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Lipid somersaults: Uncovering the mechanisms of protein-mediated lipid flipping

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ABSTRACT

Membrane lipids diffuse rapidly in the plane of the membrane but their ability to flip spontaneously across a membrane bilayer is hampered by a significant energy barrier. Thus spontaneous flip-flop of polar lipids across membranes is very slow, even though it must occur rapidly to support diverse aspects of cellular life. Here we discuss the mechanisms by which rapid flip-flop occurs, and what role lipid flipping plays in membrane homeostasis and cell growth. We focus on conceptual aspects, highlighting mechanistic insights from biochemical and *in silico* experiments, and the recent, ground-breaking identification of a number of lipid scramblases.

Contents

Abbreviations: ABC transporter, ATP-binding cassette transporter; bCM, bacterial cytoplasmic membrane; DLO, dolichol-linked oligosaccharide; ER, endoplasmic reticulum; GPCR, G protein-coupled receptor; GPD, glucose-P-dolichol; GPI, glycosylphosphatidylinositol; LPS, lipopolysaccharide; MPD, mannose-P-dolichol; NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; OST, oligosaccharyltransferase; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine.

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1. Introduction

The defining feature of a biological membrane is its bilayer structure, a sandwich of two monolayers of phospholipids visible as a trilaminar track by thin-section electron microscopy. The two monolayers are coupled, a fact that is easily revealed by noting that the membrane bends if the number of lipids on one side exceeds that on the other. This is akin to the bending of a bimetallic strip in which the coupled metals have different coefficients of thermal expansion. Gorter and Grendel provided the first experimental evidence for bilayer organization in 1925 when they compared the area occupied by lipids extracted from red blood cells with the predicted area of the cell membrane using surface chemistry approaches [1,2]. Sheetz and Singer proposed the bilayer couple hypothesis in 1974 [3].

Bilayer membranes form spontaneously when phospholipids are dispersed in water, a consequence of the hydrophobic effect discussed by Tanford [4]. The bilayers formed in this way are usually nested, one within the other, to form a multilamellar structure like the layered skin of an onion. These structures can be dispersed into individual unilamellar vesicles by sonication, or by extrusion through filters after freezing and thawing. The resulting vesicles can be quite small, <50 nm in diameter, in which case there are more lipids in the outer leaflet than in the inner leaflet. For large unilamellar vesicles, >150 nm in diameter, the number of lipids in each monolayer is almost identical. Phospholipids within an individual leaflet of the bilayer are very dynamic. They undergo a number of intramolecular motions that can be so great as to sometimes bring the methyl end of their acyl chains into the vicinity of the glycerol moiety. They also exhibit rapid rotational and lateral diffusion (Fig. 1). Thus, a lipid spins around its own axis, normal to the plane of the membrane, with a characteristic time of ~1 ns, and can diffuse laterally to occupy the position of a neighboring lipid (a displacement on the order of ~1 nm) within ~100 ns. In contrast to these fast movements, phospholipid exchange between the two leaflets of the bilayer occurs only slowly. Thus, reorientation of a phospholipid across the ~3 nm thickness of a membrane has a characteristic time of ~100 h.

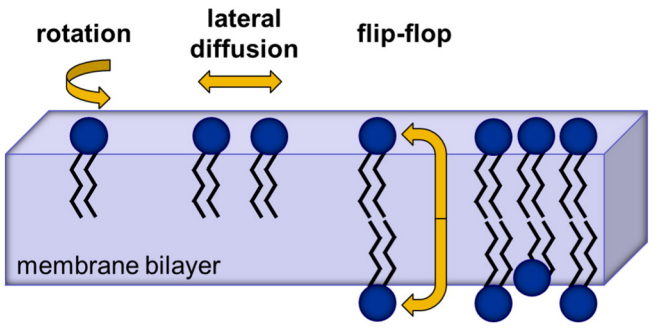

Fig. 1. Phospholipid motions in a membrane. Phospholipid bilayers are two-dimensional fluids. Individual lipid molecules have a cross-sectional area of ~0.7 nm^2. In each monolayer of the membrane bilayer they can rotate very rapidly around their head-to-tail axis with a characteristic time of 10^{-9} s, and diffuse laterally within the plane of a membrane leaflet with a translational diffusion coefficient of ~10^{-8} cm^2 s^{-1}, i.e. the time taken for a phospholipid to move ~1 nm to replace a neighboring phospholipid is ~100 ns. In contrast, spontaneous exchange of phospholipids between leaflets (flip-flop) is slow, taking typically ~100 h. The energy barrier that must be overcome in order to move the phospholipid headgroup through the hydrophobic interior of the membrane is >20 kcal mol^{-1}. Adapted from Mouritsen 'Life—As a Matter of Fat' [5].

The low frequency with which phospholipids flip spontaneously across pure lipid bilayers was first reported in 1971 by Kornberg and McConnell [6]. They reconstituted trace quantities of spin-labeled phospholipid analogues into synthetic vesicles, chemically reduced all labeled lipids in the outer leaflet with ascorbate to generate asymmetric vesicles in which the non-reduced lipid probes were located only in the luminal leaflet, and then monitored the translocation of the probes from the inner to the outer leaflet again using ascorbate. They reported a frequency of translocation of ~10^{-5} s^{-1} at 30 °C. By carrying out the measurement at different temperatures they could deduce the activation energy E of the translocation process and also estimate the prefactor A in the Arrhenius rate equation (rate = $A \cdot \exp(-E/k_BT)$, where k_B is the Boltzmann constant and T is the absolute temperature): E~20 kcal mol^{-1}, A~10^9 s^{-1}. The prefactor is typical for reactions that occur in liquids whereas the huge activation barrier, roughly equivalent to the energy derived from hydrolysis of 3 ATP molecules to ADP under standard conditions, is readily attributed to the fact that polar, ionic, or zwitterionic phospholipid headgroups have to traverse the highly hydrophobic interior of the bilayer as the lipid reorients from one leaflet to the other. An initially asymmetric bilayer is therefore relatively stable, with decay of its asymmetry occurring only very slowly over a time frame of 100 hours.

While transbilayer movement of polar lipids only rarely occurs in synthetic systems, fast flip-flop is crucial for cellular life. Thus, constitutive flip-flop of phospholipids is necessary to meet the demands of cell growth, while regulated flip-flop events are needed to sculpt the cellular responses to physiological challenges. Transbilayer translocation of lipids is also needed for membrane homeostasis, to control transbilayer lipid asymmetry in membranes, and to coordinate with protein machinery in generating intracellular transport vesicles. In almost all cases, these transport events are mediated or regulated by proteins without which flip-flop rates would be too slow to match physiological demands. Historically, the role of these proteins was defined through activity measurements that distinguished two types of translocation events: those that required ATP to move lipids vectorially across a membrane and those that were ATP-independent. Although these activity measurements were first reported in the late 1970s and 1980s, the identity of the proteins themselves remained mysterious. Only recently have members of both protein categories been identified and their activities verified by reconstitutions into phospholipid vesicles. The discovery of the molecular identity of the transporters has come hand in hand with fresh insights into the mechanisms of transbilayer translocation of lipids. This has been supplemented by informative *in silico* approaches using molecular dynamics methods to understand lipid translocation. Our objective in this review is to structure and highlight this explosion of new information.

2. Parameters influencing spontaneous lipid flip-flop

Although flip-flop of typical membrane lipids is slow, not all lipids flip-flop slowly. Lipids with a simple hydroxyl headgroup (ceramide, diacylglycerol, and cholesterol) have a very high spontaneous rate of