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Review

# Glycolipid transfer proteins and membrane interaction

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ABSTRACT

The glycolipid transfer protein is found from animals and fungi to plants and red micro-alga. Some eukaryotes that do not encode the glucosylceramide synthase like the yeast *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* do neither produce glycolipid transfer like proteins. On the other hand yeast like *Eremothecium gossypii* that do synthesize glucosylceramide also express glycolipid transfer protein. Based on this novel genetic relationship it is not far fetched to assume that there must be a strong correlation between the synthesis of the glycolipid precursor and the glycolipid transfer protein. Because the glycolipid transfer protein is localized in the cytosol it is unlikely that it would participate in events associated with lipid rafts or caveolar structures, since they are found on the outer leaflet of the plasma membrane. Rather, GLTP is likely to be involved in events at the cytosolic side of the plasma membrane or the endoplasmic reticulum, maybe function as a reporter or sensor of glycolipid levels. A similar function has been proposed for other proteins with affinity for lipids like the oxysterol binding proteins and phosphatidylinositol transfer proteins that are thought to be able act as lipid sensors. Recent discoveries in the glycolipid transfer protein field are discussed.



**Contents**



## 1. Introduction

The glycolipid transfer protein (GLTP) is a 24 kDa protein that transfer glycolipids from one bilayer membrane to another *in vitro* [1]. The precise biological function is still unknown. However, its intracellular localization is cytosolic [2]. GLTP enhances only the transfer of glycolipids with a β-linked sugar residue to its ceramide or glycerolipid backbone. Both neutral and anionic glycolipids are transferred by GLTP but not phospholipids, sphingomyelin or neutral lipids. A glycolipid with an α-linked sugar is not a substrate, like the marine sponge glycolipid α-galactosylceramide (KRN7000) known to immunostimulatory and antitumor activity [3]. The presence of GLTP in the cell cytosol limits its potential role in cells involving glycolipids, since most of the known glycolipid metabolic and catabolic processes take place in subcellular compartments, such as in the Golgi apparatus, endoplasmic reticulum and the lysosomes [4–6]. The composition and properties of the membranes harboring the glycolipids are very important for the transfer activity of GLTP. GLTP senses whether the membrane is tightly or loosely packed [7,8]. It is also sensitive to the lipid packing nature, such as if the membrane is composed of sphingomyelin or phosphatidylcholine [9]. Insight into the biological role of GLTP is emerging. When GLTP was overexpressed in D6P2T rat Schwann cells it was indirectly shown that the transport of glucosylceramide from the Golgi complex to the plasma membrane was to some extent enhanced [10]. Further, when GLTP was knocked-

*Abbreviations:* GLTP, glycolipid transfer protein; FAPP2, four phosphoinositol 4-phosphate adaptor protein-2; PH, pleckstrin homology

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down in the same cells, the transport of glucosylceramide to the plasma membrane was not seen, in the presence of brefeldin A that fuses the Golgi stack to the ER [10]. Warnock and coworkers also showed in 1994 that when vesicular transport was inhibited with brefeldin A, glucosylceramide still reached the cell surface, and speculated this to be caused by GLTP [11].

