J. Membrane Biol. 146, 1–14 (1995)

The Journal of
Membrane
Biology

Topical Review

Lipids in Biological Membrane Fusion

L. Chernomordik[1], M.M. Kozlov[2], J. Zimmerberg[1]

[1]Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bldg. 10, Rm. 10D04, 10 Center Drive, Bethesda, MD 20892

[2]FB Physik, Freie Universitat Berlin, WE 2, Arnimallee 14, 1000 Berlin 14195, Germany

Received: 1 January 1995/Revised: 22 March 1995

Introduction

Fundamental cell processes as diverse as constitutive secretion, triggered exocytosis, membrane recycling, protein trafficking, fertilization, and enveloped virus infection involve a rearrangement of biological membranes—membrane fusion. Two membranes merge into one with a joining of the aqueous volumes they initially separated. Impressive progress has been achieved on the isolation and characterization of some of the components of the machinery that brings membranes into contact and fuses them in a tightly regulated time- and site-specific manner [7, 106, 132].

It is thought that biological membrane fusion is mediated by specialized 'fusion' (or 'fusogenic') proteins, which change their conformation upon interaction with specific triggers [132]. The "simplest" class of fusion reactions is used by enveloped viruses to enter host cells. In 'viral' fusion only one specialized envelope protein (e.g., the well characterized influenza hemagglutinin (HA)) is required to mediate fusion of bound membranes [132]. Some specific mutations of these fusion proteins cause complete loss of their ability to mediate fusion. For a number of nonviral biological fusions, where the specific fusion proteins remain unknown, fusion is inhibited by treatments that modify membrane proteins [106, 123]. Proteins involved in triggered and constitutive exocytosis and other fusion reactions are rapidly being identified [106]. However, even a thorough knowledge of each player's personality does not yield an understanding of the rules of a game. Although the crystal structure of the ectodomain of influenza HA is known for both neutral and low pH forms [7, 134], the molecular mechanism(s) underlying the merger of two membranes in hemagglutinin-mediated fusion is (are) still unclear. We do not know if membrane fusion in disparate biological processes proceeds via different mechanisms having different structural intermediates, each completely dependent on the specific fusion protein involved. Can fusion be described only in terms of protein conformational changes and protein-protein interactions, with lipid acting as a passive lubricant? Or, is there some stage of membrane fusion that is dependent on the properties of membrane lipid bilayers and common to different biological fusion reactions? The data that we will review in this work support the latter hypothesis.

We will show that diverse biological and model fusion reactions may be modulated by altering membrane lipid composition. The effects of different lipids on membrane fusion correlate with their ability to support the formation of different nonbilayer structures. We will describe a theoretical model of membrane fusion as a consecutive formation of transient and local nonbilayer intermediates. The energetics of these intermediates will be shown to depend on the bending elastic properties of lipid monolayers. The concept developed here is that while biological fusion is driven mainly by proteins, there is an unavoidable stage of the actual merger of membranes which is modulated by the properties of lipid

Correspondence to: L. Chernomordik

Key words: Membrane fusion — Lipid bilayer — Bending elasticity — Fusion intermediates — Lysolipid — Exocytosis

bilayers. At this stage, different fusion reactions converge to the same types of structural intermediates, driven by the same physical forces.

Membrane Lipid Composition Modulates Biological Fusion

Biological membranes are dynamic structures with a lipid composition known to be very asymmetric and heterogeneous. Although the exact lipid composition of the membrane site involved in membrane fusion is hardly ever known, there are a number of approaches that allow a significant alteration of the net lipid composition of biological membranes or at least outer membrane monolayers [18, 48, 49, 104, 108]. The simplest and most universal way of changing lipid composition of practically any membrane, including those expressing fusion proteins, is to add exogenous lipids which incorporate into membranes.

We studied the effects of lipids on four diverse biological fusion processes: sea urchin egg cortical exocytosis, mast cell degranulation, rat liver microsome—microsome fusion and syncytia formation of baculovirus infected insect cells triggered by Ca^{2+}, GTP-γ-S, GTP and H^+, respectively [16]. The addition of lysolipids (a class of naturally-occurring phospholipids) to the medium brings about fast (within 5 min), dose-dependent, and potent inhibition of all four fusion reactions (Fig. 1). At inhibiting concentrations, incorporated lysolipids comprise approximately 5–10% of the membrane lipids.

Lysophosphatidylcholine (LPC) also inhibit myoblast fusion [108], protein trafficking between successive cisternae of the Golgi stack [37], chromaffin cell degranulation (R. Holz, *personal communication*) and fusion of liposomes with Sendai virus [136] and Semliki Forest virus (J. Wilschut, *personal communication*). Paradoxically, for a long time lysolipids were considered to promote rather than inhibit fusion. However, 'fusogenic' effects of lysolipids are observed mainly in the nonphysiological erythrocyte-erythrocyte fusion and only at higher, lytic concentrations of LPC [100]. Interestingly, lysolipids, even at lytic concentrations, never promote Ca^{2+}, low pH, or GTP-dependent biological fusion in the experimental systems studied [16].

Fusion inhibition can not be attributed to any specific chemical moiety of lysolipids [16]. Zwitterionic LPC and lysophosphatidylethanolamine, and negatively charged lysophosphatidylserine and lysophosphatidylinositol, as well as a set of LPCs varying in the lengths of hydrocarbon chains and extent of unsaturation all had

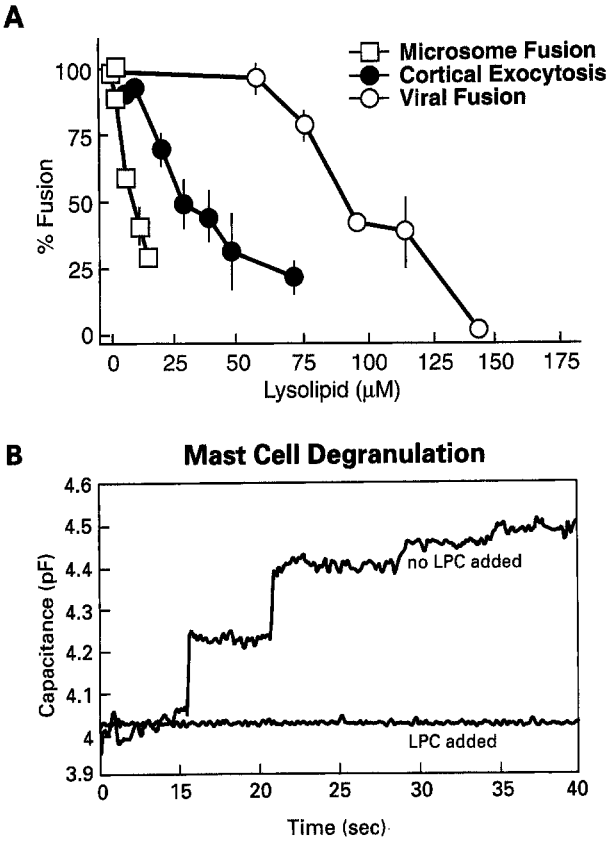

Fig. 1. Inhibition of biological membrane fusion by lysolipids. (*A*) We show oleoyl LPC-induced inhibition of GTP-dependent fusion of rat liver microsomes (□); calcium-triggered cortical exocytosis (●); and pH-induced fusion of insect cells infected by baculovirus (○). Membrane fusion in these experimental systems was quantified by dequenching of fluorescent lipid, by light scattering assay, and by counting of cells in syncytia, respectively. Each point is mean ± SE, n = 3, normalized to fusion response in the absence of exogenous lipid. (*B*) Capacitance traces of mast cells in the presence and absence of 9.6 μM oleoyl LPC in the pipette solution. The time at which the whole-cell configuration was established is taken as zero. Adapted from [16].

similar effects on exocytosis and viral fusion. In addition to these natural lipids, the synthetic surfactant Tween 80, having a nonionic polar head quite different in structure from that of lysolipids, also inhibits fusion. Experiments with synthetic surfactants show that this inhibition is not caused by any products of lysolipid biological transformation.

Not all lipids inhibit cell-cell fusion when added exogenously. Dioleoyl and dicapryl phosphatidylcholines and palmitic acid have no visible effects [16]. *Cis*-unsaturated fatty acids actually promote biological fusion. A 5-min incubation of cells infected by baculovirus with arachidonic or oleic acid (AA and OA, respectively) results in a significant increase (up to 40%) in the number of cells forming syncytia upon subsequent application of low pH medium [13]. *Cis*-unsaturated fatty acids also promote endosome-endosome fusion

Abbreviations: LPC, lysophosphatidylcholine; OA, oleic acid; AA, arachidonic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; HA, influenza virus hemagglutinin.

[79], Ca^{2+}-triggered fusion of chromaffin granules [19], and GTP-dependent fusion of microsomes [96].

Both inhibition and promotion of biological fusion by exogenous lipids are completely reversible. Removal of the short-chain lysolipids (e.g., lauroyl LPC) by simply washing the membranes with fresh buffer, restores the original fusion competence of the membranes [16, 124]. The fusion competence of membranes treated with oleoyl LPC or AA can be restored only by perfusion with buffer containing fatty acid-free bovine serum albumin [16, 124]. Presumably, these lipids are harder to extract from membranes because of their longer hydrocarbon chains. Thus, both inhibition and promotion of fusion by specific changes in membrane lipid composition are not mediated by any irreversible effects such as cell lysis, irreversible inactivation, or solubilization of membrane components.

