Greasing Membrane Fusion and Fission Machineries

Koert N.J. Burger

Department of Molecular Cell Biology, Institute of Biembranes, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
K.N.J.Burger@bio.uu.nl

Biological membrane fusion is a local-point event, extremely fast, and under strict control. Proteins are responsible for the mutual recognition of the fusion partners and for the initiation of biomembrane fusion, and thus determine where and when fusion occurs. However, the central event during membrane fusion is the merger of two membranes, which requires a transient reorganization of membrane lipids into highly curved fusion intermediates. This review focuses on the potential role of lipids in the generation of membrane curvature, and thus in the regulation of membrane fusion and fission.

Key words: Fission, fusion, lipid polymorphism, lysophosphatidic acid, membrane curvature, phosphatic acid, type-II lipids

Received 15 May 2000, revised and accepted for publication 22 May 2000

Membrane fusion is a universal event in the functioning of a living organism. It is crucial for the formation of the mature muscle fiber, for exocytosis and endocytosis, and for intracellular membrane traffic, and is used by enveloped viruses to enter and infect cells (1–3). Membrane fusion is energetically unfavorable and does not occur spontaneously. Instead, specific molecules are responsible for the induction of biomembrane fusion in vivo. Proteins determine the specificity of biomembrane fusion, and there is little doubt that proteins are also directly involved in the induction of membrane fusion. However, it has proven extremely difficult to discriminate fusogenic factors directly involved in the induction of membrane fusion from secondary factors only involved in processes that precede or follow actual membrane coalescence. Only in the case of virus–membrane fusion, have the fusogenic proteins directly responsible for the induction of membrane fusion been identified unequivocally (4).

Important clues on the general mechanism of biomembrane fusion were obtained using morphological and electrophysiological methods, which are among the few techniques in membrane fusion research that can be applied to the intact biological system. In 1979, new electron microscopy (EM) techniques became available in which the structure of a biological specimen is stabilized by fast freezing avoiding the changes in sample ultrastructure induced by the chemical pretreatments used during conventional EM techniques (5). The large-scale removal of membrane proteins from the contact area and the large single-bilayer diaphragms observed during conventional EM analysis of biomembrane fusion proved to be artifacts (6). Using fast freezing, biomembrane fusion is consistently observed as a local-point event that involves an area of the interacting membranes of less than 20 nm in diameter [reviewed in (7)]. This local-point fusion concept of biomembrane fusion was later confirmed by patch-clamp analysis, showing that fusion starts with the formation of a minute fusion pore with a diameter of only 1–3 nm (8). Importantly, the local-point fusion concept also applies to pure lipid model membrane fusion, where EM analysis revealed the presence of very small fusion intermediates, ‘lipidic particles’, suggesting a common lipidic intermediate in model and biological membrane fusion (9,10). The fact that membrane fusion is a local-point event, implies that fusion only requires a local restructuring of the interacting lipid bilayers, and that relatively few lipid molecules (hundred to a few thousand) are directly involved in the fusion process. Since there is no large-scale removal of proteins from the contact area, proteins can be directly involved in biomembrane destabilization and the induction of membrane fusion. The regulation of biomembrane fusion is dominated by proteins, but the central event during membrane fusion is the merger of two membranes, which requires a transient reorganization of membrane lipids into highly curved fusion intermediates. The potential role of lipids in the generation of these intermediates and thus in the regulation of membrane fusion and fission is discussed below.