## 2. GLTP and GLTP-like proteins

The human GLTP is encoded by a single-copy GLTP gene found in both chromosomes 11 and 12 [12]. The locus 11p15.1 gene is a transcriptionally inactive intronless silent gene exhibiting 94% homology with the full-length coding sequence, still it contains key amino acids involved in glycolipid recognition, but the gene product does not show glycolipid transfer activity. The gene encoded in locus 12q24.11 on the other hand has 5 exons and 4 introns and is highly conserved in mammals and other vertebrates [12]. This gene product is the same protein first discovered in membrane-free cytosolic extracts of bovine spleen, and was in 1980 called the cerebroside transfer protein by Raymond Metz and Norman Radin [13,14]. Later the protein was shown to be specific for glycolipids and was named the glycolipid transfer protein by Akira Abe et al. [15]. Since then, GLTP has been isolated from various mammalian sources [16–20]. Gene products with glycolipid transfer activity have also been found in other eukaryotes, for instance the HET-C2 from the fungus *Podospora anserina* [21], and AtGLTP1 from the plant *Arabidopsis thaliana* [22] are both glycolipid transporters *in vitro*. Their *in vivo* role is not well known. However, valuable information of the function of the *het* gene families gives clues to which processes GLTPs might be involved. The fungal *het* genes are involved in a complex set of heterokaryon-incompatibility and associated network of cellular machinery responsible for the acceptance or rejection of partners in parasexuality [23,24]. Recently we suggested that the role of the *A. thaliana* GLTP may be in directing glycolipid transport to specific areas of the plasma membrane, or play a role in maintenance of glycolipid membrane domains [22]. Also another *A. thaliana* protein called ACD11 (accelerated cell death 11) that has a similarity to GLTPs (about 30%) is able to accelerate the transfer of sphingosine, but not ceramide and galactosylceramide, between membranes [25]. The lethal recessive knockout of ACD11 shows activation of programmed cell death, and transgenic expression with human GLTP partially suppresses the phenotype of the *acd11* null mutant, resulting in a later onset of programmed cell death development [26]. Together these findings suggest that the biological role of GLTP could also be related to similar events in eukaryotic cells, such as cell–cell contact and cell growth and survival.

GLTPs from porcine, bovine and human contain 209 highly conserved amino acids and the proteins have no similar structural folds to other lipid transfer proteins [27–29]. The phosphoinositol 4-phosphate adaptor protein-2 (FAPP2) contains a GLTP domain connected to a pleckstrin homology domain (PH), and transfers glycolipids both *in vivo* and *in vitro* [10,30] (Fig. 1A). In addition, the 519 amino acid FAPP2 isoform has a C-terminal putative PDZ binding motif (DEVV) that is absent in the 507 amino acid FAPP2 isoform [30]. (Fig. 1B).

In a screening of PH domain-containing proteins the FAPPs were described as proteins that selectively binds to PtdIns(4)P with their PH domain [31]. FAPP1 (PLEKHA3) interacts strongly with PtdIns(4)P and is involved in the synthesis or breakdown of PtdIns(4)P in cells [32]. Initially FAPP2 (PLEKHA8) was described as an effector of Arf1 (ADP ribosylation factor) and PtdIns(4)P that is involved in trans-Golgi network to plasma membrane trafficking [33]. FAPP2 also has a crucial