Modulation of biological fusion by membrane lipids is also found when other techniques are utilized to alter membrane lipid composition. Replacing phosphatidylcholine (PC) with phosphatidylethanolamine (PE) promotes both virus—liposome [6, 51] and sea urchin egg cortical granule—liposome fusion (S. Vogel and L. Chernomordik, *unpublished results*). In many cases, fusion is facilitated by inclusion of cholesterol into the liposome composition [91, 122].

Biological fusion involves the establishment of proper membrane contacts; activation of fusion proteins upon interaction with a specific effector (trigger), e.g., Ca^{2+}, H^+, and GTP, or by receptor binding; merging of membranes either following or preceding the opening of the fusion pore, and finally; the expansion of this pore. What stage of the fusion reaction is lipid sensitive? This question was specifically addressed in [124]. Because lysolipids inhibit fluorescent membrane dye redistribution between fusing membranes, for H^+-triggered viral fusion, for Ca^{2+}-triggered exocytosis [124] and for GTP-dependent microsome-microsome fusion [16], lipids affect biological membrane fusion at a step prior to actual membrane merger. We know also that LPC arrests mast cell degranulation at a stage preceding fusion pore formation [16]. In *beige* mouse mast cells internally perfused with LPC, there is no indication of even a single conductance flicker of a fusion pore—the transient aqueous pathway between two membrane compartments during their fusion (Fig. 1*B*). On the other hand, the lipid-sensitive stage follows the triggering step. In these experiments, short-chain lauroyl LPC is present during a ~1 min application of a specific trigger (low pH for baculovirus-mediated fusion and Ca^{2+} for cortical granule exocytosis), and most of the fusion is inhibited. Fusion, however, commences upon withdrawal of lysolipids [124], demonstrating that triggering causes the formation of an 'activated state' which is lipid sensitive but does not require the continued presence of trigger to later develop into the fully fused state. The reversible character of lysolipid inhibition allows us to functionally uncouple triggering from steps involving membrane merger.

The conclusion that the lipid-sensitive stage follows the triggering event was further substantiated by the observation that exogenous lipids do not need to be present during triggering in order to affect fusion. Lysolipids and *cis*-unsaturated fatty acids inhibit and promote baculovirus-mediated fusion, respectively, even when added immediately after application of low pH pulse [13, 124]. Finding that lipids affect biological fusion downstream of triggering explains why the effects of the lipids are common for very diverse fusion reactions.

To summarize, membrane lipid composition dramatically affects biological fusion at a trigger-independent stage prior to actual merger of membranes. The same lipids inhibit (e.g., LPC) and promote (e.g., AA) such contrasting biological fusion reactions as calcium-triggered exocytosis and low pH-triggered viral fusion, which vary in characteristic rates [1] and in the type of membrane leaflets which make the initial contact (''endoplasmic'' *vs.* ''exoplasmic'' fusion [119]).

Lipids Affect Biological Fusion and Lipid Bilayer Fusion Similarly

Lipids govern some important biological reactions via direct interactions with proteins [3, 135]. Lipids may modulate biological fusion acting as specific receptors or even as cofactors changing the conformation and activity of the proteins involved. For effects of this type, one may expect specific dependence of fusion on small amounts of particular lipids, and this is true for dependences of membrane fusion of Semliki Forest virus on the presence of cholesterol [6, 58, 131] and sphingolipids [89] in the target membrane. Lipids could also affect the lateral association of fusion proteins into multimolecular complexes presumably required to drive membrane merger [38, 60], and alter the mode of insertion of the fusogenic peptide into the target membrane [78]. Fusion inhibition and promotion may be mediated by lipid interaction either with membrane proteins or other lipids. The latter hypothesis is supported by a number of studies carried out on model lipid bilayers.

The relative simplicity of model systems based on purely lipidic bilayers invites their use to study molecular mechanisms of membrane fusion. Some of these experimental models and, in particular, planar lipid bilayer fusion to phospholipid vesicles (liposomes) [34] or to other planar lipid bilayer [15], allow one not only to control the total lipid composition of membranes but also to independently alter the lipid composition of different monolayers of the membranes. Hereafter, the lipid monolayers which make the initial contact of two membranes will be referred to as 'contacting monolayers'.

The monolayers which are not exposed initially toward the gap between the membranes will be called 'distal monolayers'.

Fusion of planar lipid bilayers includes two distinct stages [15]. First, lipids of the membranes' contacting monolayers merge to form a single bilayer in a contact region. This step of membrane interaction, referred to as 'monolayer fusion' or 'hemifusion', occurs before (or sometimes without) the second and subsequent step, complete fusion, when an aqueous pathway (fusion pore) develops between aqueous volumes initially separated by the membranes. The walls of this pore are formed by the merged distal monolayers of the membranes. The existence of the hemifusion stage in the fusion of planar lipid bilayers was proven by a number of experimental approaches [15, 74, 88] including the demonstration that the specific capacitance of the contact region coincides with that of a single bilayer [15]. Hemifusion was also reported for liposome-liposome [2, 24, 27, 105], liposome-planar lipid bilayer interaction [98], and lipid bilayers formed on the surface of mica cylinders [46]. As discussed in detail below, hemifusion may also occur with biological membranes.

We found that hemifusion and complete fusion of lipid bilayers are actually controlled by the composition of different membrane monolayers. In [11], liposomes were labeled by porin ion channels and a fluorescent lipid at self-quenching concentrations. The incorporation of porin channels into planar bilayers at each fusion event during osmotically driven complete fusion was observed as conductance jumps. Merger of membranes, for either hemifusion or complete fusion, are observed as flashes of dye redistribution. Combining electrical measurements and fluorescence microscopy allowed us to distinguish different stages of fusion. Spontaneous, osmotically-independent hemifusion of liposomes to planar lipid bilayers was promoted by the presence of PE in the liposome membrane and dramatically inhibited by adding LPC to the contacting monolayers of membranes. No inhibition was observed when AA was used instead of LPC in similar experiments. Addition of LPC to modify the distal monolayer of the planar bilayer also had no effect on monolayer fusion but promoted complete fusion. In contrast, modification of the distal monolayer of planar bilayer by AA resulted in a dramatic inhibition of complete fusion. Similar results were reported for the model of two planar bilayers: LPC inhibits hemifusion if added to membranes' contacting monolayers, and promotes complete fusion if added to the distal membranes monolayers (Fig. 2) [15].

In general, the lipid dependence of fusion described for the purely lipid bilayers is very similar to those reported for biological fusion. In addition to protein-mediated fusion (*see above*), PE supports fusion much better than PC in liposome-liposome [25, 133], liposome-planar bilayer [17], and planar bilayer-planar bilayer [14, 15] fusion. Cholesterol promotes fusion of lipid bilayers [14, 133]. LPC inhibits fusion of model lipid bilayers (Table) along with diverse biological fusion reactions. LPC promotion of PEG-induced liposome-liposome fusion (73a) is apparently related to the LPC effects on distal membrane monolayers (73b). Unsaturated fatty acids promote liposome-liposome fusion and biological fusion if present in the contacting membrane monolayers (Table). In contrast, inhibition of liposome-planar bilayer fusion by AA added to the distal monolayer of the planar bilayer imitates the inhibition of cortical granule exocytosis in sea urchin eggs by adding AA to the outer leaflet of the egg plasma membrane (topologically, corresponding to the distal membrane monolayer) [26]. Thus, in biological fusion, as in lipid bilayer fusion, the same lipids may promote or inhibit membrane fusion if added to different membrane monolayers (Table).

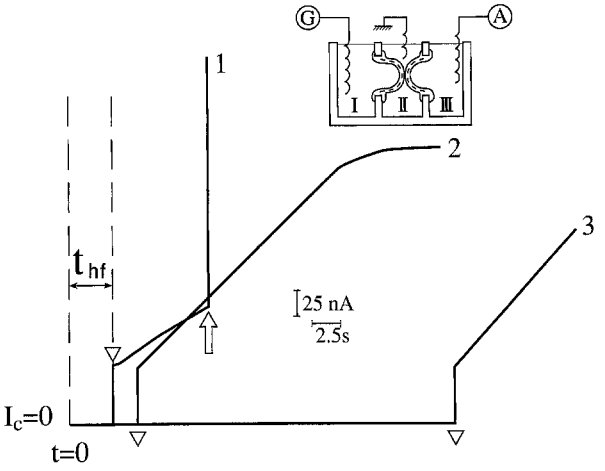

Fig. 2. Adding LPC to different monolayers of planar lipid bilayers affects different stages of membrane interaction. Current response of two solvent-free planar lipid bilayers of PE to a linear voltage sweep of 100 *V/s* with an amplitude ±20 mV applied by the generator *G* was recorded at the output of the operational current amplifier *A* with grounded electrolyte solution in compartment II. At $t = 0$ membranes were brought into contact. The arrowheads indicate the initiation of hemifusion characterized by a drastic increase in the capacitive current and by a qualitative change in the character of the capacitive response [15, 88]. The following increase of the current corresponds to steady growth of the contact bilayer area (curves 1, 2 and 3). Fusion pore formation (arrow in curve 1) is characterized by many orders of magnitude increase in the current measured, again with simultaneous change of the character of current oscillogram. 5 μM egg LPC added into peripheral compartments of the experimental cell (compartments I and III) to incorporate into distal membranes' monolayers caused the spontaneous transformation of hemifusion into complete fusion (curve 1). 0.5 μM egg LPC added between membranes (compartment II) increased the waiting time, t_{hf}, of hemifusion (curve 3) in comparison with the control experiment, where no LPC was added (curve 2). Addition of LPC to compartment II to higher final concentrations further increased t_{hf} [14]. (Courtesy of Dr. Grigory Melikyan).