Molecular Models of Membrane Fusion

In the end, membrane fusion requires close apposition and coalescence of a small area of the lipid bilayers. A progressive force is required to drive two bilayers closer than 3 nm together (11). Two forces dominate, a repulsive hydration force arising from water tightly bound to the lipid headgroups, and an attractive hydrophobic force between the hydrocarbon interiors of the membranes. At a pressure of about 100 atm, the attractive hydrophobic force locally bypasses the repulsive hydration force and the bilayers semi-fuse. The introduction of membrane defects exposes more of the membrane interior and substantially reduces the pressure required for semifusion. This is likely to be a general theme in all membrane fusion events. During biomembrane fusion, defects in membrane lipid packing could arise from extreme membrane curvature, local changes in lipid composition, or the insertion or de-insertion of a stretch of hydrophobic amino acids (1,2,12).
With few exceptions [see (13,14)], membrane fusion is envisaged as a ‘non-leaky’ process involving three distinct stages: membrane adhesion, semifusion, and pore formation (or vice versa, during membrane budding and fission). At the semifusion stage of membrane fusion, the outer (cis) lipid monolayers of the two membranes are continuous but the inner (trans) lipid monolayers are still separated. Experimental support for the existence of a semifusion intermediate in both model and biological membrane fusion has been obtained (15–18). Two main molecular models have been proposed for the semifusion intermediate. In both models, the semifusion intermediate has a strong net negative (concave) monolayer curvature. In the first model, two membranes join to form an inverted lipid micelle at the semifusion stage (19), while the second, and currently prevailing model, predicts the formation of a so-called stalk (20). In a modification of the stalk model (21,22), the stalk develops into a trans monolayer contact, which is short-lived and ruptures resulting in the formation of a small pore (Figure 1). It is important to note that the evaluation of the different models of membrane fusion is dominated by theoretical considerations, and that direct data on the structure and composition of the fusion intermediates (in the end in biomembrane fusion) are absolutely required before a definite answer concerning the molecular nature of the semifusion intermediate can be given. Nonetheless, the stalk mechanism is compatible with a wide range of observations on model membrane fusion, and there is increasing evidence that similar intermediates, with a net negative monolayer curvature, mediate biomembrane fusion (23).

**Figure 1: Stalk model for the intermediate stages during membrane fusion (20, 21): Adhesion (A), semifusion (B), and pore-formation (C).** Semifusion starts with the formation of a stalk (B-1), which develops into a trans monolayer contact (B-2). Hydrophobic interstices are shaded.

**The Role of Lipids in Biomembrane Fusion**

During patch-clamp analysis of biomembrane fusion, the early fusion pore is often seen to reversibly open and close several times, observed as capacitance flickering, before expanding irreversibly. A key question concerns the nature of the initial, transient pore. Is the pore formed by a multimeric protein complex, or is the pore a mixed protein-lipid or an entirely lipidic structure (8,24)? Because direct data on the structure and composition of the fusion pore are lacking, a definitive answer to this question cannot be given. However, lipids are likely to play a key role in the induction of biomembrane fusion for several reasons. First, proteins are not an absolute prerequisite for membrane fusion; pure lipid model membranes can be induced to fuse in the absence of added proteins. Second, important characteristics of biomembrane fusion such as capacitance flickering are also observed in pure lipid model systems, and the activation energies of the molecular rearrangements involved appear to be very similar (25). Finally, low (sublytic) concentrations of LPC not only inhibit model membrane fusion but also biomembrane fusion in an efficient and reversible manner, and in biological systems as different as the low pH-induced fusion of virus-infected cells, and exocytosis in patch-clamped mast cells or isolated sea urchin cortices (23). The latter suggests that model and biological membrane fusion both involve a lipidic intermediate with a net negative monolayer curvature [for a critical discussion on the effects of lysolecithin (LPC) on membrane fusion [see (23), and Stegmann, this issue of Traffic]. Together, these findings support the idea that the membrane lipid composition is an important determinant of the willingness of a membrane to fuse, and suggest that specific lipid species may play an active role in the local destabilization and fusion of membranes.