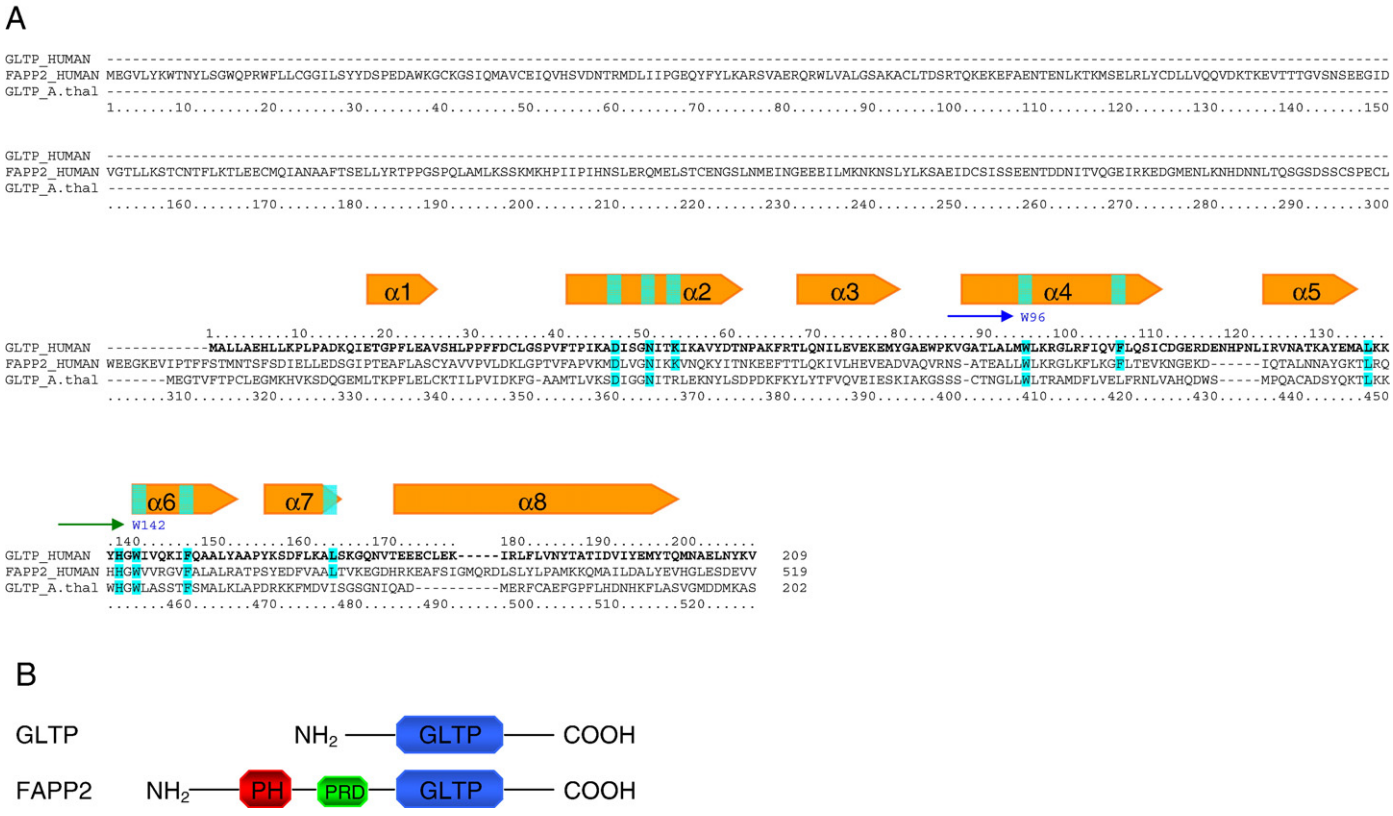


**Fig. 1.** (A) Alignment of sequences of human GLTP, FAPP2 and the *Arabidopsis thaliana* GLTP1. The eight alpha-helices in the human GLTP are depicted in orange, and the conserved amino acids important for the glycolipid binding activity shown in turquoise. Tryptophan 96 crucial for the glycolipid recognition and transfer activity of GLTP is conserved in FAPP2 as tryptophan 407 (blue arrow). The tryptophan at position 142, which is also conserved (green arrow), is likely to be involved in the membrane interaction. (B) Schematic presentation of mammalian proteins with a glycolipid transfer protein (GLTP) domain. FAPP2 has an N-terminus pleckstrin homology (PH) domain, a proline rich domain (PRD) and a GLTP-homology domain.

role in cargo delivery to the apical plasma membrane in polarized cells [34]. Recently, FAPP2 has been demonstrated to be a glycolipid transfer protein that is involved in the transport of glucosylceramide from its site of synthesis, the cytosolic leaflet of the Golgi complex, to its site of conversion into more complex glycosphingolipids, the later Golgi compartments [30]. There FAPP2 is crucial for the synthesis of more complex glycolipids, depending on the production of PtdIns(4)P and small GTPase Arf1 in the Golgi complex. Furthermore, FAPP2 takes part in the retrograde pathway of glucosylceramide transport from the Golgi complex to the ER [10].

## 3. Comparison of the structure of GLTP and GLTP-like proteins

The three dimensional structures of the known GLTPs from different species all reveal a double-layered all-alpha-helical conformation with a ligand-binding site consisting of a sugar recognition center and a hydrophobic channel specific for hydrocarbon chains [35–38] (Fig. 2). This unique GLTP fold differs considerably from that of other known lipid transfer protein structures. In general, lipid transfer protein structures and structures that bind lipids are dominated by β-sheet motifs, such as β-barrels, β-cups and β-grooves. Most lipid transfer proteins are also structurally stabilized by disulfide bridges between alpha-helical bundles, such as the saposins and non-specific lipid transfer proteins (nsLTP), phosphatidylglycerol-, ceramide-, fatty acid and plant lipid transfer proteins [39–42]. In comparison to GLTP lipid transfer proteins from plants are generally much smaller with molecular mass around 10–12 kDa [42].

