Transmembrane Lipid Trafficking: Facts and Speculations

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Abstract

The transmembrane distribution of phospholipids in eukaryotic cells is under the control of specific proteins or "flippases". These proteins either permit the rapid equilibration of phospholipids between both bilayer halves or accumulate specific lipids on one side and, hence, create lipid domains. The role of flippases during blood coagulation and apoptosis is well established. At a more speculative level it can be inferred that flippases, because they can generate membrane invaginations, are implicated in endocytosis. Finally, by modulating the surface tension, they could regulate the activity of other membrane proteins.

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Introduction

All biological membranes contain a mixture of many different lipids. For example, it has been estimated that about 400 chemically different lipids coexist in the erythrocyte membrane. Although functional membranes are in a fluid state which *a priori* favours lipid mixing, there are many experiments which prove the existence of transverse and lateral lipid segregations in biological membranes, most likely due to lipid-protein interactions. The life-time, size and origin of lateral domains in fluid biomembranes are often matters of controversies. By contrast, stable transmembrane segregation of lipids in the plasma membrane of eukaryotic cells is a well established fact that has been carefully evaluated. Briefly, aminophospholipids (phosphatidylserine, PS and phosphatidylethanolamine, PE) as well as phosphoinositides are essentially located in the inner monolayer while the choline-containing phospholipids (phosphatidylcholine, PC and sphingomyeline, SM) as well as glycolipids are mainly located in the outer monolayer.

This asymmetry, first discovered in human erythrocytes, seems to be a ubiquitous property of animal cells. Inner membranes, as well, are probably asymmetrical although the evidence are less

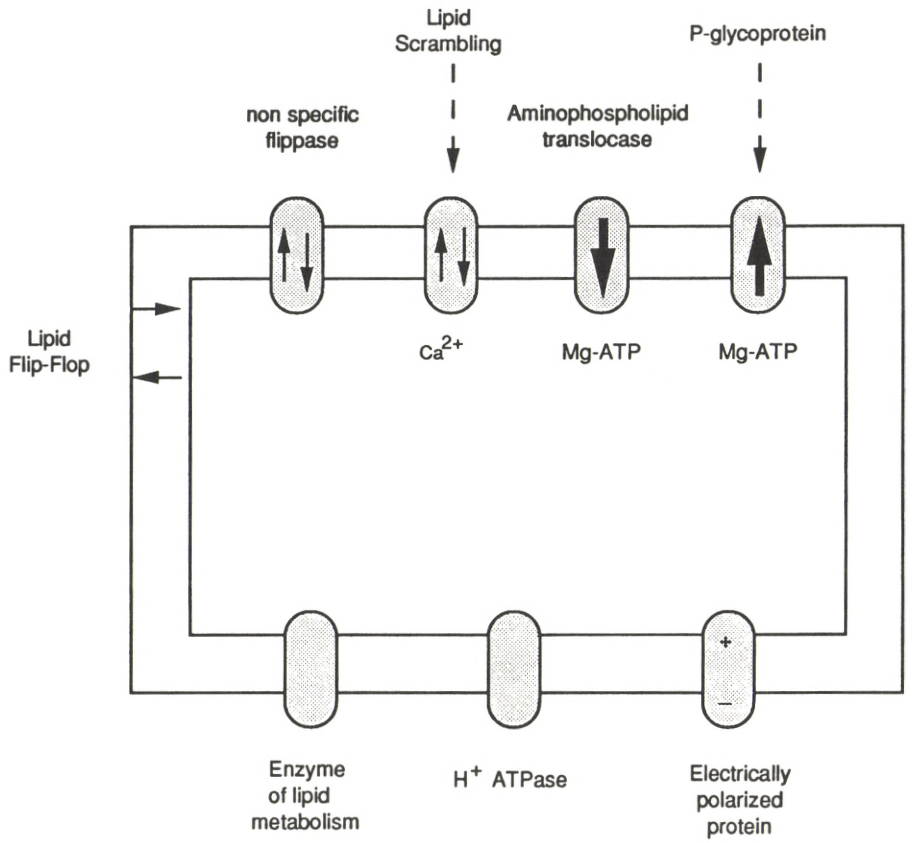


Figure 1. Schematic representation of the various proteins involved in transmembrane orientation of lipids.

compelling. Sonication of a lipid mixture with different head groups gives rise to asymmetrical bilayers because of the high curvature of small vesicles. However, transverse lipid segregation in biological systems is essentially due to the activity of proteins, some of which are enzyme involved in the synthesis of phospholipids, other proteins called "flippases" are transport-proteins that catalyze the exchange of lipids in both directions or transport lipids selectively from one leaflet to the other. In the latter case, ATP hydrolysis is required as in the case of ion carriers since such a process maintains a membrane in an out-of-equilibrium situation. Note that the stability of the asymmetrical distribution of phospholipids is facilitated by the very slow spontaneous flip-flop of phospholipids which is of the order of several hours or even several days (Devaux, 1991; Devaux, 1993; Devaux and Zachowski, 1994; Williamson and Schlegel, 1994; Diaz and Schroit, 1996).

