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# In Search of Lipid Translocases and Their Biological Functions

**In plasma membranes, lipids distribute asymmetrically across the bilayer, a process that requires proteins. Recent work identified novel lipid translocators in yeast, and their activity was functionally correlated to endocytosis, thus boosting investigations on identity, mechanism, and function of lipid translocases.**

The asymmetric distribution of lipids in plasma membranes (PM) of eukaryotic cells is well known. Whereas neutral phospholipids, like phosphatidylcholine (PC), sphingomyelin, and (glyco)sphingolipids (GSL), are largely localized in the outer leaflet, the aminophospholipids phosphatidylethanolamine (PE) and phosphatidylserine (PS) reside predominantly in the inner leaflet. Cholesterol is almost equally distributed over both leaflets and, in the outer leaflet, is often found in association with GSL, thereby forming microdomains ("rafts"). Mechanisms underlying the maintenance and/or regulation of this distinct lateral and transbilayer lipid distribution are still poorly understood. Because of thermodynamic constraints, the involvement of integral membrane proteins in translocating lipids across bilayers appears obvious. Potential lipid translocators include an ATP-dependent aminotranslocase (P-type ATPase), a specific inward-directed pump for aminophospholipids; a lipid nonspecific $Ca^{2+}$-dependent scramblase, which mediates bidirectional translocation; and members of the ATP binding cassette (ABC) transporter family, which mediate outward lipid migration. As these properties were mostly revealed through the use of fluorescent or spin-labeled lipids, direct demonstration of translocation of natural lipids is as yet poorly supported. Furthermore, incomplete protein purification and poor functional reconstitution has frustrated the functional identification of lipid translocases.

Blood cells and, more recently, the genetically tractable yeast *Saccharomyces cerevisiae* are most often employed to study general principles of the mechanism and regulation of phospholipid translocation. In yeast, following their initial insertion in the outer PM leaflet, fluorescent NBD (nitrobenzoxa-diazole) derivatives of PE and PS, as well as PC, are transported across the bilayer in a protein- and energy-dependent, but endocytosis-independent, manner (Grant et al., 2001). Interestingly, internalization of PC and PE is inhibited in anterograde *sec* mutants, suggesting that inward translocation requires continuous transport and recycling of the translocase(s) and/or accessory elements to the cell surface. Although it was questioned in early work (Chen et al., 1999), it now becomes apparent (Gomes et al., 2000; Pomorski et al., 2002) that Drsp2, a member of a subfamily of P-type ATPases, is one of the key players. Mutated *drs2* generates an aminophospholipid transport-defective phenotype, but a plant homolog from *Arabidopsis*, ALA1, can complement for the deficiency in PS translocation (Gomes et al., 2000). Although mainly localized to the Golgi, Drs2p is rather dynamic and cycles between PM and late Golgi (Hua et al., 2002; Pomorski et al., 2002). In an elegant contribution, Pomorski et al. (2002) now identify two novel *drs2*-related P-type ATPases, Dnf1p and Dnf2p, as lipid translocators for PS, PE, and PC in the yeast PM. Overall, a dynamic picture emerges of cycling translocases in the endosomal (Dnf) and Golgi-PM secretory track (Drs2p) (Hua et al., 2002; Pomorski et al., 2003), rationalizing previous observations that secretory mutants display an inhibition of lipid translocation (Grant et al., 2001). Most importantly, Pomorski et al. show that, in *dnf1*/*dnf2* deletion mutants, *endogenous* PE accumulates at the cell surface and further increases when *drs2* is also deleted, suggesting that the ATPases may act in concert as a lipid translocation machinery. It is yet not yet clear where Drs2-mediated PE translocation activity is expressed, i.e., at the Golgi, at the PM, or at both. Neither can it be excluded that, in the *drs2* mutant, vesiculation at the Golgi, which is facilitated by Drs2p, might have been impaired (Gall et al., 2002). As a result, PM-directed transport of accessory compounds of the translocation machinery, which may include "activator proteins" not related to ATPases, e.g., Rosp3, could have been impeded. Interestingly, the translocation capacity, but not the apparent affinity for either lipid class (i.e., PE, PC, and PS), of both translocators differed, with Dnf1p, relative to Dnf2p, displaying by far the highest activity. Although the noted lipid preference disqualifies these proteins as specific aminophospholipid translocases (but natural PC as substrate has yet to be determined), sphingolipids, phosphatidic acid, and phosphatidylglycerol are not substrates (Pomorski et al., 2003). Further work on regulation, including the potential homo- or heterooligomerization, will be important to solve issues as to why there should be multiple translocases, displaying similar affinities for

distinct lipids, and how lipid species, subject to translocation, are discriminated.