The sugar, glucose in most glycosphingolipids and galactose in galactosylceramide and sulfatide, linked to ceramide is firmly anchored within the GLTP recognition center. The center is located in the beginning of the hydrophobic tunnel. The glycolipid is bound to specific amino acids by a network of hydrogen bonding and hydrophobic tethering interactions. The hydrogen bonding involves: aspartic acid 48, asparagine 52, and lysine 55 of alpha-helix 2, and tyrosine 207 near the C-terminus. Tryptophan 96 (W96) on alpha-helix 4 serves as a platform for the first sugar, and cannot be replaced by another amino acid. Tryptophan 96 forms a hydrogen bond between the OH-2 and OH-3 groups of the sugar and the D48 and N52 (Fig. 3A). Depending on the placement of the ligand acyl chains, the complexes formed by GLTP and a glycolipid can take tree different forms. (1) The apo-GLTP form has a closed hydrophobic tunnel. (2) A maximally expanded hydrophobic tunnel with both acyl chains inside, a “sphingosine-in” conformation. This conformation can be observed for instance with 18:1-lactosylceramide. (3) With longer acyl chains (24:1) only one chain is inside the hydrophobic tunnel and sphingosine is on the outside interacting with hydrophobic amino acids on the surface near the tunnel entrance [35–37]. This is also the likely form when a fluorescently labeled glycolipid, such as the anthrylvinyl- or BODIPY-glycolipids is bound to GLTP. The larger bulkier chain carrying the fluorophor would occupy a larger volume inside the tunnel forcing the sphingosine chain to be on the outside.

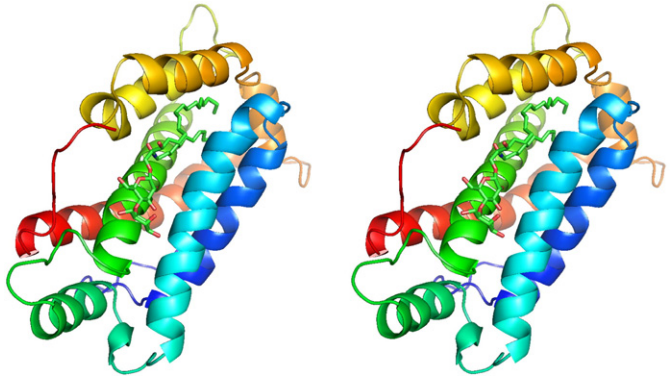

**Fig. 2.** Stereo view of the bovine GLTP (1TFJ) in a ribbon representation with lactosylceramide bound. The unique GLTP fold differs considerably from that of other known lipid transfer protein structures. GLTPs have a double-layered all-alpha-helical conformation with a ligand-binding site consisting of a sugar recognition center and a hydrophobic channel specific for hydrocarbon chains.

