diameter from 75 to 450 nm and consist like LDs of a core of TAG and cholesteryl ester surrounded by a monolayer of lipids (phospholipids and small amounts of non-esterified cholesterol) to which specialized proteins (apolipoproteins) assemble. Thus, the organization and presumably the generation of chylomicrons is very similar to that of LDs (Figure 2).

After a meal, dietary fat is digested into fatty acids and MAGs by gastric and pancreatic lipases, and neutralized into micelle or liposome form by bile acids to reach the intestinal lumen. The mechanism of the intestinal lipid uptake still remains elusive and controversial. It is assumed that this uptake occurs via two mechanisms in a concentration-dependent manner, a carrier-dependent process facilitated by the transporter CD36 at low concentrations and passive diffusion at high concentrations of lipids in the intestinal lumen [37]. The absorbed lipids (fatty acids and MAGs) bind to the intracellular FABP (fatty-acid-binding protein), and are delivered to the ER for re-esterification (Figure 3). However, the detailed mechanism by which the absorbed lipids travel to the ER is not clearly understood. TAG produced within the ER lumen bind to ApoB (apolipoprotein B) as a first step of chylomicron formation. The pre-chylomicron assembly is initiated by transcription of ApoB that is cotranslationally translocated into the ER lumen (Figure 3). Here the MTP (microsomal triacylglycerol transfer protein) facilitates the transport of

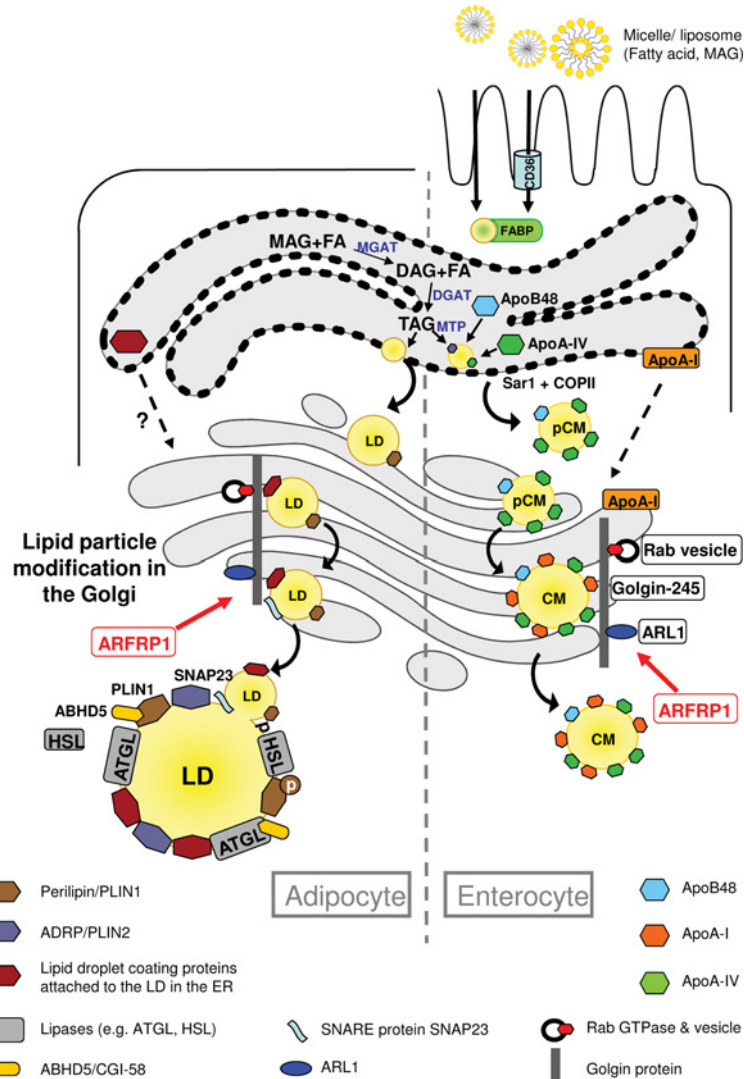


Figure 3 Proposed model of the action of the ARFRP1-ARL1-Golgin-Rab cascade on LD formation (left part) and chylomicron maturation (right part)