The study by Pomorski et al. may strongly facilitate work aimed at revealing correlations between functional and biological activities of translocases. Although a physiological role for translocators has been poorly defined, their involvement in vesiculation and fusion events, as during cell division (Emoto and Umeda, 2000), when major changes in lipid distribution occur and membranes adjust their radii of curvature, are readily envisaged. Translocase activity is also thought to be of relevance in lipid-modulated functioning of proteins, cellular signaling, cold tolerance in plants and yeast, apoptotic events, and/or cell removal by macrophages. However, a direct link between lipid translocation and cellular functioning has not been demonstrated.

Pomorski et al. (2002) report intriguing observations on the inhibition of endocytosis in Δ*dnf1*Δ*dnf2*, but most prominently in Δ*dnf1*Δ*dnf2*Δ*drs2* cells, again supporting their concerted action. Since Drs2p facilitates the biogenesis of clathrin-coated vesicles at the trans-Golgi network (Gall et al., 2002) and transport from endosomes to the vacuole (Chen et al., 1999) and considering the translocase activity of Dnf1 and Dnf2 (Pomorski et al., 2002), the data implicate Drs2p-related P-type ATPase-controlled bilayer asymmetry in the formation of transport vesicles. Key steps in clathrin-coated vesicle formation include the assembly of coat proteins, membrane budding, and vesicle scission from the donor membrane. Some of the numerous proteins involved bind to specific lipids or exhibit lipid-modifying enzymatic activities, indicating an intimate functional relationship between proteins and lipid composition in vesicle formation. Regulated lipid translocation across the bilayer may add a new level of regulation. High local concentrations of aminophospholipids in the cytoplasmic leaflet could modulate the recruitment of peripheral proteins, such as ARF, clathrin, amphiphysin, and endophilins. The latter may participate in lipid-specific membrane deformation and regulate vesicle budding (Farsad et al., 2001). In fact, asymmetric changes in lipid content, as such, in a monolayer surface area may cause perturbations that will change intrinsic bilayer curvature, an event that also seems to require a sufficient level of cholesterol. Aminophospholipid translocases may thus help to generate specific lipid microdomains in the cytoplasmic leaflet, which likely involves a more complex cascade of local phospholipid metabolism. The bilayer couple hypothesis (Sheetz and Singer, 1974) proposes that an imbalance of surface area between inner and outer leaflet governs membrane bending, which may facilitate vesicle budding. Thus, vesiculation in endocytosis may require the translocation of lipid from outer to inner leaflet, since clathrin polymerization by itself is not sufficient. This is supported by the observed vesiculotubulation of giant liposomes induced by the asymmetric transmembrane distribution of phospholipids (Farge et al., 1999). Proteins with aminophospholipid translocase activity can induce such bilayer asymmetry. This is completely in concert with the inhibition of endocytosis in Δ*dnf1*Δ*dnf2*Δ*drs2* cells, described by Pomorski et al. (2002). However, for clarification of whether a single or a multicomplex of Dnf/Drs2 proteins suffice for translocation activity and/or vesicle formation, reconstitution of the entire machinery in liposomes is needed at conditions that avoid high lateral pressure, since that might affect translocation per se. It also remains to be determined whether the inhibition of endocytosis in Δ*dnf1*Δ*dnf2*Δ*drs2* cells is due to an inhibition of coat recruitment, membrane deformation (budding), or vesicle scission. Further electron microscopy studies may be useful to define in which step(s) (amino)phospholipid translocases act. Yet, the work has set the stage for new strategies and directions for investigating the mechanism(s) and functions of lipid translocases.

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