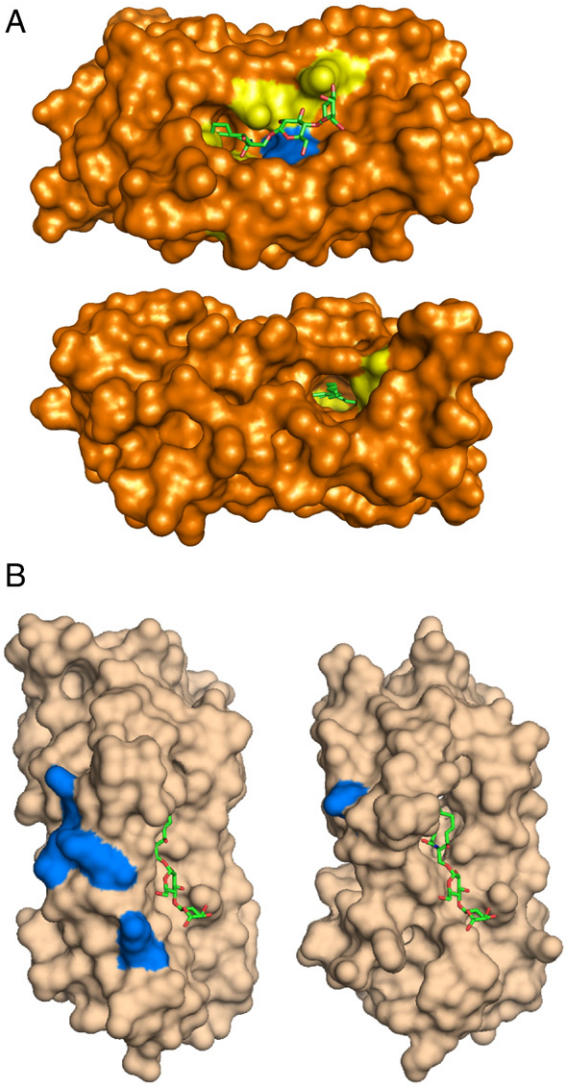


**Fig. 3.** (A) Surface structure of bovine GLTP with bound lactosylceramide. The amino acids within the sugar recognition centre are shown in yellow and tryptophan 96 in blue. In the 180° rotation (lower image) the bottom of the hydrophobic tunnel can be seen. (B) Surface properties of bovine GLTP (left) and *Podospora anserina* HET-C2 (model, right). The positively charged four lysines form a locally charged area on the surface of GLTP. Only one residue is conserved in HET-C2 giving it other properties when binding to charged membranes.

A large positively charged area formed by four lysine residues, K87, K137, K138 and K208 is located on the surface of GLTP in the vicinity of the sugar-binding pocket (Fig. 3B). This positively charged surface area is not conserved in HET-C2 and ACD11. Only one lysine residue (K137) is conserved in HET-C2 and none in ACD11. As a result, the binding to membrane surfaces and transfer of glycolipids of HET-C2 is different compared to GLTP [43]. ACD11 does not transfer glycolipids despite the structural similarity [25,26].

## 4. GLTP interaction with membranes

GLTP associates with the membrane surface in the presence or absence of glycolipid, the binding is transient and not membrane perturbing [44,45]. The binding process is strongly influenced by the

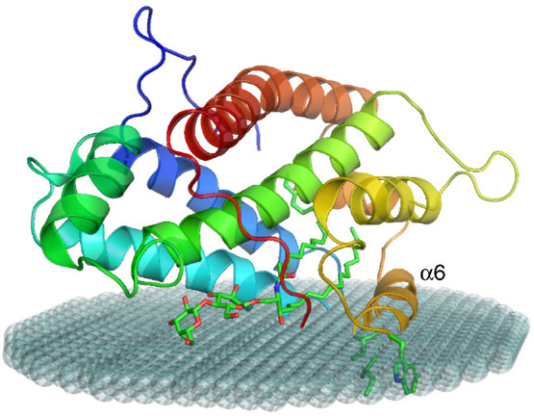


**Fig. 4.** Putative membrane interaction of GLTP, with tryptophan 142 and the two isoleucine residues (143 and 147) in alpha-helix 6 penetrating into the interfacial region. The alpha-helix 6 is most likely displaced outward about 2.5 Å when the glycolipid inserts into the tunnel. The sphingosine chain is suggested to enter last and to be the first to depart GLTP upon interaction with a membrane. The figure is constructed based on experimental data discussed in this review, as well as predictions from the Orientations of Proteins in Membranes (OPM) database [61,62].

membrane lipid composition and is thought to involve a membrane interaction site that contains tryptophan 142, together with nearby solvent exposed hydrophobic residues isoleucine 143 and 147 [46,47]. Recent photochemically induced dynamic nuclear polarization (CIDNP NMR) data provide direct evidence that the solvent exposed W142 residue is sensitive to the membrane environment, and that the protein does not need to penetrate deeply into the membrane interface to allow binding of a glycolipid [47].

