Membranes of the world unite!

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Despite diverse origins, cellular fusion mechanisms converge at a pathway of phospholipid bilayer fusion. In this mini-review, we discuss how proteins can mediate each of the three major stages in the fusion pathway: contact, hemifusion, and the opening of an expanding fusion pore.

Introduction

The ubiquitous and dynamic remodeling of membranes through fusion and fission defines cellular compartmental organization (Jahn et al., 2003; Shemer and Podbilewicz, 2003; Kielian and Rey, 2006). Controlling membrane dynamics in developmental intercellular fusion, intracellular trafficking, and cell invasion by enveloped viruses and parasites may lead to new strategies for quelling diseases. An understanding of membrane remodeling at the physicochemical level that might guide the development of such strategies requires interdisciplinary investigation of protein–lipid interactions. Only proteins have sufficient complexity and information content to organize and regulate membranes, whereas fusion and fission ultimately unite and separate membrane lipids.

In this mini-review, we focus on the hypothesis that all fusion is essentially lipidic at its core (Cohen et al., 1980; Zimmerberg et al., 1980). The hemifusion–fusion or stalk–pore pathway of membrane fusion was identified and explored first in theoretical work and experiments on artificial protein-free bilayers (Kozlov and Markin, 1983; Chernomordik et al., 1987; Lee and Lentz, 1997), and then in viral fusion (for review see Chernomordik and Kozlov, 2003), in intracellular fusion (Chernomordik et al., 1993; Lu et al., 2005; Reese et al., 2005; Xu et al., 2005), and, most recently, in developmental cell fusion (Podbilewicz et al., 2006). This pathway starts with hemifusion, a stalklike connection between the contacting membrane leaflets where the distal leaflets and the aqueous inner contents remain distinct (Fig. 1, C and D). Hemifusion is followed by the opening of an expanding lipidic fusion pore to complete the fusion reaction (Fig. 1, E and F). Each of the essential stages of the pathway will be first described for lipid bilayers, and then for biological membranes with an emphasis on the mechanisms by which proteins may drive each stage. An alternative pathway (Breckenridge and Almers, 1987; Jackson and Chapman, 2006), featuring a proteinaceous gap junction–like fusion pore, is discussed in Chernomordik and Kozlov (2005).

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Abbreviations used in this paper: FP, fusion peptide; HD, hemifusion diaphragm; LPC, lyso PC; PC, phosphatidylcholine; OA, oleic acid; TMD, transmembrane domain.

Contact between membranes

Contact between lipid bilayers. Contact of two lipid bilayers is determined by the thickness of a layer of water separating the polar heads of lipids at equilibrium (Luzzati planes; Rand and Fuller, 1994). For lipid bilayers without surface electric charge, this equilibrium distance is set by the interplay between intermembrane interactions, such as long-range Van der Waals attraction, short-range repulsive interactions referred to as hydration forces (Rand and Parsegian, 1989), and an effective repulsion originating from bilayer undulations (Helfrich, 1988). Based on x-ray measurements, the characteristic values of the interbilayer distances for most biologically ubiquitous lipids, such as phosphatidylcholine (PC), are 2–3 nm (Rand and Parsegian, 1989). These distances are only a few times larger than the dimensions of the lipid polar groups. Hence, they are comparable to the scale of membrane surface roughness.

Contact between fusing biological membranes. Initial contact between fusing biological membranes is fundamentally different from that between two protein-free bilayers. First of all, the distance between bilayers of biological membranes is as wide as 10–20 nm, and the contact is almost always mediated by tethering molecules. The contact zone is crowded with membrane-associated proteins, including those involved in membrane binding and fusion (Fig. 1 A). For some membranes, such as the envelopes of alphaviruses, protein networks are very tight and coat the membrane surface.