ARFRP1 is necessary to recruit ARL1 to the Golgi; ARL1 binds to the scaffolding protein Golgin-245, which itself interacts with Rab proteins. Left part of the cartoon indicates the steps required for LD fusion and the regulation of lipases in adipocytes. Small lipid-carrying particles are transported from the ER to the Golgi where more TAGs are loaded and additional proteins are attached. These small LDs can fuse with larger cytosolic LDs by SNARE-mediated processes. Fusion and degradation of LDs by lipolysis are regulated by the localization as well as the phosphorylation of LD-coating proteins (e.g. perilipin/PLIN1, ADRP/PLIN2) and lipases (e.g. HSL, ATGL). Right part of the cartoon demonstrates the chylomicron formation in the ER and the Golgi in enterocytes. Fatty acids and MAG from micelles or liposomes are taken up from the intestinal lumen by diffusion or transporters (e.g. CD36). In the cytosol their transport to the ER is mediated by binding transport proteins (e.g. FABP). In the ER resynthesized TAGs (generated by subsequent enzymatic esterification and transfer: MGAT/DGAT, MTP) are incorporated into ApoB48-containing pCM (pre-chylomicrons). Subsequently, ApoA-IV binds to the pCM, which are then released to the cis-Golgi. ApoA-I is attached to the chylomicrons within the Golgi. ApoA-I loaded chylomicrons are then transported through the Golgi, released on the trans-site and finally secreted into the lymph. We propose that the ARFRP1-ARL1-Golgin-Rab cascade is needed for an appropriate chylomicron assembly of ApoA-I and its transport through the Golgi. The role of the cascade for regulating sorting of proteins to the LD required for their fusion and for inhibiting lipolysis needs to be clarified.

ApoB-free LDs to ApoB during its translation. These droplets fuse with nascent ApoB-containing particles to form pre-chylomicrons [38]. Since there is one ApoB molecule per lipid particle, secretion of chylomicrons can be determined by measuring ApoB release.

ApoB circulates in two distinct forms: ApoB100 and ApoB48. Human ApoB100 is the product of a large mRNA (messenger RNA) encoding 4536 residues. ApoB48 arises from a C-to-U deamination of a single cytidine base in the nuclear ApoB transcript, introducing a translational stop codon, a process, referred

to as ApoB RNA editing [38]. In humans, ApoB100 is exclusively secreted by the liver in VLDL (very-low-density lipoprotein), ApoB48 is secreted by the intestine. In several other species such as mice, ApoB100 is generated and released in both liver and intestine [39,40].

In the next step of chylomicron formation, ApoA-IV is added to the surface of the ApoB48 containing particle allowing the formation of a larger particle. It is believed that ApoA-IV stabilizes and/or maintains the surface of pre-chylomicrons in the ER allowing additional core lipidation. The pre-chylomicrons exit the ER in a specialized transport vesicle, the PCTV (pre-chylomicron transport vesicle) to the *cis*-Golgi and this transport was shown to be the rate-limiting step in the intracellular transit of TAG across the enterocyte [41]. The ER to Golgi trafficking of pre-chylomicrons appears to be unique from that of other cargo, such as proteins. The budding is independent of COPII (coating protein II) proteins, but trafficking and fusion with the *cis*-Golgi are dependent on COPII proteins, Sar1 and Sec23/24, as well as a soluble SNARE fusion complex composed of VAMP7, syntaxin 5, Bet1 and vti1a [42]. In the Golgi, the pre-chylomicrons are further processed and transported to the basolateral membrane via a separate vesicular system for exocytosis into the intestinal lamina propria [43–45]. Little is known about post-ER maturation and transport process of chylomicrons in the Golgi. We have recently demonstrated that the ARFRP1–ARL1–Golgin–Rab cascade is needed for further lipidation of chylomicrons as well as for the assembly of ApoA-I to the chylomicron in the Golgi (see below) [46].

In general, chylomicrons function to transport dietary fat and fat-soluble vitamins via the lymph to the blood. The size heterogeneity of the secreted particles depends on the rate of fat absorption as well as on the type and amount of absorbed fat. The fatty acid composition of TAG present in chylomicrons reflects the composition of dietary fat. Differences in the fatty acid compositions are not the result of differential incorporation of dietary fatty acids into different lipids but are mainly due to different pools of lipids used for chylomicron assembly [46].