Once the protein is at the interface, it must scoot for the glycolipid and recognize the carbohydrate moiety on the glycolipid. The mechanism of glycolipid entry and exit from GLTP is thought to function with a cleft-like gating mechanism. Two interhelical loops and one alpha-helix conformational change facilitate entry and exit of the lipid chains in the membrane-associated state when the glycolipid sugar is bound to the recognition center [37]. When a protein–glycolipid complex is formed, it is released into the aqueous environment. This mechanism would be consistent with a carrier mechanism, as GLTP would have to desorb from the surface completely to accomplish GSL transfer (Fig. 4). GLTP would not function with the same mechanism as the ceramide transporter CERT. CERT can facilitate movement at the contact sites between the endoplasmic reticulum and trans-Golgi cisternae by a 'neck-swinging' movement of the START domain (steroidogenic acute regulatory protein-related lipid transfer) [41,48,49]. The transfer of ceramide by CERT from the endoplasmic reticulum to the Golgi apparatus in a critical step in sphingomyelin synthesis [50].

For an optimal protein–lipid complex to form within the membrane interfacial environment, lateral diffusion of either lipid or protein or both is required. The rapid lateral diffusion rates of lipids in fluid-phase membranes and the formation of locally concentrated glycolipid clusters would increase the probability of GLTP to associate with a glycolipid molecule. Once a glycolipid cluster is found by GLTP in the membrane matrix the transfer would further be enhanced, because the glycolipid pool is directly available in close proximity. Examples of such enriched glycolipid environments in biological membranes are rafts and caveolae.

As pointed out earlier, the positively charged surface of GLTP (Fig. 3B) has effects on how well glycolipids are moved between artificial membranes. When neutral donor vesicle membranes were compared to negatively charged vesicles (5 or 10 mol% negative lipids) a significant decrease in the glycolipid transfer rate from the negatively charged membranes was seen [51]. Introduction of the same amount of negative charge into the acceptor vesicle membrane however, did not impede the transfer rate as effectively. Also, positive charges in the donor vesicle membrane are not as effective at retarding the glycolipid transfer as is a negative charge in the donor vesicle. It is assumed that since GLTP is positively charged at neutral pH (pI=9.0) it would associate with the negatively charged donor membrane through electrostatic interactions between the protein and