Table. Effects of lysophosphatidylcholine (LPC) and arachidonic acid (AA) on membrane fusion depend on which side of the membranes the exogenous lipid is added

	Contacting monolayers		Distal monolayers	
	Biological fusion	Model bilayers	Biological fusion	Model bilayers
LPC	Inhibition: Exocytosis [16, 124] Viral fusion [16, 124, 136] Intracellular fusion [16, 37, 96]	Inhibition: BLM-BLM[a] [14] LS-BLM[b] [11] LS-LS[c] [78, 136]	Promotion: Exocytosis [75]	Promotion: BLM-BLM [15] LS-BLM [11]
AA	Promotion: Exocytosis [19] Viral fusion [13] Intracellular fusion [79, 96]	Promotion: LS-LS [80] (but no effect LS-BLM [11])	Inhibition: Exocytosis [26]	Inhibition: LS-BLM [11]

[a] BLM-BLM stands for the experimental system of two planar lipid bilayers.
[b] LS-BLM stands for liposome fusion to planar lipid bilayer.
[c] LS-LS stands for liposome-liposome fusion.

Biological Fusion Involves Formation of Local and Transient Intermediates

Ultrastructural analysis of exocytotic events show fusion pores of 20–150 nm in diameter [8, 9, 21, 61, 95]. These pores have smoothly-fractured walls indistinguishable from those of fractured bilayer lipid membranes. Electrophysiological recordings of exocytosis and viral-induced syncytia formation yielded kinetic information on smaller fusion pores. After fusion is triggered, a delay is encountered, lasting as short as 100 μsec in synaptic transmission and as long as 100 sec in influenza HA-mediated fusion [81, 82, 116, 140]. Following this delay, a small fusion pore forms connecting the lumens of two membrane compartments [5, 115, 142, 140]. The initial conductances of the smallest, initial fusion pores varies between 20–1000 pS suggesting that pore diameters range from 1 to 7 nm. After formation, the fusion pore conductance varies continuously in time [21, 84, 115, 142]. It either closes completely or continues to fluctuate in the 0.6 to 20 nS range with a mean around 3 nS [21, 87, 139] eventually opening at a relatively rapid rate.

Fusion pore formation has also been inferred from the rates of movement of aqueous dyes through the fusion junction. In HA-mediated cell-cell fusion, NBD-taurine flux is explained by multiple small pores, since hemoglobin did not redistribute for several minutes [107]. Lipid dye is always seen to move prior to aqueous dye. These data support the notion that the fusion junction may be comprised of several small pores, having a significant perimeter for lipid dye flux, while impeding aqueous dye [140].

Is the initial fusion pore proteinaceous [1], or is the wall of the pore formed at least partially by lipids [83, 141]? The latter hypothesis is supported by an analysis of the conductance distributions of exocytotic fusion pores [87], temperature dependency of the rate constants for fusion pore formation [93] and the similarity between some characteristics of fusion pores and those developed in exocytotic granule membrane under high electric field [92]. We also know that fusion pores form even when HA-expressing cells fuse with purely lipid bilayers and these pores are qualitatively similar to those observed in HA-mediated cell-cell fusion [81, 82].

Is aqueous continuity established first, or does it follow the formation of lipidic continuity? Lipid flux during fusion pore formation and growth has been measured with simultaneous electrical and fluorescent experiments [120, 140]. In both studies, membrane dye (Di-I or R-18) was originally only on one of two cells that were to fuse, and only after fusion pore conductance grew to more than its initial value did dye move, sometimes minutes later. These results argue against hemifusion as an early stage of biological fusion.

On the other hand, strong evidence for a hemifusion intermediate prior to fusion pore formation is reported in a recent paper by Kemble et al. [57]. These authors have replaced the transmembrane domain of HA with a glycosylphosphatidylinositol, GPI, a lipid that anchors HA in the external leaflet of cell plasma membrane. Upon application of low pH this mutant HA mediates only hemifusion i.e., redistribution of lipid dye with no flux of aqueous dye for as long as detected (one hr). Interestingly, GPI-anchored mutants of HIV gp120/41 are also unable to mediate syncytia formation [128]. Earlier findings were suggestive of hemifusion in other biological systems [99, 114, 117]. The fact that the extracellular domains of a fusion protein have the power to bring the external leaflets of two cells together and merge them to form a lipidic junction is highly supportive of the hypothesis that the wild type HA acts first in the same manner, and the transmembrane domains act next to merge the distal leaflets to open the fusion pore. If this

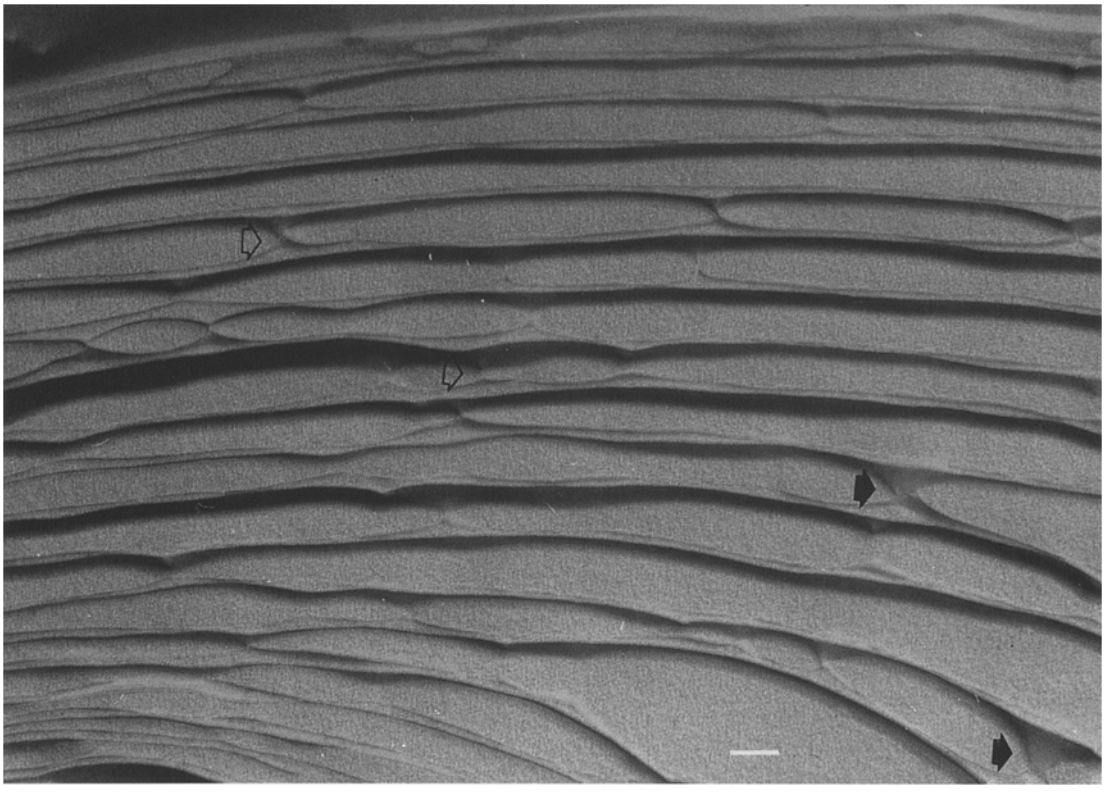

Fig. 3. Freeze fracture electron micrograph of phospholipid bilayer lamellae induced to fuse by freezing and thawing. At some of the numerous sites of close membrane contact (open arrows) the hydrophobic fracture faces of adjacent bilayers are seen to be connected. Solid arrows point to possible extended areas of intermembrane contact. Scale bar = 100 nm. For experimental details see [54]. Reprinted with permission from Hui, S.W., Stewart, T.P., Boni, L.T., Yeagle, P.L. 1981. Membrane fusion through point defects in bilayers. *Science* **212:**921–923. Copyright 1981 American Association for the Advancement of Science.

is true, then the lag in lipid flux after fusion pore formation in the wild types (*discussed above*) must reflect retardation of lipid dye flux by some other mechanism such as immiscibility of lipids, transmembrane domain interaction to form a barrier to lipid flux, etc. [143].

Theoretical Models of Lipid Intermediates of Membrane Fusion

Thus, the lipid composition of membranes similarly modulates diverse biological fusion reactions, where fusing membranes comprise complex mixtures of different lipids and proteins, and fusion of purely lipid bilayers. These findings suggest that any membrane fusion includes a distinct stage mediated by lipids and common for fusion in biological and artificial systems. In addition, there are some indications that the initial fusion pore in biological fusion may be a lipidic pore.