Biomembranes typically contain some 100 different lipid molecules, which, upon isolation, show dramatic differences in physicochemical properties. Early studies suggested that LPC, a micelle-forming (‘type-I’) lipid, was involved in biomembrane fusion (26). However, as outlined above, recent data have shown that lysophospholipids do not stimulate but inhibit model and biological membrane fusion. Acidic phospholipids, especially phosphatidylinositol (PI) and phosphatidylserine (PS), have also been proposed to play a central role in the induction of biomembrane fusion, in conjunction with Ca$^{2+}$ (27). Extensive model membrane studies have shown that divalent cations can induce membrane fusion of acidic phospholipid-containing membranes, probably by a combination of charge neutralization, cross-linking of membranes, local dehydration, and the induction of local defects in lipid packing. However, in view of the high concentration of Ca$^{2+}$ required for direct membrane destabilization, a general role for acidic phospholipids and Ca$^{2+}$ in biological membrane fusion seems unlikely. Instead, Ca$^{2+}$ probably exerts its effect by inducing a conformational change in Ca$^{2+}$-binding proteins such as annexins (28). A possible exception is the fusion of fully primed and docked secretory vesicles with the plasma membrane during regulated exocytosis where fusion occurs in the immediate proximity of calcium influx.
and the fusion machinery is transiently exposed to high local calcium concentrations (29). Finally, inverted nonbilayer-prefering lipids (‘type-II lipids’) have been proposed to play a key role in biomembrane fusion (9,30). In contrast to type-I lipids, which are virtually absent from biomembranes, type-II lipids are present in significant amounts in almost every biomembrane (31,32). One of the nonbilayer lipid phases formed by these lipids is the inverted hexagonal-II (H_{II}) phase. Almost any biological membrane contains lipids that, upon isolation, will form an H_{II} phase under physiological conditions of ionic strength, pH and temperature. Examples are the unsaturated phosphatidylethanolamines (PEs) present in all eukaryotic membranes (in the plasma membrane, typically 25 mol% of total phospholipid, of which 80% is present in the cytosolic leaflet). The lipid molecules in the H_{II}-phase are organized in hexagonally arranged cylinders, with the polar headgroups lining a central aqueous channel (Figure 2). The tendency of type-II lipids to form inverted nonbilayer lipid structures can be explained by considering their overall dynamic shape (33,34). Type-II lipids are cone-shaped, they have a small headgroup area as compared with the cross-sectional area of the acyl chains. In contrast, type-I lipids, such as most lysophospholipids, have an inverted cone-shape, while bilayer-prefering lipids are best described as having the shape of a cylinder. It is important to note that the terms inverted cone- and cone-shaped have not been used consistently in literature, and that confusion can be avoided by using the terms type-I and type-II for lipids with a positive- and negative-curvature preference, respectively. Despite its simplicity, the shape-structure concept of lipid polymorphism has proven to be very powerful in predicting the phase behavior of many membrane lipids and their mixtures. This is best illustrated by the fact that an equimolar mixture of a type-I and a type-II lipid, LPC and unsaturated PE, forms bilayer structures (35). On the other hand, the concept should not be oversimplified. In particular, it should be realized that the phase-preference or spontaneous curvature not only depends on the molecular structure of the lipid in question, but also on its dynamic properties, the hydration of the lipid headgroup, as well as on intra- and intermolecular interactions. Thus, the curvature preference of lipids is greatly influenced by factors such as temperature, pH, salt and cation concentrations. For example, LPC is organized in bilayers at temperatures below the gel-to-liquid crystalline phase transition temperature.

A biomembrane consists of a mixture of bilayer-prefering and type-II lipids, but despite the presence of type-II lipids, its basic structure is a lipid bilayer. The type-II lipids are forced to remain in the lipid bilayer, a situation which is often referred to as ‘frustration’. In prokaryotes where the lipid composition of the membrane can be easily manipulated, the ratio of bilayer to nonbilayer preferring lipids was shown to be under strict metabolic control, indicating that the propensity of the membrane to form type II structures is of great importance for cellular functions (36,37). The ratio is an important determinant of the interfacial properties of the bilayer as well as membrane stability, and thus influences the activity of integral and peripheral membrane proteins (38) and determines the propensity of the membrane to undergo fusion. Model membrane studies show that physiologically relevant factors such as diacylglycerol (DG), unsaturated fatty acids, and hydrophobic proteins can induce a bilayer to H_{II}-phase transition in physiologically relevant mixtures of bilayer-prefering and type-II lipids (31,39). The transition of a bilayer to an H_{II}-phase occurs through membrane fusion, and the same nonbilayer semifusion intermediates (Figure 1) are most likely involved in fusion and H_{II}-phase formation (22). The total lipid extract of several biological membranes forms an H_{II}-phase upon hydration, suggesting that, in vivo, these membranes will be eager to form nonbilayer lipid structures such as those involved in membrane fusion. Thus, the willingness of a biomembrane to fuse may, at least in part, be determined by the ratio of bilayer to nonbilayer-prefering lipids present in the membrane.