THE ARFRP1–ARL1–GOLGIN–RAB CASCADE

It has been suggested that the *trans*-Golgi Golgin proteins play important roles in maintaining the *trans*-Golgi structure and in regulating membrane trafficking [7]. Disruption of GCC185 in HeLa cells caused fragmentation of the Golgi apparatus [47]. In addition, the GRIP domains of both Golgin-245 and Golgin-97 associate with tubular membrane extensions of the *trans*-Golgi [48]. Moreover, Lock et al. [48] reported that Golgin-97 is involved in trafficking of E-cadherin containing vesicles out of the *trans*-Golgi. Golgin-97 was shown to play an important role in vesicular transport between the *trans*-Golgi and the endosome *in vitro* [49]. The fact that on the one hand ARL1 interacts with Golgins [50], and on the other hand its disruption also resulted in a block in transport from the endosome to the *trans*-Golgi in

HeLa cells, suggested that the ARL1–GRIP Golgins pathway is a key regulatory process for endosome-*trans*-Golgi traffic [49]. Presumably the GTPase ARFRP1, which associates with the *trans*-Golgi in its GTP-bound state [11] acts upstream of the ARL1–Golgin cascade. There it is responsible for recruiting ARL1 and its effectors (Golgin-245 and Golgin-97) to Golgi membranes [9–11].

The Rab family of small G proteins contains key regulators of membrane trafficking in eukaryotic cells. They play important roles in delivering cargos to correct destinations and controlling vesicle budding, as well as uncoating and mobility of vesicles [51]. The recent finding that the Golgin proteins bind to Rab proteins has increased the speculation that some Rab proteins locating at the Golgi (Rab1, Rab2, Rab6, Rab19 and Rab30) tether incoming transport carriers and other membrane-bound structures to the *trans*-Golgi via bridging by GRIP domain Golgin proteins (Figure 2) [12]. However, these interactions were found in yeast and up to now the binding between Rab proteins and Golgins has not been validated in mammalian cell lines. Furthermore, it has been reported that Rab2 is involved in the regulation of vesicle trafficking between the ER and the *cis*-Golgi [52], and Rab6 is within the Golgi apparatus [53,54]. Thus the question arises as to whether Rab2 and Rab6 exhibit a broader distribution within the Golgi apparatus and if they are also present at the *trans*-Golgi where they interact with Golgin proteins.

Our recent work indicated for the first time that the Golgin-245/Rab2 pathway plays a role in vesicular trafficking in the intestinal epithelium [46]. We have shown at least a functional link between ARFRP1, ARL1, Golgin-245 and Rab2 because each single knockdown resulted in a similar reduction in TAG release in an intestinal cell model (Caco-2 cells), suggesting that ARFRP1–ARL1–Golgin–Rab act in a cascade mediating the lipidation of pre-chylomicrons in the Golgi (see below). We therefore believe that ARFRP1 is necessary to translocate ARL1 and Golgin-245 to the Golgi, followed by recruiting Rab2-bound vesicles to this compartment (Figure 3). It can be speculated that Rab2 regulates the trafficking of lipid containing vesicles during the chylomicron formation in the intestine. When Rab2 is not recruited to the Golgi properly (e.g. in *Arfrp1* knockout models or after suppressing *Arfrp1*, *Arll1* or *Golgin*), lipid vesicles might not be delivered to the Golgi, thereby causing less lipidation of pre-chylomicrons in the Golgi [46]. However, currently, direct evidence for a physical association of Golgin proteins and Rab proteins that is needed for the regulation of vesicular trafficking or the molecular mechanism of vesicular traffic by this cascade is still lacking.

THE ROLE OF ARFRP1 IN LD FORMATION

The first hint that the Golgi apparatus as well as ARFRP1 and its downstream targets play a role in LD formation came from data obtained by characterizing the fat-specific *Arfrp1* null mutant (*Arfrp1^{ad-/-}*), which exhibited a lipodystrophic phenotype [34].

The mice showed markedly reduced amounts of brown adipose tissue and nearly absent white adipose tissue, which was also reflected by massively reduced plasma leptin levels. Histological analysis indicated a smaller size of LDs in brown adipose tissue. Possible explanations for this phenotype could involve a disturbed lipogenesis, alterations in LD storage as well as an elevated lipolysis [34].