In formulating a theoretical model of lipid bilayers fusion, we can benefit from the results of morphological studies suggesting how the fusion intermediates may look. Light microscopy of swollen multilamellar structure formed by PC in water reveal necklike structures (''passages'' in [42]) connecting adjacent bilayers). Structures mediating fusion of lipid membranes were studied by electron microscopy in a system of closely packed PC/PE bilayers [54]. The lipid connections (''contact points'' in [54]) between contacting lipid monolayers separated by a thin film of water (opened arrows in the Fig. 3) observed in this study were interpreted by Hui and coauthors as early fusion intermediates. Similar structures of the early intermediates of fusion or lamella—H_{II} phase transition (*see below*) were reported in some other electron microscopy studies [77, 112]. The development of these connections results in the formation of intermembrane contacts consisting of a single bilayer (solid arrows in the Fig. 3). Fusion pore development in this contact bilayer completes the reaction by the formation of passages.

A stalk-pore model of fusion intermediates developed in [12, 14, 15, 65, 67, 77, 73] is close to the morphological pictures and simple enough to allow reasonable physical estimates. In the initial state, the membranes are separated by a thin layer of water (Fig. 4*a*). An average distance between the bilayers is determined by the balance between the Van der Waals attraction [97] and the whole set of repulsive interactions including hy-

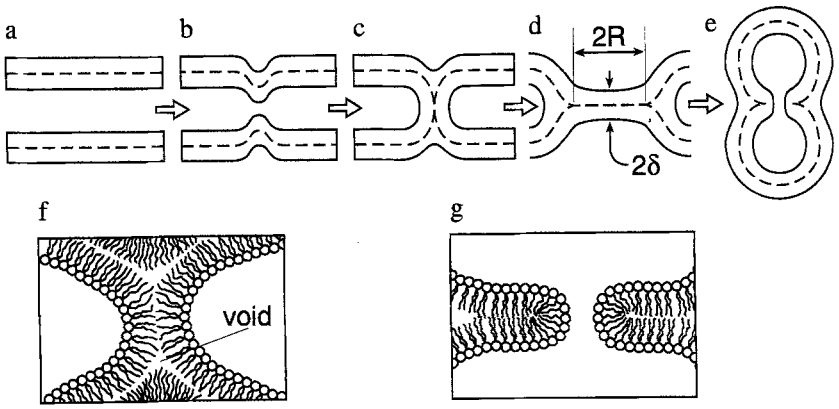

Fig. 4. Stages of lipid bilayer fusion in a stalk-pore model [15, 65, 67, 73]. (*a*) two opposed membranes; (*b*) fluctuation resulting in a local close contact between membranes; (*c*) a stalk formation; (*d*) stalk expansion resulting in formation of a contact bilayer; (*e*) formation of a pore in the contact bilayer completing the fusion process; (*f*) enlargement of a stalk; (*g*) enlargement of a pore. Dashed lines show the boundaries of the hydrophobic surfaces of two monolayers. 2δ and 2R are the thickness of the lipid bilayer and the diameter of the contact bilayer, respectively.

dration repulsion [102], undulation forces [44] and possibly electric repulsion for the charged lipids. Characteristic values of these distances for neutral monolayers of different lipids lie in the range of 1.2–2.2 nm [102]. While being on average parallel, the membranes exert bending fluctuations (undulations) towards each other (Fig. 4*b*). The first fusion intermediate in the model, a stalk, is a necklike structure connecting only the contacting monolayers of the membranes (Fig. 4*c,f*). An alternative model suggests the formation of inverted micelles as fusion intermediates [110, 121]. However, the most recent analysis indicates that this type of intermediate is energetically less probable than stalks [111].

The second stage of the fusion process in stalk-pore model is a radial expansion of the stalk resulting in formation of a contact bilayer (Fig. 4*d*). The contact bilayer consists of distal monolayers of fusing membranes. Formation of a fusion pore in the contact bilayer is considered the final stage of the fusion process (Fig. 4*e,g*). The pore edge is assumed to be covered by polar heads of lipid molecules preventing contact between the hydrophobic interior of the membrane and the surrounding water. To simplify theoretical estimations, we approximate the shapes of the stalk and the curved parts of the monolayers appearing at the further stages of the fusion process by rotating the segments of circles about a common vertical axis (Fig. 4*d,e*).

The essential property of all of these fusion intermediates is a strong bending of the portions of lipid monolayers which form them (Fig. 4*c,d,e*). Therefore, we assumed that the main contribution to the energy of fusion intermediates is related to this bending and we calculated the bending energies of the lipid structures at different stages of fusion [12, 14, 65, 67, 73].

Lipids Affect Bending Energy of Monolayers

The theory of bending elasticity of membranes was developed by Helfrich in 1973 [43] for nearly flat membranes and then extended [40, 59, 66, 68] to describe strong bent lipid monolayers. The state of bending of a lipid monolayer is characterized in a most general way by its two principal curvatures c_1 and c_2, or (what is equivalent) by the principal radii of curvature $R_1 = 1/c_1$ and $R_2 = 1/c_2$, which are determined at every point of the monolayer surface. The monolayers in a stalk and in a fusion pore are bent so strongly that the radii of curvature are comparable with the monolayer thickness (Fig. 4*c–e*). The material properties of the monolayer are characterized by the bending rigidity of the monolayer κ, and by the spontaneous curvature of the monolayer J_s. While κ has the usual physical meaning of Hookean rigidity relating stress and deformation, the spontaneous curvature J_s is a unique feature of an elastic surface which accounts for its shape in a stress-free state. The whole description of the monolayer bending elasticity is related to the so-called neutral surface which keeps its area constant with bending deformation [68].

In a simplified form (with the effects of the Gaussian curvature being neglected), the bending energy per unit area of the monolayer is given by [43]

$$\gamma = \frac{\kappa}{2}(c_1 + c_2 - J_s)^2 \qquad (1)$$

The whole energy is obtained by integrating the energy density (1) over the neutral surface of the monolayer.

The bending rigidity of lipid monolayer has the same order of magnitude for different lipids and can be estimated as $10\,kT$ [68], where k is the Boltzman constant and T is the absolute temperature ($1\,kT \sim 0.7$ Kcal/mol). In contrast to the bending rigidity, the spontaneous curvature depends drastically on the kind of lipids in the monolayer [41, 62, 76, 101, 109]. The spontaneous curvature manifests itself by the shape of the lipid monolayer in a mesophase formed spontaneously by lipid in a fully hydrated state[1]. At the same temperature and electrolyte composition, different lipids form different mesophases such as micelles, which can have the shape of cylinders or spheres with hydrophobic interiors (Fig. 5*A*)

[1] The propensity of different lipids to form monolayers of different shapes is also discussed in the literature using the term 'effective molecular shape of lipid' [20, 53, 55, 70].

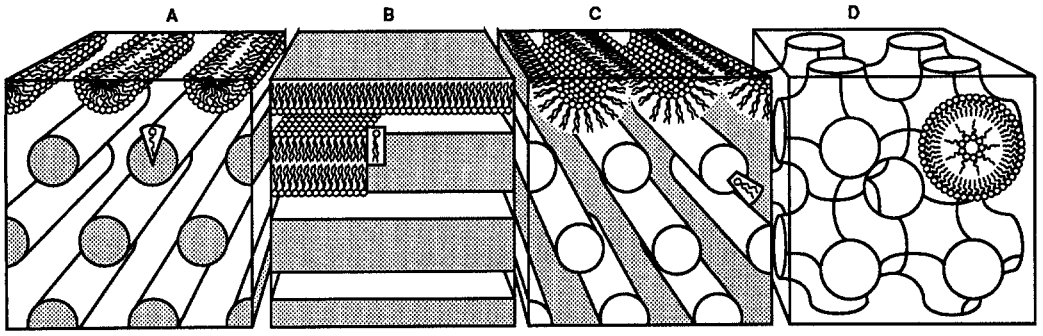

Fig. 5. The structure of some liquid-crystalline phases formed spontaneously by lipid molecules in water [20, 76, 103]. (*A*) normal hexagonal phase, H_I,—(cylinder micelles)—hydrocarbon chains fill the interior of the cylinders, water is outside; (*B*) lamellar phase, *L*, (an alternate sequence of planar bilayers and water layers); (*C*) inverted hexagonal phase, H_{II},—water is in the interior of the cylinders and hydrocarbon chains fill the gap between cylinders; (*D*) bicontinuous cubic phase. (Adapted with permission from [103].)

[36], lamellar phases composed of flat monolayers (Fig. 5*B*) [76], inverted hexagonal H_{II} phases formed by cylindrically curved monolayers with water enclosed inside the cylinders (Fig. 5*C*) [41, 76, 101], or bicontinuous cubic phases, built up by monolayers having a saddlelike shape (Fig. 5*D*) [62, 76, 109]. The curvature of the monolayers in the spontaneously formed structures mentioned above can, in a first approximation, be considered as spontaneous curvatures. Since lipid monolayer can be bent in different directions (Fig. 5*A,C*); we have to define the sign of spontaneous and geometrical curvatures. Following the convention used earlier, we will define the curvature of a monolayer in the inverted hexagonal H_{II} phase as negative. The curvature of a micelle monolayer is positive. Lipids which form lamellar phases (e.g., PC) have spontaneous curvature close to zero. Lipids which form inverted hexagonal H_{II} phase (e.g., unsaturated PE or cardiolipin in presence of Ca^{2+} ions [20]) or promote its formation when added to other lipids (e.g., AA, OA [33, 52] and cholesterol [10, 30]) have high negative spontaneous curvatures of the radii comparable to the thickness of the monolayer. Micelle-forming lysolipids (e.g., LPC) have a high positive spontaneous curvature [28, 71].