Because biomembranes contain large amounts of type-II lipids, on one hand, and a correlation exists between their presence and membrane fusion activity in model systems, on the other hand; type-II lipids are attractive candidates for a direct involvement in biomembrane fusion. By virtue of their low headgroup hydration, type-II lipids would be expected to enhance membrane adhesion by reducing the hydration repulsion, while their negative curvature preference should facilitate the formation of highly curved concave semifusion intermediates and stimulate membrane fusion. The idea that biomembrane fusion may take advantage of the phase-preference of type-II lipids is supported by studies on the lipid dependency of biomembrane fusion. External addition or local generation of cis-unsaturated fatty acids, oleic acid and arachidonic acid, stimulates chromaffin granule fusion (40,41), ER-derived microsome fusion (42), and virus-

<table>
<thead>
<tr>
<th>Lipid Species</th>
<th>Molecular Shape</th>
<th>Lipid Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysocephosphatidylglycerol</td>
<td>Inverted Cone / Wedge</td>
<td>Micellar</td>
</tr>
<tr>
<td>Phosphatidylcholine (PC)</td>
<td>Cylinder</td>
<td>Bilayer</td>
</tr>
<tr>
<td>Phosphatidylethanolamine (PE)</td>
<td>Cone</td>
<td>Hexagonal II</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatidylserine-cholesteryl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatidylglycerol (PG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatidylcholine-cholesteryl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiolipin (CL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipalmitoylglycerol (DAG)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2: Shape-structure concept of lipid polymorphism** (33,34). Note that unsaturated fatty acids, ceramide, diacylglycerol, and cholesterol will themselves not form an H_{II}-phase, but promote H_{II}-phase formation in mixed-lipid systems. For details see text.
induced cell–cell fusion (43). This stimulatory effect can be prevented by co-incubating with LPC, and is fully reversible by albumin extraction of the fatty acids (23). Second, inhibitors of phospholipase-A2 impede endosome fusion both in permeabilized and intact cells, while in permeabilized cells, fusion activity can be partially restored by the addition of arachidonic acid (144); also see (45). In addition to lipid breakdown products, fusogenic proteins may promote membrane fusion by enhancing the negative curvature tendency of the lipid system; synthetic viral fusion peptides promote the formation of type-II lipid structures and model membrane fusion (46). The same mechanism may be used in vivo by the fusion peptide of viral fusion proteins, or a hydrophobic entity of a fusogenic protein in general. Unfortunately, the best characterized biomembrane fusion protein, the hemagglutinin glycoprotein (HA) of influenza virus, mediates exoplasmic fusion with the endosomal membrane; the initial interaction occurs between exoplasmic membrane leaflets containing only small amounts of type-II lipids. Although HA-mediated membrane fusion appears to involve a stalk-like semifusion intermediate, the formation of this intermediate may depend primarily upon specific lipid–protein interactions (14,47). Future experiments will have to show whether a fusion machinery acting on membrane leaflets rich in type-II lipids, for example during synaptic vesicle exocytosis may be designed to gain more profit from the presence of type-II lipids in the membrane.

**Membrane Domains and Transbilayer Area Asymmetry**

There are ample indications for the existence of membrane microdomains with a specific lipid and protein composition in vivo (55,56), and model membrane studies have shown that microdomain formation can drive membrane deformation and fission (57–59). In this domain-induced budding concept (Figure 3A-1), the edge of a membrane domain has an energy proportional to the length of the edge, and membrane bending is a spontaneous process governed by a competition between a decrease in edge energy and an increase in bending energy.

An alternative driving force for membrane bending originates from the fact that membranes are very flexible but can hardly be stretched. Thus, a difference in lateral pressure between the two membrane leaflets immediately results in membrane deformation [bilayer couple hypothesis; (60)]. Both in erythrocytes and in pure lipid model systems, the insertion of additional lipid (equivalent to 0.1–1% of total lipid) in one of the membrane leaflets induces dramatic shape changes (61,62). Thus, energy-driven lipid translocases may induce transbilayer area asymmetry and regulate membrane bending in vivo (Figure 3A-2). Indeed, inward translocation of short-chain PS and PE by the ATP-driven aminophospholipid translocator present in the plasma membrane of human erythrocytes cells, stimulates endocytosis (63). Moreover, deletion of the DRS2 gene, which encodes a putative aminophospholipid translocator present in late Golgi of yeast, results in a decrease in clathrin-coated vesicles budding from the Golgi complex (64). Transbilayer area asymmetry can also be induced by lipid metabolism when this results in a change in the molecular area or biophysical properties of the membrane lipid. ATP depletion of human erythrocytes is accompanied by breakdown of phosphatidylinositol-4,5-bisphosphate shrinking the inner membrane leaflet by ~0.6%, and probably explaining the spiny appearance of human erythrocytes after ATP depletion (65). In contrast to phospholipids, the spontaneous transbilayer movement (flipflop) of several phospholipid breakdown products, such as ceramide and DG, is very rapid. If lipid metabolism is confined to one of the membrane leaflets, equilibration of the breakdown products