Structural analysis of LDs in brown adipocytes of wild-type mice revealed membranous structures and small electron-dense particles in close proximity or attached to the surface of the LDs. These small electron-dense vesicles most likely present small lipid-carrying particles that deliver TAG to the larger storage LDs and consequently lead to the growth of LDs by fusion. The enlargement of LDs by fusion is mediated by members of the SNARE family [23], which are also involved in membrane fusion events. In wild-type adipose cells, the SNARE protein SNAP-23, which is described to be involved in LD fusion, was associated with small LDs, indicating fusion events and therefore LD growth [34]. In contrast, in adipose tissue of *Arfrp1^{ad-/-}* mice the membranous structures and electron-dense particles on the surface of the LDs were less abundant, which resulted in a smoother surface. In addition, SNAP-23 was predominantly located in the cytosol and at the plasma membrane in adipose tissue of *Arfrp1^{ad-/-}* mice. These findings suggest that the Golgi apparatus and ARFRP1 mediate LD growth via organizing the membranous structures at the LD, the transport of TAG containing particles through these membranous structures and/or finally the sorting of SNAP-23. Valdez et al. [55] described SNAP-23 to be associated with the *trans*-Golgi network in a complex with syntaxin 11 providing a link between the *trans*-Golgi apparatus, SNAP-23 sorting and LD fusion. As a further link, the assembly and specificity of the SNARE complexes are described to be regulated by Rab GTPases (reviewed in [56]) of which several are potential downstream targets of the *trans*-Golgi-associated cascade ARFRP1-ARL1-Golgin-Rab and could therefore influence LD fusion [45].

Besides growth, LD size is influenced by the rate of degradation by lipases. To protect LDs from lipolysis, they are surrounded by proteins of the PAT family (perilipin/PLIN1, adipophilin/PLIN2, TIP47/PLIN3) [57,58]. In times of an increased energy demand, LDs in adipose tissues are broken down to provide energy for the peripheral tissues. This is mainly mediated by two lipases, HSL and ATGL, that are tightly regulated as stated above. An altered *trans*-Golgi function as induced by the adipocyte-specific deletion of *Arfrp1* led to an elevated lipolysis in adipocytes marked by an increased amount of activated phosphorylated HSL and a more pronounced localization at the surface of LDs [34]. Therefore, it can be speculated that ARFRP1 and presumably its action on ARL1, Golgins and Rab proteins, modulates location of the LD associating proteins (PAT proteins and lipases) or one of their regulators, e.g. ABHD5. Under basal conditions, ABHD5 is located at the surface of LDs in association with perilipin, thereby inhibiting lipolysis. When perilipin is phosphorylated it changes its conformation, releases ABHD5, which interacts with ATGL leading to an activation of ATGL and lipolysis [59]. One possible explanation of how the *trans*-Golgi and ARFRP1 influence LD size might be that it is needed: (i)

for sorting of ABHD5 to the LD, (ii) for providing the interaction between ABHD5 and perilipin/PLIN1, (iii) or the release of ABHD5 from ATGL, thereby turning off lipolysis. Accordingly, the total amount as well as the association of the ATGL with LDs was enhanced in the adipose tissue of *Arfrp1^{ad-/-}* mice indicating that lipolysis was activated. The fact that siRNA induced knockdown of *Arfrp1* in 3T3-L1 adipocytes increased basal lipolysis and association of ATGL to LDs demonstrated that elevated lipolysis in *Arfrp1^{ad-/-}* mice is not a secondary effect but rather a direct consequence of ARFRP1 loss [34].

Besides the involvement of the *trans*-Golgi and ARFRP1 in the sorting of LD-associated proteins, it was already described by Guo et al. [31] that proteins involved in vesicular trafficking, ARF1/COPI, participate in LDs morphology and lipolysis. This proposes additional alternative pathways in which trafficking at the Golgi apparatus and proteins such as ARFRP1 could play so far unexplored roles for lipid storage.

In adipose tissue, the *trans*-Golgi and the GTPase ARFRP1 are essential for targeting of proteins to the surface of LD. The lack of ARFRP1 and its downstream partners at the *trans*-Golgi are responsible for the lipodystrophic phenotype of the fat-specific knockout mice by preventing the normal enlargement of LDs via fusion events and activating lipolysis.

THE ROLE OF ARFRP1 FOR CHYLOMICRON FORMATION

The impact of ARFRP1 on chylomicron formation was noticed during the characterization of the *Arfrp1* intestine-specific knockout mice (*Arfrp1^{vil-/-}*). These mice displayed severe growth retardation due to reduced fat absorption [46]. We could clearly demonstrate that the disruption of *Arfrp1* expression did not impair the uptake of fatty acids but caused a dramatic decrease in lipid release from the intestinal epithelium to the lymph and blood.