The theory of membrane bending elasticity based on the concept of bending rigidities and spontaneous curvature [43] successfully describes the elastic properties of both nearly flat and strongly bent monolayers [36]. In particular, Eq. 1 quantitatively describes deformations of the cylindrical monolayer of the inverted hexagonal H_{II} phase under controlled osmotic pressure [64, 69]. The values of the bending rigidity for these strongly bent monolayers were fairly close to those measured for flat monolayers [86]. In addition, the theory of membrane elasticity explains [64] the recent paradoxical finding that application of osmotic pressures to lipid monolayers of the H_{II} phase of dioleoyl PE changes not only the radii of the cylinders but also may drive the hexagonal-lamellar-hexagonal sequence of the phase transitions [35]. The model describing the competition between the elastic energy (Eq. 1) of the H_{II} phase monolayers and the energy of interaction of membranes in the lamellar phase gives an excellent quantitative description of the phase diagrams obtained by two different experimental methods without use of any unknown parameters. The successful application of the theory of bending elasticity for strongly bent lipid monolayers justifies its application to the analysis of membrane fusion intermediates.

Stalk has a Net Negative Curvature

The surface of the monolayer forming the stalk (Fig. 4*c*) has a saddlelike shape characterized by two principal curvatures, one of which (meridional) is negative and the other of which (parallel) is positive [67]. The negative meridional curvature seen at the cross section of the stalk (Fig. 4*c,f*) is comparable with the curvature of H_{II} phase cylinders all along the stalk membrane. On the other hand, the parallel curvature changes all along the stalk surface from a large positive value in the middle to zero at the transition from the stalk to flat monolayer. The competition between two principal curvatures determines the sign of the net curvature of the stalk.

If the thickness of the water gap separating contacting monolayers of fusing membranes is assumed to be equal to the thickness of a lipid monolayer, δ, [102] and the neutral surface is located at half of the monolayer thickness (Fig. 4*c,d*), the energy of the stalk, F is given by the following expression

$$F = 2\pi\kappa \left\{ J_s\delta[\pi(\rho + 1.5) - 4] + 2\frac{(\rho + 1.5)^2}{[(\rho + 1.5)(\rho + 2.5)]^{1/2}} \arctan\frac{(\rho + 2.5)^{1/2}}{(\rho + 1.5)^{1/2}} - 4 \right\} \quad (2)$$

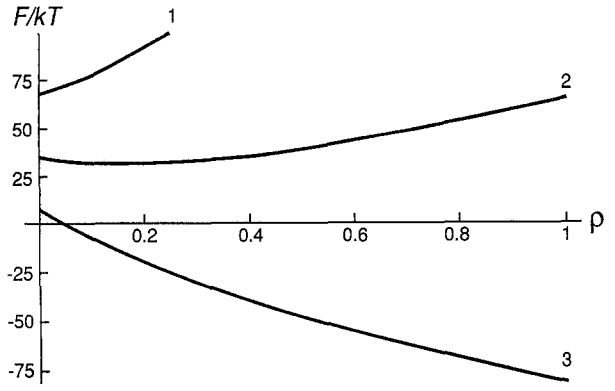

Fig. 6. Dependence of the energy of a stalk on the radius of the contact bilayer. The bending rigidity of the monolayer is $\kappa = 10\ kT$. Parameter in the curves is the spontaneous curvature of monolayer: $J_s = 0.6/\delta$ (curve 1); $-0.2/\delta$ (2); $-0.7/\delta$ (3), where δ is a monolayer thickness of ~2 nm. Dimension-less radius of the contact bilayer ρ equals R/δ, where R is the radius of the contact bilayer.

where ρ is a dimensionless radius of the contact bilayer ($\rho = R/\delta$, where R is the radius of the contact bilayer). Shown in Fig. 6 are the dependences of the stalk energy F on ρ for some values of spontaneous curvatures of lipid monolayers, which roughly correspond to those of the mixture of PC + LPC (4:1) (curve 1, $J_s = 0.6/\delta$, estimated from [70]); dioleoyl PC (curve 2, $J_s = -0.2/\delta$ [113]); and dioleoyl PE (curve 3, $J_s = -0.7/\delta$ [101]) are shown in Fig. 6. The energy, F_O, of a formed but not yet expanded stalk ($\rho = 0$), decreases with the decrease of spontaneous curvature and becomes negative if $J_s \leqslant -0.88/\delta$. For $J_s = -0.7/\delta$ the energy of a stalk is negative starting from small values of ρ (curve 3). If the spontaneous curvature is not negative enough, the energy, although changing nonmonotonically, remains positive for all values of ρ (curve 2). For $J_s < -0.5/\delta$, the energy F monotonically decreases with the radius and becomes negative, meaning that stalks will appear and grow spontaneously. For positive spontaneous curvature, the energy of a stalk monotonically increases with its radius and is relatively high for all values of ρ (curve 1). Due to thermal fluctuations, the stalk may be formed within a reasonable time (~1 sec) for $F_0 \sim 40\ kT$. Note that this estimate based on the value of the characteristic frequency of bilayer fluctuations, f, $\sim 10^{29}\ s^{-1}\ m^{-2}$ [127] and membrane contact area, a, ~1 μm^2, is actually rather insensitive to the accuracy of the values of f and a, since these characteristics contribute only to the pre-exponential factors.

The qualitative conclusion of the calculations presented is that modifications of the spontaneous curvature of the monolayer change the energy of the stalk and, thus, control its formation and expansion. Shifting the spontaneous curvature of the monolayers to more negative values by adding H_{II}-phase-supporting lipids should promote stalk formation. In contrast, changing the monolayer spontaneous curvature to more positive values by adding micelle-forming lipids should inhibit stalk formation or even prevent it.

An important further development of this theoretical model has been suggested recently by D. Siegel [111]. The three intermediates considered in the modified model were a stalk, a trans monolayer contact (TMC) (analogous to the contract bilayer of a very small area in [12, 65]) and, finally, a fusion pore. Formation of any intermediate structure between two membranes has to be accompanied by the peeling apart of the hydrophobic sides of lipid monolayers to create hydrophobic voids within the intermediate (Fig. 4*f*). The modified stalk model (111) takes into account not only the elastic energy of fusion intermediates but also accounts for additional energetic contributions of these hydrophobic voids which play the central role in this model. The hydrophobic voids of fusion intermediates were considered to have energies per unit volume equal to those of the hydrophobic interstices of the H_{II} phases [111]. These estimates gave very high values for the energies of voids and the related energy of bending of the distal monolayers assumed to dimple, reducing the volumes of hydrophobic voids [111]. As a result, the total energy of the nonexpanded stalk has very large positive values, although dependent on the spontaneous curvature in a way similar to that described by our model. Still higher energy is the price for transformation of the stalk into a TMC. Even for the highly negative spontaneous curvature $J_s = -0.32\ nm^{-1}$ ($J_s \sim -0.7/\delta$) the energies of stalk and TMC obtained by Siegel [111] were still about 60 kT and 150 kT. Let us note that the value of the bending rigidity used in [111] (20 kT), while in agreement with some experimental results, is twice as large as the bending rigidity of strongly curved monolayers recently determined for the H_{II} phases [66, 68, 69]. A correction of the bending rigidity value results in a twofold decrease in the estimated energies of fusion intermediates. The energy of the stalk in the modified model approaches the value we obtained; however, the energy of TMC remains very high (about 75 kT in the most favorable case). Relief of the energy of voids and distal monolayers in TMC came only with the formation of a fusion pore having negative energy. Thus, to fuse, membranes must overcome a very high energy barrier corresponding to the energy of the TMC and caused by the enormous energy of hydrophobic voids. We do not see any real source of energy which would let the lipid bilayers overcome such a barrier. However, we know that model lipid bilayers do fuse.

Both the modified stalk model of Siegel and our model suggest in an implicit way that all lipid molecules in membranes are identical. In such a system, there is only one way (energetically very expensive) to fill hydrophobic voids: to stretch the acyl chains of the lipids

bordering them. However, even model lipid bilayers formed from individual synthetic lipid of >99% purity contain minor impurities. Note that voids in a stalk may be filled by just a few well suited lipid molecules, in contrast to the H_{II} phase, where a high portion of these lipids is required to fill the hydrophobic interstices. Filling the hydrophobic voids with traces of lipids with differing hydrocarbon chain lengths or with apolar lipids [111, 126] would drastically reduce the energies of the fusion intermediates. The energy cost is related to the entropy of the redistribution of these molecules and can be estimated as 1 kT per one molecule. Just a few of such lipids, while representing a small fraction of all lipids bordering the hydrophobic void, may dramatically decrease the void energy in comparison with that assumed in [111]. Filling of the voids by the impurities not only reduces the energy of the voids but also leads to relaxation of a very strong bending of the outer monolayer assumed in [111]. The energy contribution of hydrophobic voids should be even less significant for biological membranes known to consist of very complex mixtures of lipids including apolar ones.