**The Role of Lipids in Biomembrane Fission**

Although both fission and fusion require close membrane contact, in the case of fission this requires strong membrane bending and the formation of a highly constricted neck. An additional complication in exoplasmic membrane fission concerns cytosolic factors involved in the regulation of fission, which now act on the membrane leaflets opposite to the membrane leaflets that initially interact and semifuse. Thus, a key question concerns the mechanism by which the extreme curvature in the neck region is generated, and the contacting membrane leaflets are destabilized and induced to (semi) fuse.

In model membrane systems, budding and fission can occur in the absence of added proteins, and some intracellular membrane budding events in vivo might not require a supramolecular complex of coat proteins. For example, endocytosis occurs via a clathrin- and dynamin-independent pathway involving buds and vesicles that lack a visible coat (48). However, coat proteins appear to be crucial in most biomembrane budding/fission events, although the precise role of membrane coats in shaping highly constricted membrane buds and, even more so, in semifusion and fission, is not completely understood. Membrane coats are probably multifunctional, playing a role in the formation of the vesicle, determining its size, selecting and concentrating its contents, and preventing fusion prior to the completion of budding (49,50). In a number of elegant in vitro studies, coat proteins turned out to be sufficient for efficient vesicle formation (51–53). Recently, the formation of COPI-coated vesicles was reconstituted in a liposomal system and, although coatomer was absolutely required, vesiculation was remarkably insensitive to the lipid composition of the liposomes suggesting that lipids are not part of the general core mechanism for budding of COPI-coated vesicles (54). These studies show that membrane coats are essential for Golgi membrane budding in vitro, but do not formally prove that coat assembly is the direct driving force for bud formation and membrane fission in vivo (see below). Moreover, local high concentrations of specific lipids are likely to facilitate (protein-mediated) membrane constriction and fission in vivo. The potential role of lipids in membrane bending and the induction of membrane fission is discussed below. 

Burger
will induce a relative increase in the lateral pressure in the opposite membrane leaflet and induce membranes to bend (Figure 3A-3). Microinjection of sphingomyelinase into sphingomyelin-containing giant liposomes results in the asymmetric production of ceramide and the outward budding of vesicles (66), while the treatment of macrophages with externally added sphingomyelinase results in ATP-independent endocytosis through the formation of large endocytic vesicles lacking discernable coats (67); note that these treatments also affect the spontaneous monolayer curvature, see below).

Both yeast and mammalian cells contain cytosolic proteins capable of exchanging one lipid species for another between membranes in vitro, and these proteins are likely to affect, directly or indirectly, the (local) membrane lipid composition in vivo and play a role in the regulation of membrane bending and fission (Figure 3A-4). The yeast Sec14 gene product preferentially binds phosphatidylinositol (PI) (68). It is structurally and functionally related, but not homologous, to mammalian PI-transfer proteins (PITP). Sec14 yeast mutants are defective in protein transport from the Golgi to the plasma membrane, a defect which can be partially rescued by expressing the mammalian PITP. In a recent in vitro study, PITP was found to be essential for the formation of COPI-coated vesicles from the trans-Golgi network isolated from dog kidney cells (69); also see (70). Only the PI-loaded form of Sec14p could substitute for mammalian PITP in the budding reaction, suggesting that the delivery of PI to the budding sites is essential for vesicle formation. In the absence of cytosol, PI-loaded PITP vesiculates uncoated Golgi membranes in an uncontrolled fashion, but in its presence, the action of PITP is restricted to the sites where COPI-coated buds are formed. A local change in lipid composition in the neck region due to the recruitment of PI and its conversion into DG (or PA, see below) could be directly responsible for membrane fission in vitro. Translocation of DG to the exoplasmic leaflet of the Golgi membrane could drive the final constriction of the neck region while the type-II character of DG would be expected to facilitate semifusion and fission. The same mechanism of membrane fission may also be operational in vivo: studies performed in yeast indicate that Sec14p regulates the Golgi DG pool, although PI binding and transfer appear to be dispensable for this function (71). Finally, transbilayer area asymmetry and membrane bending may result from asymmetric insertion of proteins into the membrane (see below).

