

# A lipid transfer protein that transfers lipid

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Very few lipid transfer proteins (LTPs) have been caught in the act of transferring lipids *in vivo* from a donor membrane to an acceptor membrane. Now, two studies (Halter, D., S. Neumann, S.M. van Dijk, J. Wolthoorn, A.M. de Maziere, O.V. Vieira, P. Mattjus, J. Klumperman, G. van Meer, and H. Sprong. 2007. *J. Cell Biol.* 179:101–115; D'Angelo, G., E. Polishchuk, G.D. Tullio, M. Santoro, A.D. Campli, A. Godi, G. West, J. Bielawski, C.C. Chuang, A.C. van der Spoel, et al. 2007. *Nature.* 449:62–67) agree that four-phosphate adaptor protein 2 (FAPP2) transfers glucosylceramide (GlcCer), a lipid that takes an unexpectedly circuitous route.

Lipids are made by membrane-embedded synthases, and then they move in the secretory pathway by *en bloc* inclusion in membrane vesicles and by two additional mechanisms unique to lipid traffic. First, they translocate (flip) between the two leaflets of lipid bilayers. For lipids with hydrophilic headgroups, the bilayer presents a strong diffusion barrier, and this traffic is mediated by flippases or pumps. Because lipid synthases have their active sites on one side of the membrane only, the two leaflets of most lipid bilayers *in vivo* are asymmetric. Second, LTPs bind lipids in a hydrophobic pocket and carry them across the aqueous environment of the cytoplasm. LTPs were all originally identified from *in vitro* activities, and it has been hard to generate strong evidence for lipid transfer in living cells. A benchmark was recently set by the ceramide transporter (CERT), which shuttles ceramide between the ER and TGN (Hanada et al., 2007). Now, two new studies show that FAPP2 is also an authentic LTP (see Halter et al. on p. 101 of this issue; D'Angelo et al., 2007).

Lipid transfer by FAPP2 has not been studied previously, but it contains a domain that is also found in glycolipid transfer protein (GLTP), which transfers simple glycolipids *in vitro* (Brown and Mattjus, 2007). FAPP2 had been shown to contribute to an aspect of membrane traffic from the TGN to the plasma membrane (Godi et al., 2004), but the precise roles of both FAPP2 and GLTP are unclear, possibly because there is no apparent need for the cytoplasmic transfer of glycolipids.

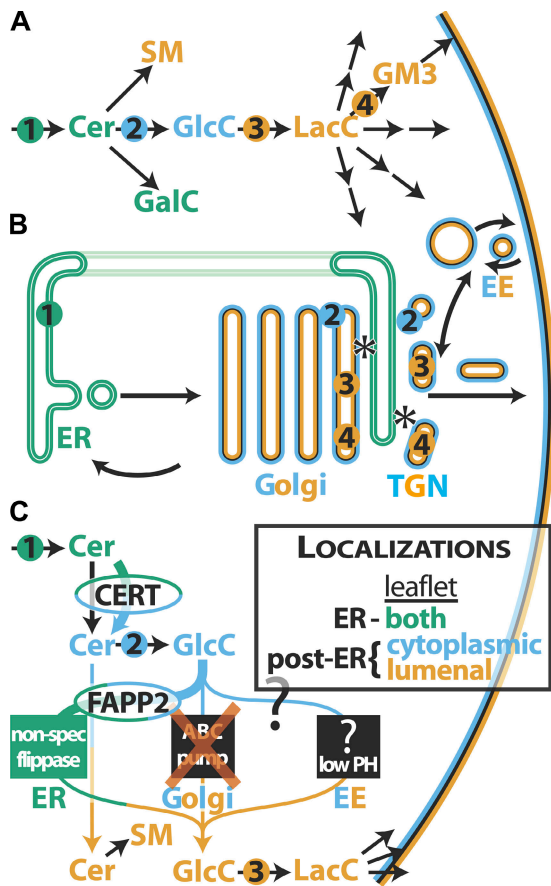
Their precursor is ceramide, which is made in the ER and delivered to the Golgi by a combination of CERT and vesicular traffic. Almost all other enzymes involved in glycolipid metabolism are in the Golgi complex, adding sugars step-wise to generate many hundreds of complex glycolipids (Fig. 1 A). Importantly, the first enzyme, GlcCer synthase, has its active site on the cytoplasmic leaflet, whereas all of the others have active sites on the luminal leaflet (Fig. 1 B).

D'Angelo et al. (2007) and Halter et al. (2007) both find that decreasing FAPP2 levels by RNA interference reduces the conversion of GlcCer to lactosylceramide (LacCer) and downstream glycolipids. D'Angelo et al. (2007) also show that FAPP2 has GlcCer transfer activity *in vitro*. The implication of this shared finding is surprising. Why would GlcCer need to traffic away from the Golgi if it is both synthesized and consumed there? Maybe the assignments of where the enzymes are localized are wrong, as these were made before the genes were cloned. To address this, Halter et al. (2007) revisit the localization of three enzymes at the start of the pathway. All three synthases are enriched in the latter half of Golgi stacks, overlapping extensively (Fig. 1 B), so the role of FAPP2 is not explained by physical separation of the enzymes.