The intimate contact of fusion requires an opening of protein-depleted patches in the opposed membranes (Fig. 1 B). This may additionally crowd the proteins outside of these patches. Interactions between membrane proteins and the cytoskeleton can restrict protein mobility along the membrane surface, hindering the displacement of proteins. The subcortical actin meshwork itself can prevent direct contact of protein-free patches (Eitzen, 2003). These obstacles must be removed or considerably weakened to enable an appropriate bilayer contact. If these obstacles cannot be removed, fusion is inhibited. Clinically, inhibitors of this protein-displacement fusion stage

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can act as potent and broad-range antiviral agents. For instance, multivalent lectins of the innate immunity system block membrane fusion during enveloped virus entry by cross-linking sugar moieties of membrane-surface proteins (Leikina et al., 2005). The resulting network of immobilized glycoproteins decreases the access of membrane bilayers to each other, inhibiting fusion. This strategy may be exploited for the discovery of antifusion drugs.

In productive fusion, plasma membrane patches partially depleted of proteins can be generated by a local disruption of the cytoskeleton network adjacent to the membrane, resulting in partial shrinkage of the otherwise stretched network to reposition many integral membrane proteins (Kozlov et al., 1990).

A more general way of facilitating protein displacement is to produce a very limited area of tight contact between bilayers of biological membranes. Fusion proteins may bring two bound membranes into very close contact by acting on only one of them. For instance, in fusion mediated by a homotrimeric influenza virus HA, insertion of its functionally essential amphipathic "fusion peptide" (FP) domains (Tamm, 2003) into the viral membrane and subsequent restructuring of the protein generate deformation of the viral bilayer in the vicinity of HA trimer. To minimize the energy of deformation, trimers assemble into ringlike clusters and dimple the viral envelope with protein-depleted top toward the target membrane (Kozlov and Chernomordik, 1998).

Fusion proteins may also pull bilayers together by acting on both of the membranes (Fig. 1 B). Viral fusion proteins are anchored in the viral envelope by their transmembrane domains (TMDs) and, under fusion conditions, insert their FP into the target membrane. As a result, the two membrane-inserted domains of the protein are positioned in different membranes. Further conformational changes "zipper" the protein into a hairpin-like shape with TMD and FP at the same side of a rigid structure, thus, bringing the two membranes into close proximity. The bilayer contact of intracellular fusion involves a formation of hairpin structure composed of membrane proteins anchored in opposing membranes (Jahn et al., 2003). For both viral and intracellular fusion, the interbilayer distance reached by bridging membranes with hairpin structure can be close to the hairpin thickness constituting several nanometers, and might be further decreased by insertion of the membrane-proximal regions of the hairpin into membranes (Kweon et al., 2003).

Hemifusion

Hemifusion of lipid bilayers. Establishment of a protein-free contact, although a prerequisite, is insufficient for hemifusion, even when the membranes are separated by only 2–3-nm gaps (Rand and Parsegian, 1989). Hemifusion is observed only for specific lipid compositions and specific ions in the aqueous bathing solution or upon dehydration of the intramembrane contact (Lee and Lentz, 1997; Chernomordik and Kozlov, 2003). Special conditions that promote hemifusion are characterized by a common property; in the initial state, the membrane monolayers accumulate energy, which is released upon hemifusion.

Hemifusion-driving energy will accumulate if the curvature of the contacting membrane monolayers differs from their spontaneous curvature. The spontaneous curvature of a lipid is determined as the preferred curvature of a monolayer formed by this lipid (Chernomordik and Kozlov, 2003). The spontaneous curvature characterizes the effective shape of lipid in a monolayer that fully reflects interactions of lipid molecules between themselves, as well as with the bathing solution. A monolayer that tends to bulge spontaneously toward the layer of polar heads is seen to consist of molecules having an effective shape of inverted cones, and its spontaneous curvature is conventionally defined as positive. A lipid monolayer that bulges spontaneously toward the hydrocarbon tails has a negative spontaneous curvature, and is described as consisting of cone-shaped lipid molecules. Based on experimental studies, lyso PC (LPC) has positive spontaneous curvature, whereas cone-shaped phosphatidylethanolamine, oleic acid (OA), diacylglycerol, and probably cholesterol at a moderate membrane concentration, have negative spontaneous curvature. If the curvature of the monolayer in the bilayer deviates from its spontaneous curvature, the monolayer is under elastic stress and, if allowed, would