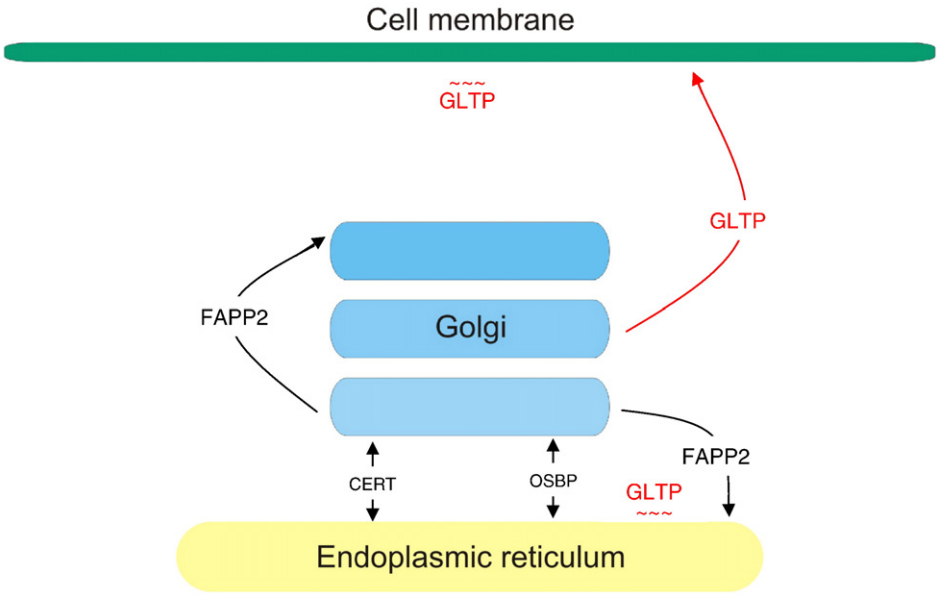


**Fig. 5.** Glucosylceramide, which is synthesized from ceramide in the early Golgi compartment is transported to the distal Golgi compartments by FAPP2 [10,30]. FAPP2 is also able to transport glucosylceramide back to the ER. A potential role for GLTP could be in the transport of glucosylceramide from the Golgi complex to the plasma membrane [10]. Another role could be as a sensor for the glucosylceramide levels of the ER membrane, or perhaps at the plasma membrane. Ceramide reaches Golgi from the ER via CERT. Ceramide is converted into sphingomyelin by sphingomyelin synthase in the Golgi lumen, or it is glycosylated to glycosylceramide on the cytosolic surface of Golgi by glucosylceramide synthase. Glucosylceramide is converted to more complex glycosphingolipids in the lumen of the later Golgi compartments. How glucosylceramide is translocated to the Golgi lumen prior to the glycosylation steps remains unclear. CERT and FAPP2 are targeted to the Golgi complex via their pleckstrin homology domain binding to ARF1-GTP and phosphatidylinositol-4-phosphate. CERT possess an ER-targeting motif FFAT, two phenylalanins in an acidic tract that can interact with the VAP proteins in the ER. Oxysterol-binding protein (OSBP) also interacts with VAP through a similar ER-targeting motif, and with Golgi through its pleckstrin homology domain, and is believed to act as a sterol sensor.

the membrane surface [43,51]. This results in a slow GLTP off-rate from the donor surface, and consequently results in a diminished rate of GLTP mediated transfer.

Experimentally it has been shown that GLTP mediated delivery of a fluorescently labeled galactosylceramide (anthrylvinyl acyl chain label) was very effective to membranes that were composed of a raft-like equimolar mixture of palmitoyl sphingomyelin and palmitoyl-oleoyl phosphatidylcholine. The delivery rates were much slower if the acceptor vesicles where made of either of the phospholipids in majority (not raft-like) [52,53]. In theory this would suggest that GLTP might be sensitive if tightly packed domains are starting to build-up. Transfer of glycolipids from tightly packed environments, such as sphingomyelin containing membranes are very slow compared to membranes containing other phospholipids [7,9,51,52], again suggesting that raft-like domains might not be the source of glycolipids for GLTP in natural membrane environments. Because GLTP is localized in the cytosol it is unlikely that it would participate in raft and caveolar structure, since they are found to a large extent on the outer leaflet of the plasma membrane. Rather, GLTP is likely to be involved in events at the cytosolic side of the plasma membrane. Maybe GLTP could function as a reporter or sensor of glycolipid levels on the inner plasma membrane leaflet. If high levels of glycolipids would form on the inner leaflet GLTP would be a potential candidate sensing these changes.

## 5. Concluding remarks

It is tempting to raise the question whether the role of GLTP *in vivo* is to bind glycolipids, but not to transfer them. Is the binding of glycolipids by GLTP linked to other mechanisms that are regulating glycolipid homeostasis, and the protein would function as a glycolipid sensor? A similar function has been proposed for the oxysterol binding protein (OSBP) family that are thought to be able to act as sterol sensors [54–56]. CERT and oxysterol binding proteins bind to ER via their FFAT motif (two phenylalanines in a acidic tract) that allows them to associates with VAP, vesicle-associated membrane-protein associated protein [57]. Other proteins that have affinity for lipids are the phosphatidylinositol transfer proteins (PITP). PITPs bind and move phosphatidylcholine and phosphatidylinositol between membranes [58]. The class II PITPs RdgBa1/Nir2 has been suggested to control diacylglycerol levels at the Golgi complex by acting on the CDP–choline pathway [59]. In addition to its N-terminal PITP domain the RdgBa protein contains a FFAT motif that targets proteins to the endoplasmic reticulum via association with VAP.

GLTP could also be part of these types of proteins that bind and sense different intracellular lipid levels, but not necessarily transfer them *in vivo*. More evidence for the importance of glycolipid binding rather than transfer by GLTP is that it was found that GLTP was inefficient in releasing the glycolipid to phosphatidylcholine acceptor vesicles [60]. In other words there is a clear tendency of the bound glycolipid to remain associated with GLTP, even in the presence of excess membrane (Fig. 5). For GLTP to be regard as a lipid sensor, the processes that it may regulate are yet to be discovered.

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