We considered above the energetics of fusion intermediates starting from the nonexpanded stalk. A question arises, however, about the mechanism of stalk formation from initially separated membranes (Fig. 4*b*). To approach each other, membranes have to overcome a strong mutual repulsion. Breaking of the continuity of contacting monolayers results in some exposure of the hydrophobic interior of the monolayers to the surrounding water. In [73] we hypothesized that the energy price for close approach of the membranes and breaking the contacting monolayers is paid by the out-of-plane thermal fluctuations. These fluctuations were found to be powerful enough locally to overcome the hydration forces and push the membranes very close to each other (Fig. 4*b*). In this model, the mechanism of the rupture of contacting monolayer is related to the competition between the hydration forces and the hydrophobic energy of contact between the monolayer interior and water. Estimates show that at small distances between membranes the energy of hydration repulsion becomes so large that it is energetically more favorable to replace it by the hydrophobic energy rupturing the contacting monolayers [73].

Another model developed recently by Helm et al. [47] suggests that the energy for lipid bilayer hemifusion comes solely from long range attractive forces of the hydrophobic interaction between the interiors of the membranes. To support this model experimentally, the authors have shown that stretching of membranes adhered on mica cylinders promotes their hemifusion [46, 47, 72]. These results are consistent with earlier observations on the important role of tension in the promotion of lipid bilayer fusion [34, 94].

Fusion Pore has a Net Positive Curvature

Since monolayers in the same membrane cannot lose contact with each other, stalk expansion results in the compression of contracting monolayers and the extension of distal ones. It brings the contact bilayer under mechanical tension, which in some cases may superpose with preexisting membrane tension such as the one generated by osmotic pressures [34, 94]. To complete fusion, a lipidic fusion pore of critical radius has to form and expand in the contact bilayer (Fig. 4*e,g*). The geometry of a lipidic fusion pore is very similar to that of a stalk but the directions of the lipid monolayer bending in these two structures are opposite. The meridional curvature of the monolayer seen at the cross section of the pore (Fig. 4*e*) is strongly positive and constant all along the pore edge monolayer. This curvature is similar to that of a cylindrical micelle (Fig. 5*d*). In contrast, the parallel curvature is negative and changes its value along the pore edge. Since meridional curvature dominates, the net curvature of the pore edge is positive. Thus, the more positive the spontaneous curvature of the monolayer forming the pore (distal monolayers of the fusing membranes), the more favorable the formation of this structure.

The energetics of a lipidic fusion pore was analyzed in [65], assuming that the elastic energy of the bent lipid monolayer at the edge of the pore provides the main contribution to the overall energy of the pore. Depending on the spontaneous curvature of the monolayer forming its edge, fusion pores can be energetically unfavorable; i.e., have some minimal energy at a finite radius, or tend to expand infinitely. At zero membrane tension and zero spontaneous curvature of the monolayer, the energy of the pore edge is about 10 kT/nm. However the more positive the spontaneous curvature, the more favorable is pore formation and expansion [65].

Recently, the formation and energetics of the fusion pore have been considered both experimentally and theoretically by Nanavati et al. [87]. Detailed investigation of the electrical conductance of fusion pores formed during exocytosis in peritoneal mast cells supported the stalk-pore model of the last stage of the fusion process (Fig. 4*d,e*). Using the theoretical approaches developed earlier [67], Nanavati and coauthors demonstrated that fluctuations of the fusion pore conductance may reflect the fluctuations of the radius of a lipidic pore in the contact bilayer (Fig. 4*e*) when different lipids get into the pore edge. However, interpretation of some theoretical results in [87] is questionable. Indeed, the conclusion of the work that the inverted H_{II} phase-forming lipids with negative spontaneous curvature should promote pore formation is in disagreement with experimental results [14, 129] as well as with theoretical considerations presented above. The formal reason for this result in [87] is related

to the use in one calculation of two opposite conventions concerning the signs of the geometrical and spontaneous curvatures.

Experimental Results are Consistent with Stalk-Pore Model

According to the model, to be best suited for fusion, membranes should be asymmetrical, with the contacting monolayers containing H_{II} phase-promoting lipids and distal monolayers containing micelle-forming lipids. Little is known about lipid compositions of different monolayers of membranes which take part in the highly efficient intracellular fusion reactions of exocytosis and protein trafficking. PE (negative spontaneous curvature) is located predominantly in the outer (contacting) monolayer of the secretory chromaffin granules [138]. The inner (distal) monolayer of the chromaffin granules has a very significant amount of lysolipids in it (up to 17% of the total phospholipid [125, 130]). The inner (distal) monolayer of the Golgi apparatus membranes contains up to 10% of lysolipids [85]. Thus, the lipid asymmetry in these membranes, which are presumably specialized for fusion, is consistent with the predictions of the stalk-pore model on 'ideally fusable' membranes.

The lipid effects on membrane fusion discussed in this work are consistent with the model. Merger of contacting monolayers prior to fusion pore formation was reported both for artificial lipid bilayers and for biological membranes. Lipids of negative spontaneous curvature (AA, OA, PE, cholesterol), which should facilitate stalk formation, promote fusion, and LPC (positive spontaneous curvature) inhibits fusion, if these lipids are present in contacting monolayers of membranes. In contrast, for lipids added to distal monolayers, promotion of fusion by LPC, and its inhibition by AA, is readily explained by the net positive curvature of a fusion pore.

In [14], hemifusion and pore formation were studied in parallel for planar lipid bilayers of PE in the presence of different concentrations of LPC. The exponential increase of the waiting time of hemifusion and linear decrease of the linear tension of pores with LPC concentration, and even the actual ratio between the slopes of these dependences were in quantitative agreement with the theoretical model.

The spontaneous curvature of the lipid monolayer can be modulated by its interaction with amphiphilic peptides. A specific correlation was found by Epand, Yeagle and others ([29, 31, 32, 56, 137], but *see* [118]) between the effects of some peptides on the lamellar to inverted hexagonal H_{II} phase transition and the effects of these peptides on viral fusion and fusion of model membranes. The peptides that shift the spontaneous curvature of lipid monolayers toward positive values (same direction as LPC), inhibited fusion. The peptides that promoted H_{II} phase formation ($J_s < 0$, the effect qualitatively similar to that of AA), promoted fusion as well. Thus, amphiphilic peptides affect fusion in a manner consistent with the model discussed.

Thus, the stalk-pore model of fusion, founded mainly on an analysis of the elasticity of membrane lipid monolayers, is consistent with experimental results and suggests a natural explanation for the known effects of lipid composition on biological fusion and the fusion of purely lipid bilayers. However, three important assumptions of the stalk-pore model: (i) the existence of a hemifusion stage of fusion i.e., merger of membranes prior to fusion pore development; (ii) the specific effects of the composition of distal leaflets of the membranes on fusion pore formation stage; and (iii) the presence of lipids in the edge of the initial fusion pore require additional experimental verification for biological fusion.

Conclusions

The results reviewed suggest that membrane fusion in diverse biological fusion reactions involves formation of some specific intermediates: stalks and pores. Energy of these intermediates and, consequently, the rate and extent of fusion depend on the propensity of the corresponding monolayers of membranes to bend in the required directions.

Proteins and peptides can control the bending energy of membrane monolayers in a number of ways. Monolayer lipid composition may be altered by different phospholipases [50, 85, 90], flipases and translocases [4, 50]. Proteins and peptides can change monolayer spontaneous curvature or hydrophobic void energy by direct interaction with membrane lipids [20, 32, 111]. Proteins may also provide some barriers for lipid diffusion in the plane of the monolayer [83, 141]. If diffusion of lipids at some specific membrane sites (e.g., in the vicinity of fusion protein) is somehow hindered, the energy of the bent fusion intermediates would reflect the elastic properties of these particular sites rather than the spontaneous curvature of the whole monolayers. Proteins may deform membranes while bringing them locally into close contact. The alteration of the geometric (external) curvature will certainly change the elastic energy of the initial state and, thus affect the energetic barriers of the formation of the intermediates [143]. In addition, the area and the energy of the stalk can be reduced by preliminary bending of the contacting membranes [111]. The possible effects of proteins and polymers on local elastic properties and local shapes of the membranes have been recently analyzed [22, 39, 45, 63]. These studies may provide a good basis for future development of theoretical models of protein-mediated fusion.

Various models for biological fusion have been presented as hypothetical sequences of intermediate confor-

mations of proteins, with membrane lipids just covering the empty spaces between the proteins. Although the results discussed above do not allow us to draw an all-explaining cartoon of the fusion mechanism, they do indicate which properties of membrane lipid bilayers (if modified by fusion proteins) would get these bilayers to fuse. In addition, these data suggest a specific geometry to bent fusion intermediates (stalks and pores) and imply a contribution by lipids to the energy of these intermediates. We think that the synthesis of rapidly developing structural information on fusion proteins with the analysis of the physics of membrane rearrangement may soon yield a real understanding of the fascinating and fundamental phenomenon of membrane fusion.

Special thanks to Drs. G. Melikyan, S. Leikin and P. Blank for their valuable input and critical readings of the manuscript; and to Dr. G. Melikyan for kindly providing his previously unpublished results, presented in Fig. 2. Figure 3 was kindly provided by Dr. S. Hui. We would also like to thank Dr. D. Siegel for very helpful discussions. We are grateful to Drs. R. Holz and J. Wilschut for allowing us to cite their unpublished results. Finally, Lynn Kelly provided invaluable help with preparation of the manuscript.