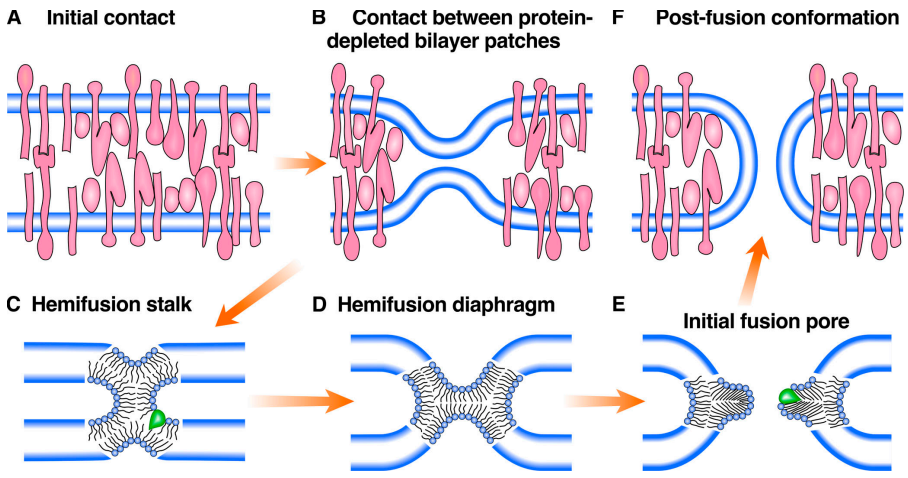

Figure 1. **Membrane fusion through hemifusion intermediates.** At the state of initial contact (A), lipid bilayers of biological membranes are covered by membrane proteins (pink shapes) including, among others, proteins that mediate membrane binding and fusion. Membrane-associated proteins move apart to allow local close contact between two membrane bilayers (B) and a merger of their contacting leaflets into a stalklike hemifusion connection (C) that expands into a small HD (D). A lipidic fusion pore opens in a HD (E). This pore gives rise to an hourglass fusion pore (F), expansion of which completes the fusion reaction. Blue lines show the bilayer surfaces formed by lipid polar heads. When present in contacting membrane leaflets, inverted cone–shaped lipids such as LPC (shown in green) do not fit into the curvature of the lipid monolayer forming a stalk intermediate (C) and inhibit hemifusion. When added to distal leaflets, the same lipid fits the curvature of the fusion pore edge (E) and promotes pore opening.

release this stress by bending toward its spontaneous curvature. Theory indicates that negative spontaneous curvature of monolayer favors hemifusion, and that positive spontaneous curvature hinders hemifusion (Chernomordik and Kozlov, 2003). These predicted effects were borne out in experiments on the dependence of fusion on bilayer composition (Chernomordik and Kozlov, 2003).

Hemifusion might also be boosted by distortion of lipid monolayer packing by inclusions such as amphiphilic peptides (Tamm, 2003), which generate monolayer deformation of a complex character, including bending and tilting of the lipid hydrocarbon chains in respect to the monolayer plane (Hamm and Kozlov, 1998). Inclusions would promote hemifusion only if the latter releases the elastic stresses.

Finally, bilayers hemifuse when brought to distances much smaller than the equilibrium one by external effects. These effects might be produced by adding polyethylene glycol to draw water from the contact zone (Lee and Lentz, 1997), or by a direct dehydration of the membrane contact in a multilamellar lipid sample (Yang et al., 2003). When bilayers are separated by only 1 nm, the accumulated energy of intermembrane hydration repulsion is expected to drive fusion because formation of a fusion stalk and its expansion into a hemifusion diaphragm (HD) partially relaxes the hydration energy (Kozlovsky et al., 2004). Therefore, at these interbilayer distances, hemifusion becomes energetically favorable, as is observed experimentally (Yang et al., 2003).