At this point, Halter et al. (2007) reevaluate another long-held notion that an ABC pump in the Golgi translocates GlcCer from the cytoplasmic to the luminal leaflet. Certainly, a GlcCer analogue with the long acyl chain replaced by a more hydrophilic fluorescent group does flip by this means, but Halter et al. (2007) now find that endogenous, fully hydrophobic GlcCer does not flip at all in the Golgi or by ABC pumps. The rest of their study examines the journey of a few nanometers by which GlcCer moves from one leaflet of the Golgi to the other, discovering two new sites of GlcCer flipping (Fig. 1 C).

One pathway is revealed by collapsing the Golgi into the ER using brefeldin A (BFA). This stimulates GlcCer flipping considerably, presumably because it can access the nonspecific ER lipid flippase (Menon et al., 2000). To test the *in vivo* relevance of this pathway, Halter et al. (2007) identify GlcCer that traffics back to the ER lumen by expressing the enzyme that normally adds sulfate to galactosylceramide (but also acts on GlcCer) fused to the transporter of the sulfate donor. Although both of these enzymes are normally found in the TGN, fortuitously, the chimera never leaves the ER, so the formation of sulfated GlcCer can be used to indicate retrograde traffic. This is largely (>80%) blocked by the knockdown of FAPP2, which must mediate the retrograde traffic of GlcCer to the ER, although the volume of GlcCer passing through the ER is not assessed.

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**Figure 1. Pathways of glycolipid traffic.** (A) Ceramide is synthesized and converted into a large array of glycolipids by an ordered series of enzymatic steps. (B) Localization of ceramide synthase (1) is in the ER; Halter et al. (2007) find synthases for GlcCer (2), LacCer (3), and GM3 (4) overlap in the trans-Golgi (and presumably TGN). Lipid traffic is mediated by vesicular traffic (black arrows) and also by LTPs and flippases. A nonspecific flippase equilibrates the leaflets of the ER, but the two leaflets of post-ER membranes are highly asymmetric. A portion of ER interdigitates among trans-Golgi/TGN membranes, making ER-Golgi contacts (asterisks) ideal sites for LTP function. (C) Several other proteins are involved in these pathways. CERT is critical for the transfer of ceramide from the ER to the cytoplasmic leaflet of the TGN for sphingomyelin (SM) synthesis, but the role of CERT in GlcCer synthesis is unclear. GlcCer translocates from the cytoplasmic to the lumenal leaflet, but not (as once thought) using an ABC pump. Instead, GlcCer traffics away from its site of synthesis, either transferred by FAPP2 back to the ER or another route, possibly via early endosomes (EE). GlcCer returns to the Golgi/TGN in the lumenal leaflet for conversion to LacCer and other glycolipids. Note that ceramide flips easily in post-ER membranes, as it has no polar headgroup. Black arrows indicate enzymatic conversion or vesicular traffic; colored arrows indicate lipid traffic steps. Cer, ceramide; GlcC, glucosylceramide; LacC, lactosylceramide; GalC, galactosylceramide. Color-coded localizations: ER (both leaflets), green; post-ER, blue and orange (lumenal and cytoplasmic leaflets, respectively).

Halter et al. (2007) discover yet another pathway of GlcCer traffic that contributes to its arrival on the cell surface. The majority of this is abolished by BFA, but 30% is resistant to BFA, and this needs vacuolar ATPase function, suggesting that translocation occurs in an acidic compartment, possibly early endosomes, which continue to function in the presence of BFA. How GlcCer arrives here is unclear, and it does not appear to need transfer by FAPP2, although the model favored by D'Angelo et al. (2007) is most consis-

tent with this second pathway. This is one of many details that remain to be explained for the work of the two groups to be unified.

What might be the advantage for cells to take ceramide from the ER to the Golgi to make GlcCer, just to transfer it back to the ER? The detour is not essential, as flies avoid it by placing GlcCer synthase in the ER (Kohyama-Koganeya et al., 2004), and they also do not have a FAPP2 homologue. One possible advantage is that FAPP2 is one step in glycolipid metabolism that can be regulated easily. FAPP2 targets Golgi membranes by its pleckstrin homology (PH) domain, which binds both Arf1 and the signaling lipid phosphatidylinositol 4-phosphate (PI4P; Godi et al., 2004). It is known that the production of PI4P is essential for TGN exit in all eukaryotes, and mammalian cells have at least two different PI 4-kinases on the Golgi. D'Angelo et al. (2007) focus on this point and show that the function of FAPP2 in post-TGN traffic not only requires it to bind glycolipids but also depends on its PH domain binding PI4P. Therefore, this key molecule regulates glycolipid synthesis, among other aspects of Golgi function.

These studies confirm that LTPs do sometimes transfer lipids rather than merely sensing them. CERT has been the model LTP for a hypothesis that dual targeting to donor and acceptor is key for lipid transfer at membrane contact sites (Fig. 1 B; Olkkonen and Levine, 2004). In addition to its PH domain like FAPP2, CERT contains a domain targeting it specifically to the ER, without which it cannot acquire ceramide efficiently (Hanada et al., 2007). Can FAPP2 efficiently deliver GlcCer to the ER without specific ER targeting, or does FAPP2 target the ER by a mechanism we have yet to discover? It is even possible that PH FAPP2 carries out this function because the portion of ER within the Golgi region (Fig. 1 B) is highly enriched for PI4KII $\alpha$  (Vaughn et al., 2003), and D'Angelo et al. (2007) find that this PI 4-kinase makes the majority of PI4P that recruits FAPP2. One way or another, studies of GlcCer transfer by FAPP2 will force a reevaluation of still more ideas in the future.

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