References

1. Almers, W., Tse, F.W. 1990. *Neuron* **4:**813–818
2. Bentz, J., Ellens, H., Lai, M.Z., Szoka, F.C., Jr. 1985. *Proc. Natl. Acad. Sci. USA* **82:**5742–5745
3. Bienvenue, A., Sainte-Marie, J. 1994. *In:* Current Topics in Membranes. D. Hoekstra, editor. pp. 319–354. Academic Press, San Diego
4. Bogdanov, A., Verhoven, B., Schlegel, R.A., Williamson, P. 1993. *Biochem. Soc. Trans.* **21:**271–275
5. Breckenridge, L.J., Almers, W. 1987. *Nature* **328:**814–817
6. Bron, R., Wahlberg, J.M., Garoff, H., Wilschut, J. 1993. *EMBO J.* **12:**693–701
7. Bullough, P.A., Hughson, F.M., Skehel, J.J., Wiley, D.C. 1994. *Nature* **371:**37–43
8. Chandler, D.E., Bennett, J.P., Gomperts, B. 1983. *J. Ultrastruct. Res.* **82:**221–232
9. Chandler, D.E., Heuser, J.E. 1980. *J. Cell Biol.* **86:**666–674
10. Chen, S.Y., Cheng, K.H., Ortalano, D.M. 1990. *Chem. Phys. Lipids* **53:**321–329
11. Chernomordik, L.V., Chanturiya, A.N., Green,J., Zimmerberg, J. 1994. *Biophys. J.* **66:**A144.
12. Chernomordik, L.V., Kozlov, M.M., Leikin, S.L., Markin, V.S., Chizmadzhaev Yu, A. 1986. *Dokl Akad Nauk SSSR* **288:**1009–1013
13. Chernomordik, L.V., Leikina, E.A., Zimmerberg, J. 1995. *Virology* **69:**3049–3058
14. Chernomordik, L.V., Melikyan, G.B., Abidor, I.G., Markin, V.S., Chizmadzhev, Y.A. 1985. *Biochim. Biophys. Acta* **812:**643–655
15. Chernomordik, L.V., Melikyan, G.B., Chizmadzhev, Y.A. 1987. *Biochim. Biophys. Acta* **906:**309–352
16. Chernomordik, L.V., Vogel, S.S., Sokoloff, A., Onaran, H.O., Leikina, E.A., Zimmerberg, J. 1993. *FEBS. Lett.* **318:**71–76
17. Cohen, F.S., Akabas, M.H., Zimmerberg, J., Finkelstein, A. 1984. *J. Cell Biol.* **98:**1054–1062
18. Conti, C., Mastromarino, P., Orsi, N. 1991. *Comp. Immunol. Microbiol. Infect. Dis.* **14:**303–313
19. Creutz, C.E. 1981. *J. Cell Biol.* **91:**247–256
20. Cullis, P.R., Tilcock, C.P., Hope, M.J. 1991. *In:* Membrane Fusion. J. Wilschut, D. Hoekstra, editors. pp. 35–64. Marcel Dekker, The Netherlands
21. Curran, M.J., Cohen, F.S., Chandler, D.E., Munson, P.J., Zimmerberg, J. 1993. *J. Membrane Biol.* **133:**61–75
22. Dan, N., Berman, A., Pincus, P., Safran, S.A. 1994. *J. Phys.* **4:**1713–1725
23. Devaux, P.F. 1992. *Annu. Rev. Biophys. Biomol. Struct.* **21:**417–439
24. Duzgunes, N., Straubinger, R.M., Baldwin, P.A., Friend, D.S., Papahadjopoulos, D. 1985. *Biochemistry* **24:**3091–3098
25. Duzgunes, N., Wilschut, J., Fraley, R., Papahadjopoulos, D. 1981. *Biochim. Biophys. Acta* **642:**182–195
26. Elhai, J., Scandella, C.J. 1983. *Exp. Cell. Res.* **148:**63–71
27. Ellens, H., Bentz, J., Szoka, F.C. 1986. *Biochemistry* **25:**285–294
28. Epand, R.M. 1985. *Biochemistry* **24:**7092–7095
29. Epand, R.M. 1986. *Biosci. Rep.* **6:**647–653
30. Epand, R.M., Bottega, R. 1987. *Biochemistry* **26:**1820–1825
31. Epand, R.M., Cheetham, J.J., Epand, R.F., Yeagle, P.L., Richardson, C.D., Rockwell, A., Degrado, W.F. 1992. *Biopolymers* **32:**309–314
32. Epand, R.M., Epand, R.F. 1994. *Biochem. Biophys. Res. Commun.* **202:**1420–1425
33. Epand, R.M., Epand, R.F., Ahmed, N., Chen, R. 1991. *Chem. Phys. Lipids* **57:**75–80
34. Finkelstein, A., Zimmerberg, J., Cohen, F.S. 1986. *Annu. Rev. Physiol.* **48:**163–174
35. Gawrisch, K., Parsegian, V.A., Hajduk, D.A., Tate, M.W., Gruner, S.M., Fuller, N.L., Rand, R.P. 1992. *Biochemistry* **31:**2856–2864
36. Gelbart, W.M., Roux, D., Ben-Shaul, A. 1994. *Modern Ideas and Problems in Amphiphilic Sciences.* Springer; Berlin.
37. Glick, B.S., Rothman, J.E. 1987. *Nature* **326:**309–312
38. Golan, D.E., Furlong, S.T., Brown, C.S., Caulfield, J.P. 1988. *Biochemistry* **27:**2661–2667
39. Goulian, M., Bruinsma, R., Pincus, P. 1993. *Europhys. Lett.* **22:**145–150
40. Gruner, S.M. 1985. *Proc. Natl. Acad. Sci. USA* **82:**3665–3669
41. Gruner, S.M., Cullis, P.R., Hope, M.J., Tilcock, C.P. 1985. *Annu. Rev. Biophys. Biophys. Chem.* **14:**211–238
42. Harbich, W., Servuss, R.M., Helfrich, W. 1978. *Z. Naturforsch.* **33a:**1013–1017
43. Helfrich, W. 1973. *Z. Naturforsch.* **28c:**693–703
44. Helfrich, W. 1978. *Z. Naturforsch.* **33a:**305–315
45. Helfrich, W., Kozlov, M.M. 1994. *J. Phys.* **4:**1427–1438
46. Helm, C.A., Israelachvili, J.N., McGuiggan, P.M. 1989. *Science* **246:**919–922
47. Helm, C.A., Israelachvili, J.N., McGuiggan, P.M. 1992. *Biochemistry* **31:**1794–1805
48. Herrmann, A., Clague, M.J., Blumenthal, R. 1993. *Membr. Biochem.* **10:**3–15
49. Herrmann, A., Clague, M.J., Puri, A., Morris, S.J., Blumenthal, R., Grimaldi, S. 1990. *Biochemistry* **29:**4054–4058
50. Hinkovska-Galchev, V., Srivastava, P.N. 1992. *Mol. Reprod. Dev.* **33:**281–286
51. Hoekstra, D., Kok, J.W. 1989. *Biosci. Rep.* **9:**273–305
52. Hope, M.J., Cullis, P.R. 1981. *Biochim. Biophys. Acta* **640:**82–90
53. Hui, S.W., Sen, A. 1989. *Proc. Natl. Acad. Sci. USA* **86:**5825–5829
54. Hui, S.W., Stewart, T.P., Boni, L.T., Yeagle, P.L. 1981. *Science* **212:**921–923
55. Israelachvili, J.N., Mitchell, D.J., Ninham, B.W. 1976. *J. Chem. Soc., Faraday Trans. 2* **72:**1525–1568