The first steps of the pre-chylomicron formation and maturation, such as the assembly of ApoB48 and lipid load in the ER, were not affected by the deletion of *Arfrp1*, since *Arfrp1^{vil-/-}* mice showed no reduction of ApoB48 release and normal MTP activity. Furthermore, localization of COPII, known to be required for the transfer of pre-chylomicron transport vesicles from the intestinal ER to the Golgi [60,61], was not altered in *Arfrp1^{vil-/-}* mice [46]. The fact that (i) deletion of *Arfrp1* significantly decreased TAG levels in the chylomicron fractions in the plasma of *Arfrp1^{vil-/-}* mice and that (ii) *Arfrp1* knockdown in Caco-2 cells suppressed TAG release, resulted in the conclusion that ARFRP1 controls the chylomicron lipidation within the Golgi. TAG transfer to chylomicrons is mediated by MTP, which is not exclusively active in the ER but also present in the Golgi [62,63]. Up to now it has been believed that MTP contributes only to a minor extent to the lipidation of chylomicrons within the Golgi [45] but our data clearly indicate that a final delivery of TAG to the chylomicrons occurs in the

Golgi and that several proteins at the *trans*-Golgi are needed for an appropriate maturation process of chylomicrons [46].

The second distinctive phenotype of *Arfrp1*^{vil-/-} mice was a dramatic decrease in ApoA-I release from the enterocytes. ApoA-I can be secreted from the intestine as a component of HDL or chylomicrons. Thereby, ApoA-I generated in the ER is transported to the Golgi separately from pre-chylomicron vesicles and is added within the Golgi to the chylomicron before the mature particle is secreted into the lymph (Figure 3) [45,62].

Interestingly, the marked reduction in ApoA-I release in *Arfrp1*^{vil-/-} mice was associated with a marked accumulation of ApoA-I at the Golgi apparatus in *Arfrp1*^{vil-/-} enterocytes, where it co-localized with Rab2. In control animals, Rab2 was predominantly located in the cytosol and only partially associated with membranes of the Golgi, whereas it accumulated at membranes of the Golgi apparatus in the enterocytes of *Arfrp1*^{vil-/-} mice. We speculate that deletion of *Arfrp1* disrupts the ARL1–Golgin–Rab2 cascade resulting in a constant cytosolic localization of ARL1 and Golgin proteins that does not allow Rab2 to be released from the Golgi. In other words, in enterocytes the ARFRP1–ARL1–Golgin–Rab2 cascade is required for the final lipidation of chylomicrons and the transport of ApoA-I to the chylomicrons in the Golgi to complete the chylomicron maturation. Interestingly, the fact that on the one hand *ApoA1*^{-/-} mice do not exhibit a reduced body weight due to severe fat malabsorption [64] and on the other hand that the suppression of *ApoA1* in Caco2 cells did not result in a decreased TAG release [46] demonstrated that an appropriate ApoA-I binding to the chylomicron is not essential or the rate-limiting step for the final chylomicron lipidation in the Golgi.

All so far known impairments of chylomicron formation like familial hypobetalipoproteinaemia or abetalipoproteinaemia [36,65] are caused by alterations in pre-chylomicron formation in the ER. It is clearly understood that chylomicrons need to be transferred to the Golgi for further maturation before they are released into the intestinal lamina propria, as defects of chylomicrons docking to the Golgi result in chylomicron retention disease [66,67]. The novel finding on the role of the Golgi, particularly of the *trans*-Golgi for the growth and maturation of chylomicrons, might indicate that other genetic defects acting on this compartment might exist resulting in malabsorption or chylomicron retention disease.

The role of ARFRP1 and its downstream partners clearly show the importance of the Golgi complex for the maturation and final lipidation of chylomicrons. They are also crucial steps for normal lipid absorption, as defects in these steps affect lipid release from the mucosa and whole-body lipid homeostasis.

FUTURE DIRECTION AND CONCLUDING REMARKS

Chylomicron formation and the dynamics of LDs play a pivotal role for the entire lipid metabolism. Both organelles exhibit sev-

eral similarities and much progress has been made in understanding the molecular steps involved in their generation and breakdown. As the present review shows, besides reactions that occur in the ER, different steps taking place at the Golgi apparatus participate in the proper organization and function of chylomicrons and LDs. Future studies on other tissues like the liver will clarify the impact of Golgi processes and the ARFRP1–ARL1–Golgin–Rab cascade for the generation and release of the third class of TAG-containing particles, the lipoproteins (e.g. VLDL), and what other binding partners of the cascade are needed for its proper function. Another focus should be to identify upstream signals that initiate the cascade and activate ARFRP1 to induce its association with the *trans*-Golgi.

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