Note that local membrane bending is not necessarily due to a local difference in lateral pressure between the membrane leaflets; a global difference could drive local membrane bending with membrane microdomains acting as the nucleation sites. Coat proteins may very well regulate membrane microdomain formation, and could thus indirectly control membrane invagination and budding, and determine the rate of vesiculation as well as the chemical composition and size of the vesicles.

**Lipid Acyltransferases and Transbilayer Curvature Asymmetry**

A difference in spontaneous monolayer curvature between the two membrane leaflets, transbilayer curvature asymmetry, can also drive membrane bending. This is best illustrated by the shape changes that occur in erythrocytes upon exchange of natural, mono-unsaturated, phosphatidylcholine
(PC) present in the exoplasmic leaflet for synthetic di-saturated or di-unsaturated PC using a PC-specific exchange protein (Figure 3A–5). The PC-exchange reaction hardly affects the surface area of the exoplasmic leaflet, yet results in dramatic shape changes: the di-saturated PC increases the spontaneous curvature of the exoplasmic leaflet and results in outward membrane bending, while the opposite is observed for di-unsaturated PC (72). Lipid transfer proteins, lipid translocases, and lipid metabolic enzymes, may all influence the spontaneous curvature of the membrane leaflets by affecting membrane lipid composition and asymmetry, and thus regulate membrane bending in vivo.

The possibility that local lipid metabolism and a change in transbilayer curvature asymmetry is used in vivo to regulate membrane bending and fission is suggested by the recent discovery that phospholipid acyltransferases are part of the fission machineries involved in endocytic vesicle formation and Golgi membrane fragmentation (73,74). Strikingly, the two cytosolic proteins involved, endophilin I in endocytic vesicle formation and CtBP/BARS in Golgi membrane fission, are functionally related but show no sequence homology. Both proteins are capable of converting, in vitro, lyso-PA (LPA) into PA using oleoyl- or arachidonoyl-CoA as the acyl donor, and in both cases this activity appears to be essential for membrane fission. Because acylation of LPA selectively increases the cross-sectional area of the tail region, the role of the acyltransferases may be to reduce the spontaneous curvature of the cytosolic membrane leaflet and facilitate the formation of negative (concave) membrane curvature during constriction of the neck region (Figure 3B–1). Originally, neck constriction was proposed to result from the conversion of inverted-cone-shaped LPA into cone-shaped PA (73). However, the polymorphic phase behavior of LPA has not been studied so far and, although a conversion of LPA into PA probably reduces the spontaneous monolayer curvature, unsaturated (di-oleoyl) PA behaves as a cylindrical, bilayer-prefering, lipid at the neutral pH of the cytosol ([75,76]; K.N.J. Burger, V. Chupin, and B. de Kruijff, unpublished observations).

The idea that LPA acyltransferases regulate membrane bending and fission by changing the transbilayer curvature asymmetry is appealing, yet there are many uncertainties and alternative possibilities. First, LPA and PA can only exert their effect if they localize to specific regions of the developing bud and do not mix or diffuse out into the surrounding membrane. One possibility is that local membrane curvature drives selection of lipid molecules based on their molecular shape [e.g., see (77)], for example restricting LPA to sites with positive monolayer curvature. However, direct experimental evidence to support this notion in an experimental system that allows free diffusion of lipids between areas of different curvature has so far not been obtained [see (78)]. An alternative possibility is the formation of PA-rich microdomains. PA can act both as hydrogen bond donor and acceptor and has a high tendency to cluster (79); domains could be fixed at the neck region by protein–lipid interaction (see below). Second, the origin of the LPA used in the acylation reaction is unknown. LPA is a highly water-soluble lipid, and if LPA would be recruited from the cytosol, its acylation increases the area of the cytosolic membrane leaflet (Figure 3B–2). This would facilitate positive instead of negative membrane curvature, and might explain the fact that disruption of endophilin function in stimulated synapses blocks invagination of clathrin-coated pits (80). On the other hand, if LPA is generated in the membrane e.g. by the action of a phospholipase A2 (81), the effect on membrane curvature will depend on the fate of the fatty acids generated. Third, the extent to which LPA and PA undergo transbilayer movement, in particular in the Golgi complex, is unknown, but the neutralized form of PA undergoes rapid transbilayer movement in model membranes (82). If PA reaches the lumenal leaflet of the Golgi membrane, the mildly acidic pH and high Ca$^{2+}$ concentration in the Golgi lumen trigger type-II behavior which may well lead to membrane destabilization and fission (Figure 3B–3). Fourth, PA may be further metabolized and, for example, be converted into DG, a type-II lipid capable of rapid transbilayer movement. Finally, PA or one of its metabolites may activate downstream effectors involved in membrane bending or fission. PA is unique among anionic lipids because of its small and highly charged headgroup very close to the glycerol backbone. We recently observed that dynamin, a large GTPase regulating membrane constriction and fission during receptor-mediated endocytosis (83), is a membrane-active molecule capable of penetrating into the acyl chain region of membrane lipids, and that lipid penetration is strongly stimulated by PA (K.N.J. Burger, R.A. Demel, S.L. Schmid, and B. de Kruijff, unpublished observations). Thus, instead of promoting negative membrane curvature, the PA generated by dynamin-bound endophilin may induce deep penetration of dynamin into the membrane of the neck region, followed by membrane destabilization and fission (Figure 3B–4). Other membrane fission events may involve the generation of PA by enzymes such as CtBP/BARS (74) or phospholipase D (84,85), followed by membrane insertion of a fusiogenic protein.