Figure 1 is a schematic representation of the various type of "flippases" which have been reported in the literature. They comprise proteins that accumulate specific lipids and proteins that relax an asymmetrical situation by allowing the rapid exchange of lipids. Unfortunately only a few

of the transport proteins whose existence has been postulated, were purified to homogeneity (Tang et al., 1996) and none of them have been characterized structurally. Nevertheless, successful reconstitution experiments have been performed (Auland et al., 1994; Comfurius et al., 1996). References giving the main data concerning these proteins can be found in the reference list below. The objective of the present communication is not to discuss the experimental evidence of flippases proteins but rather to summarize the biological implications of these transmembrane movements.

Consequences of in Vivo Transmembrane Lipid Traffic

Lipid translocation and cellular traffic

In several cases it is clear that the translocation of lipids permits their subsequent transport to a different membrane within the cell. For example, PC is synthesized on the lumenal side of the ER, it then flips rapidly to the cytosolic face from where it can be shuttled to the plasma membrane or to the mitochondria by a PC exchange protein. Other lipids are accumulated by an active translocase on the external side of the plasma membrane in order to be exported out of a cell. PC in particular is extracted from the outer monolayer of the canicular membrane by bile salts. In the case of multidrug resistance, it is generally assumed that the amphiphilic character of the drugs transported by the P-gp from the inner to the outer monolayer of the plasma membrane implies automatically their solubilisation in the blood stream.

Lipid translocation and asymmetry

Does the existence of lipid pumps, such as the aminophospholipid translocase, suffice to explain the stable asymmetrical distribution of phospholipids in plasma membrane of eukaryotes? This is often assumed implicitly. Yet, there is a serious problem because the outward transport of choline containing lipids (PC and SM), if it exist, is much less efficient than the inward transport of PS and PE. There are several reports of an outward transport of PC due to proteins of the MDR family in specialized membranes like canalicular membranes (Ruez and Gros, 1994; van Helvoort et al., 1996). There is also indication that PC outward transport in red cells is faster than the steady state inward transport of the same lipid, however, the efficiency of this outward flux of PC is much slower than that of the inward flux of PS and PE. A consequence is that if lipids with a totally random distribution are sorted between the two bilayer halves with the machinery existing in red cells, the membrane will vesiculate and probably collapse. This phenomenon which is related to the "bilayer coupled effect" (Sheetz and Singer, 1974) can be demonstrated by insertion of a small percentage of amphiphilic drugs in the outer or inner monolayers of red cells. Similarly manipulation of the transmembrane distribution of lipids in liposomes causes important shape changes (Farge and Devaux, 1992; Mathivet et al., 1996). However *in vivo* the membrane asymmetry is not generated from a random distribution of lipids. Membranes of a new cell come from the division of a mother cell containing already asymmetrical membranes. In other words, asymmetry has to be reajusted progressively as new lipids are synthesized. In the latter case a difference in the kinetics of inward and outward movements may be acceptable. Moreover part of the asymmetry comes from the transmembrane orientation of the enzymes that synthesize the lipids in organelles, not in the plasma membrane. For example, it is admitted that glycosphingolipids are synthesized on the lumenal leaflet of Golgi membranes, from where they are transported to the plasma membrane by fusion of vesicles derived from the Golgi. Thus, glycosphingolipids are directed to the plasma membrane with

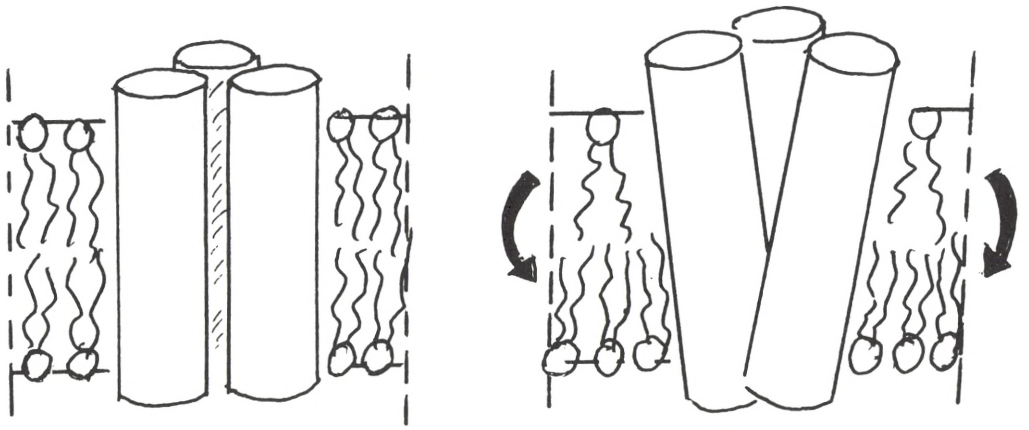
the proper transmembrane orientation. This requires the free diffusion of the precursor lipids in the Golgi membrane (Buton et al., 1997). In conclusion it is perhaps not necessary to have exact compensation of inward and outward phospholipid transports by lipid-translocators as long as the membrane is never far from its steady state lipid distribution.