56. Kelsey, D.R., Flanagan, T.D., Young, J.E., Yeagle, P.L. 1991. *Virology* **182:**690–702
57. Kemble, G.W., Danieli, T., White, J.M. 1994. *Cell* **76:**383–391
58. Kielian, M.C., Helenius, A. 1984. *J. Virol.* **52:**281–283
59. Kirk, G.L., Gruner, S.M., Stein, S.L. 1984. *Biochemistry* **23:**1093–1102
60. Klausner, R.D., Bhalla, D.K., Dragsten, P., Hoover, R.L., Karnovsky, M.J. 1980. *Proc. Natl. Acad. Sci. USA* **77:**437–441
61. Knoll, G., Braun, C., Plattner, H. 1991. *J. Cell Biol.* **113:**1295–1304
62. Koynova, R., Caffrey, M. 1994. *Chem. Phys. Lipids* **69:**1–34
63. Kozlov, M.M., Helfrich, W. 1993. *Langmuir* **9:**2761–2763
64. Kozlov, M.M., Leikin, S., Rand, R.P. 1994. *Biophys. J.* **67:**1603–1611
65. Kozlov, M.M., Leikin, S.L., Chernomordik, L.V., Markin, V.S., Chizmadzhev, Y.A. 1989. *Eur. Biophys. J.* **17:**121–129
66. Kozlov, M.M., Leikin, S.L., Markin, V.S. 1989. *J. Chem. Soc. Faraday Trans. 2* **85:**277–292
67. Kozlov, M.M., Markin, V.S. 1983. *Biofizika* **28:**255–261
68. Kozlov, M.M., Winterhalter, M. 1991. *J. Phys.* **1:**1085–1100
69. Kozlov, M.M., Winterhalter, M. 1991. *J. Phys.* **1:**1077–1084
70. Kumar, V. 1991. *Proc. Natl. Acad. Sci. USA* **88:**444–448
71. Kumar, V.V., Malewicz, B., Baumann, W.J. 1989. *Biophys. J.* **55:**789–792
72. Leckband, D.E., Helm, C.A., Israelachvili, J. 1993. *Biochemistry* **32:**1127–1140
73. Leikin, S.L., Kozlov, M.M., Chernomordik, L.V., Markin, V.S., Chizmadzhev, Y.A. 1987. *J. Theor. Biol.* **129:**411–425
73a. Lentz, B.R. 1994. *Chem. Phys. Lipids* **73:**91–106
73b. Lentz, B.R., McIntyre, G.F., Parks, D.J., Yates, J.C., Massenburg, D. 1992. *Biochemistry* **31:**2643–2653
74. Liberman, E.A., Nenashev, V.A. 1972. *Biofizika* **17:**1017–1023
75. Llanos, M.N., Morales, P., Riffo, M.S. 1993. *J. Exp. Zool.* **267:**209–216
76. Luzatti, V. 1968. *In:* Biological Membranes. D. Chapman, editor. pp. 71–123. Academic Press, New York.
77. Markin, V.S., Kozlov, M.M., Borovjagin, V.L. 1984. *Gen. Physiol. Biophys.* **3:**361–377
78. Martin, I., Dubois, M.C., Saermark, T., Epand, R.M., Ruysschaert, J.M. 1993. *FEBS. Lett.* **333:**325–330
79. Mayorga, L.S., Colombo, M.I., Lennartz, M., Brown, E.J., Rahman, K.H., Weiss, R., Lennon, P.J., Stahl, P.D. 1993. *Proc. Natl. Acad. Sci. USA* **90:**10255–10259
80. Meers, P., Hong, K., Papahadjopoulos, D. 1988. *Biochemistry* **27:**6784–6794
81. Melikyan, G.B., Niles, W.D., Cohen, F.S. 1993. *J. Gen. Physiol.* **102:**1151–1170
82. Melikyan, G.B., Niles, W.D., Peeples, M.E., Cohen, F.S. 1993. *J. Gen. Physiol.* **102:**1131–1149
83. Monck, J.R., Fernandez, J.M. 1992. *J. Cell Biol.* **119:**1395–1404
84. Monck, J.R., Oberhauser, A.F., Alvarez de Toledo, G., Fernandez, J.M. 1991. *Biophys. J.* **59:**39–47
85. Moreau, P., Morre, D.J. 1991. *J. Biol. Chem.* **266:**4329–4333
86. Mutz, M., Helfrich, W. 1990. *J. Phys.* **51:**991–1002
87. Nanavati, C., Markin, V.S., Oberhauser, A.F., Fernandez, J.M. 1992. *Biophys. J.* **63:**1118–1132
88. Neher, E. 1974. *Biochim. Biophys. Acta* **373:**327–336
89. Nieva, J.L., Bron, R., Corver, J., Wilschut, J. 1994. *EMBO. J.* **13:**2797–2804
90. Nieva, J.L., Goni, F.M., Alonso, A. 1993. *Biochemistry* **32:**1054–1058
91. Nussbaum, O., Rott, R., Loyter, A. 1992. *J. Gen. Virol.* **72:**2831–2837
92. Oberhauser, A.F., Fernandez, J.M. 1993. *Biophys. J.* **65:**1844–1852
93. Oberhauser, A.F., Monck, J.R., Fernandez, J.M. 1992. *Biophys. J.* **61:**800–809
94. Ohki, S. 1984. *J. Membrane Biol.* **77:**265–275
95. Ornberg, R.L., Reese, T.S. 1981. *J. Cell Biol.* **90:**40–54
96. Paiement, J., Lavoie, C., Gavino, G.R., Gavino, V.C. 1994. *Biochim. Biophys. Acta* **1190:**199–212
97. Parsegian, V.A., Ninham, B.W. 1971. *J. Colloid Interface Sci.* **37:**332–341
98. Perin, M.S., MacDonald, R.C. 1989. *J. Membrane Biol.* **109:**221–232
99. Pinto da Silva, P., Nogueira, M.L. 1977. *J. Cell. Biol.* **73:**161–181
100. Poole, A.R., Howell, J.I., Lucy, J.A. 1970. *Nature* **227:**810–814
101. Rand, R.P., Fuller, N.L. 1994. *Biophys. J.* **66:**2127–2138
102. Rand, R.P., Parsegian, V.A. 1989. *Biochim. Biophys. Acta* **988:**351–376
103. Rilfors, L., Lindblom, G., Wieslander, A., Christiansson, A. 1984. *In:* Membrane Fluidity. M. Kates, L.A. Manson, editors. pp. 205–245. Plenum, New York
104. Roos, D.S., Duchala, C.S., Stephensen, C.B., Holmes, K.V., Choppin, P.W. 1990. *Virology* **175:**345–357
105. Rosenberg, J., Duzgunes, N., Kayalar, C. 1983. *Biochim. Biophys. Acta* **735:**173–180
106. Rothman, J.E. 1994. *Nature* **372:**55–63
107. Sarkar, D.P., Morris, S.J., Eidelman, O., Zimmerberg, J., Blumenthal, R. 1989. *J. Cell Biol.* **109:**113–122
108. Schudt, C., Pette, D. 1976. *Cytobiologie* **13:**74–84
109. Seddon, J., Templer, R. 1993. *Phil. Trans. R. Soc. Lond. A* **344:**377–401
110. Siegel, D.P. 1986. *Biophys. J.* **49:**1171–1183
111. Siegel, D.P. 1993. *Biophys. J.* **65:**2124–2140
112. Siegel, D.P., Green, W.J., Talmon, Y. 1994. *Biophys. J.* **66:**402–414
113. Sjolund, M., Lindblom, G., Rilfors, L., Arvidson, G. 1987. *Biophys. J.* **52:**145–153
114. Song, L.Y., Ahkong, Q.F., Georgescauld, D., Lucy, J.A. 1991. *Biochim. Biophys. Acta* **1065:**54–62
115. Spruce, A.E., Breckenridge, L.J., Lee, A.K., Almers, W. 1990. *Neuron* **4:**643–654
116. Spruce, A.E., Iwata, A., White, J.M., Almers, W. 1989. *Nature* **342:**555–558
117. Stamatatos, L., Duzgunes, N. 1993. *J. Gen. Virol.* **74:**1043–1054
118. Stegmann, T. 1993. *J. Biol. Chem.* **268:**26886–26892
119. Stegmann, T., Doms, R.W., Helenius, A. 1989. *Annu. Rev. Biophys. Biophys. Chem.* **18:**187–211
120. Tse, F.W., Iwata, A., Almers, W. 1993. *J. Cell Biol.* **121:**543–552
121. Verkleij, A.J., Leunissen-Bijvelt, J., de Kruijff, B., Hope, M., Cullis, P.R. 1984. *Ciba Found. Symp.* **103:**45–59
122. Vogel, S.S., Chernomordik, L.V., Zimmerberg, J. 1992. *J. Biol. Chem.* **267:**25640–25643
123. Vogel, S.S., Delaney, K., Zimmerberg, J. 1991. *Ann. N.Y. Acad. Sci.* **635:**35–44
124. Vogel, S.S., Leikina, E.A., Chernomordik, L.V. 1993. *J. Biol. Chem.* **268:**25764–25768
125. Voyta, J.C., Slakey, L.L., Westhead, E.W. 1978. *Biochem. Biophys. Res. Commun.* **80:**413–417
126. Walter, A., Yeagle, P.L., Siegel, D.P. 1994. *Biophys. J.* **66:**366–376
127. Weaver, J.C., Mintzer, R.A. 1981. *Phys. Lett.* **86A:**57–59
128. Weiss, C.D., White, J.M. 1993. *J. Virol.* **67:**7060–7066
129. Weltzien, H.U. 1979. *Biochim. Biophys. Acta* **559:**259–287
130. Westhead, E.W. 1987. *Ann. N.Y. Acad. Sci.* **493:**92–100

131. White, J., Helenius, A. 1980. *Proc. Natl. Acad. Sci. USA* **77:**3273–3277
132. White, J.M. 1992. *Science* **258:**917–924
133. Wilschut, J. 1991. *In:* Membrane Fusion. J. Wilschut, D. Hoekstra, editors. pp. 89–126. Marcel Dekker, New York
134. Wilson, I.A., Skehel, J.J., Wiley, D.C. 1981. *Nature* **289:**366–373
135. Yeagle, P.L. 1989. *Faseb. J.* **3:**1833–1842
136. Yeagle, P.L., Smith, F.T., Young, J.E., Flanagan, T.D. 1994. *Biochemistry* **33:**1820–1827
137. Yeagle, P.L., Young, J., Hui, S.W., Epand, R.M. 1992. *Biochemistry* **31:**3177–3183
138. Zachowski, A., Henry, J.P., Devaux, P.F. 1989. *Nature* **340:**75–76
139. Zimmerberg, J. 1993. *Methods Enzymol.* **221:**99–112
140. Zimmerberg, J., Blumenthal, R., Curran, M., Sarkar, D., Morris, S. 1994. *J. Cell Biology.* **127:**1885–1894
141. Zimmerberg, J., Curran, M., Cohen, F.S. 1991. *Ann. N.Y. Acad. Sci.* **635:**307–317
142. Zimmerberg, J., Curran, M., Cohen, F.S., Brodwick, M. 1987. *Proc. Natl. Acad. Sci. USA* **84:**1585–1589
143. Zimmerberg, J., Vogel, S.S., Chernomordik, L.V. 1993. *Annu. Rev. Biophys. Biomol. Struct.* **22:**433–466