It is important to note that the principles discussed above may operate simultaneously, and together determine the bending response of a membrane in vivo. For example, lipid metabolism may result in membrane domain formation, induce transbilayer area asymmetry, as well as influence the spontaneous monolayer curvature and protein–lipid interactions. Although many factors involved in the regulation of endocytosis and intracellular membrane budding have been identified, the regulation of the central step, semifusion and fission, remains elusive. One might expect the mechanism to be similar to that of vesicle–membrane fusion, and involve a local perturbation of the contacting membrane leaflet by a protein factor. The function of coat proteins would then be to bring the interacting membrane surfaces close enough together to allow the fusogenic protein to induce membrane fission. However, this mechanism seems unlikely in view of the fact that in numerous genetic screens, a candidate fusogenic protein that might fulfill this function has not been found. Alternatively, the cooperative low-energy interactions
of coat proteins or their capacity to induce membrane microdomains may result in the extreme membrane curvature in the neck region, which in itself could be sufficient to allow spontaneous semifusion of the contacting membrane leaflet. In this scheme, membrane deformation and semifusion/fission are expected to be facilitated by specific lipid species or, possibly, by deep penetration of a protein in the non-contacting membrane leaflet.

Concluding Remarks

Lipid bilayers can fuse in the complete absence of proteins, but membrane fusion and fission in vivo are regulated by specialized proteins. There is strong experimental and theoretical support for the involvement of largely lipidic fusion intermediates and a semifusion intermediate with a net negative monolayer curvature in both model and biological membrane fusion. The most likely role of specialized proteins in biomembrane fusion is to reduce the energy barriers to fusion by facilitating the formation of these highly curved fusion intermediates. In principle, two mechanisms can be envisaged: a protein that acts as a fusogen by itself, or a protein that, after activation, produces a fusogen, for example a lipid breakdown product. Conclusive evidence for the former mechanism has been obtained: the fusogenic proteins directly responsible for virus–membrane fusion have been identified, and fusion is induced by hydrophobic peptides perturbing the lipid packing of the interacting membranes. This mechanism is likely to apply to other biomembrane fusion events as well, but it may not apply to membrane fission. Although, lipids may be merely greasing membrane fusion machineries, the recent discovery that phospholipid acyltransferases are part of two otherwise unrelated fission machineries suggests that lipids could be the key players during membrane bending and fission.

Acknowledgments

I am grateful to Thomas Pomorski, Arie Verkleij, Antoinette Killian, and Alberto Luini for helpful discussions and critical reading of the manuscript, and to FEI/Philips Electron Optics (Eindhoven, NL) for financial support. I apologize to those investigators whose contributions were not included in this review due to space limitations.

References

17. Razinkov VI, Melikyan GB, Cohen FS. Hemifusion between cells expressing hemagglutinin of influenza virus and planar membranes can precede the formation of fusion pores that subsequently fully enlarge. Biophys J 1999;77: 3144–3151.
Burger


85. Exton JH. Regulation of phospholipase D. Biochim Biophys Acta 1999;1439: 121–133.