Asymmetric Membranes: some Biological Advantages

The difference in lipid composition between each monolayers is in fact necessitated by the asymmetrical role of all cell membranes. For example the outer surface of the plasma membrane of an eukaryotic cell contains specific receptors sites, among which are glycolipids, but it should not be charged otherwise it would trap many plasma proteins. On the contrary, the inner surface contains charged lipids such as PS and PA (phosphatidic acid) to which cytosolic extrinsic proteins (G proteins, annexins) can bind but there is no glycolipids. During the cell life-time a change in the transmembrane distribution can take place to trigger a new biological event. For example, lipid scrambling in aged cells or apoptotic cells permits PS exposure on the outer monolayer and, hence, the recognition of the senescent red cells by PS receptors on macrophages. Similarly stimulation of platelets triggers the passage of PS towards the outer monolayer of platelet membranes and provides plasma prothrombinase with a PS interface needed for the conversion of prothrombin into thrombin (Williamson and Schlegel, 1994; Diaz and Schroit, 1996).

Lipid Translocation and Membrane Bending

In other cells, there is an intense flippase activity which is not justified *a priori* by the export or import of lipids. In the latter case, the asymmetrical transfer of lipids between bilayer leaflets could be used for membrane bending, a process which has been studied in liposomes (Farge and Devaux, 1992). I proposed several years ago that the lipid pumps could be responsible for the formation of membrane invaginations and, hence, be directly associated with the endocytic process (Devaux and Zachowski, 1994). Several reports indicate that, indeed, the aminophospholipid translocase activity is high in cells where there is a high endocytic activity. Recent work by Farge has shown that increasing artificially the concentration of PS, i.e. the translocase substrate, results in increased endocytosis rates in K562 cells (Farge, 1995). Note that an active outward transport of PC or SM from inside to outside would lead to the loss of membrane by shedding of vesicles in the plasma, an event which generally does not happen except in very special cases. Stimulated platelets do shed vesicles: it could be due to an active outward transport of lipids in the platelet plasma membrane.



Lipid Translocation and Surface Tension

The formation of an asymmetrical membrane with an excess of lipids on one of the monolayers (or with lipids with larger head groups) can cause another physical modification than membrane bending. Indeed, a mismatch between each monolayer creates surface tension which is detectable when the spontaneous curvature associated with the difference in lipid density of the two monolayers differs significantly from the actual curvature imposed in particular by the closure of the vesicle. That is often the case with small vesicles for which the curvature is high (Farge and Devaux, 1993). Increase in surface tension can inhibit surface undulations which constitute a repulsive force between two bilayers in close vicinity. In the absence of undulations (and of repulsive electrostatic interactions) two bilayers attract themselves by van der Waals interactions. The effect of surface tension on the aggregation of liposomes has indeed been observed (Mathivet et al., 1996). This process could be important in the preliminary step of membrane fusion *in vivo*. We can speculate also about the possible role of surface tension in the modulation of protein transconformation or as a modulator of membrane viscosity.

An Hypothetical Regulation Procedure

From the above remarks it is clear that a strict regulation of outward and inward lipid traffic is necessary to avoid unwanted membrane invaginations and/or surface tension, at least it is necessary to control the magnitude of these physical events which accompany the reorientation of lipids. As more lipid translocators are discovered, it may seem more difficult to conceive a general regulation procedure. Yet, a simple way of control could be the inhibition of the lipid translocators by the surface tension itself, see Figure 2. This could explain the difficulty that we have experienced in trying to obtain proteoliposomes with a translocase activity in LUVs with a diameter of approximately 100 nm (Auland et al., 1994). Related experiments were carried out by Cullis and his collaborators who showed that it is possible to translocate specific phospholipids through LUVs' membranes which were submitted to a transmembrane pH gradient. They showed that in order to

translocate 10% phospholipids (such as phosphatidic acid or phosphatidylglycerol) from one leaflet to the other, the LUVs had to be incubated at 60°C for half an hour whereas the translocation of a small percentage (1%) can be achieved at 20°C in a few minutes (Mui et al., 1995). This saturation effect can be explained by the building of a surface tension. Thus, lipid flipping by proteins could be regulated by the surface tension.

Conclusions

Transmembrane asymmetry forms stable separated domains of lipids in membranes. The exchange of lipids between the two pools are under the control of proteins that can accumulate against a gradient or open energy barriers to let the lipid flow. The transfer of lipids between the two pools can be a process facilitating the traffic of lipids within the cell, in particular from one membrane to the other, but it can also be a way to modify the properties of a membrane or to signal to the outside world a change within the cell. Thus, the lipid asymmetry can serve several purposes. It then becomes apparent that having a significant variety of lipids offers the possibility to use this mechanism to communicate different messages or fulfil different functions by controlling the flip-flop of specific phospholipids. Some of the ideas put forward here implicate that the translocation of lipids could be more important for eukaryotic cells than for prokaryotic cells. Therefore one is tempted to suggest that the development of flippases proteins was an important step between prokaryotes and eukaryotes.

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