Hemifusion of biological membranes. Hemifusion of biological membranes is operationally defined as lipid mixing without aqueous content mixing and/or as lipid mixing between contacting leaflets of the membranes in the absence of lipid mixing between distal membrane leaflets. Formation of a single expanding fusion pore identifies the fusion event as a complete fusion (lipid and content mixing), even if there are hundreds of hemifusion sites present. Thus, to detect hemifusion, complete fusion is inhibited by lowering temperature, modifying fusogenic proteins and decreasing their numbers (Kemble et al., 1994; Melikyan et al., 1997; Chernomordik et al., 1998). Hemifusion intermediates can be also stabilized by altering lipid composition. The effects of lipids on viral fusion, intracellular organelle fusion, and exocytosis (Chernomordik et al., 1993; Chernomordik and Kozlov, 2003; Reese and Mayer, 2005) are similar to those previously discussed for protein-free bilayers. For instance, OA in the contacting and distal membrane leaflets promotes hemifusion and, as discussed in Fusion pores in biological membranes, inhibits breaking of the hemifusion structure into a fusion pore (Chernomordik et al., 1998). Thus, adding OA to the fusing biological membranes is expected to and, indeed, facilitates detection of hemifusion.

Identification of hemifusion as lipid mixing without content mixing has several limitations, chiefly (a) the masking of hemifusion by protein–membrane interactions that restrict lipid flux in viral fusion (Chernomordik et al., 1998), and (b) the masking of complete fusion events that yield pores too transient or too small to allow detectable content mixing (Zimmerberg et al., 1994). Additional complications arise from the dynamics of hemifusion intermediates (Chernomordik and Kozlov, 2005; Giraudo et al., 2005). Because detection of hemifusion relies upon the integrated lipid flux over time, membranes may have dissociated by the time of assay. In spite of these methodological difficulties, the hemifusion phenotype has been established in many fusion reactions. Although opening of a fusion pore within a hemifusion connection awaits unambiguous demonstration, diverse lines of indirect evidence, including similar lipid dependences of biological fusion and fusion between artificial lipid bilayers, and the ability of dissimilar fusion proteins to mediate hemifusion, suggest the central place of hemifusion in protein-mediated fusion (Chernomordik and Kozlov, 2005).

To create conditions like those that promote hemifusion in protein-free bilayers, fusion proteins have to accumulate sufficient energy within the membrane bilayers and provide mechanisms that release this energy upon hemifusion. Let us consider some of the possible scenarios.

To drive hemifusion by generating the elastic stresses of the mismatch between the actual curvature of membrane leaflets and their spontaneous curvature, proteins might change the lipid composition of contacting leaflets of membranes to that with negative spontaneous curvature (Fig. 2 A). Hence, phospholipases and acyltransferases that initiate enzymatic cascades leading to increased concentrations of such lipids as diacylglycerol and phosphatidylethanolamine may promote hemifusion. Lipid-modifying enzymes have, indeed, been implicated in some intracellular fusion reactions (Barona et al., 2005). Note, the flux of lipids out of their site of synthesis must be slowed down to accumulate a sufficiently large local concentration of fusogenic lipids. Restriction of lipid flow across the fusion site is, indeed, observed at the early stages of viral fusion (Zimmerberg et al., 1994; Chernomordik et al., 1998; Zaitseva et al., 2005).

For the elastic stresses causing hemifusion to be driven by a distortion of bilayer packing, fusion proteins have to interfere with the bilayer structure. For example, fusion mediated by influenza HA critically depends on a specific boomerang-like conformation of the membrane-inserted FP that is hypothesized to produce a bilayer distortion required for hemifusion (Tamm, 2003). Note, however, that mechanisms of this kind can only work if fusion allows a relaxation of the stresses induced by the distortions. Current models do not account for this crucial step and, therefore, have to be developed further to offer a plausible scenario for fusion mediated by membrane inclusions.

Fusion proteins might drive hemifusion by producing bending stresses in bilayers (Fig. 2 B). For instance, membrane-bulging deformations that bring bilayers of two biological membranes into a very close contact also generate stresses in the protein-depleted patches of bilayers at the top of the bulges (Kozlov and Chernomordik, 1998; Kuzmin et al., 2001). Hemifusion between these bulges or between bulges and flat bilayer of the target membrane relieves the bending stress of the outer leaflets of the bulged membranes. The energy for stressing the bilayer can come from protein restructuring or protein–membrane or protein–protein interactions. Let us first estimate the minimal energy release required from one fusion protein to enable fusion. The energy of the initial fusion intermediate—the fusion stalk—is a few tens of kilocalories per mole, and its

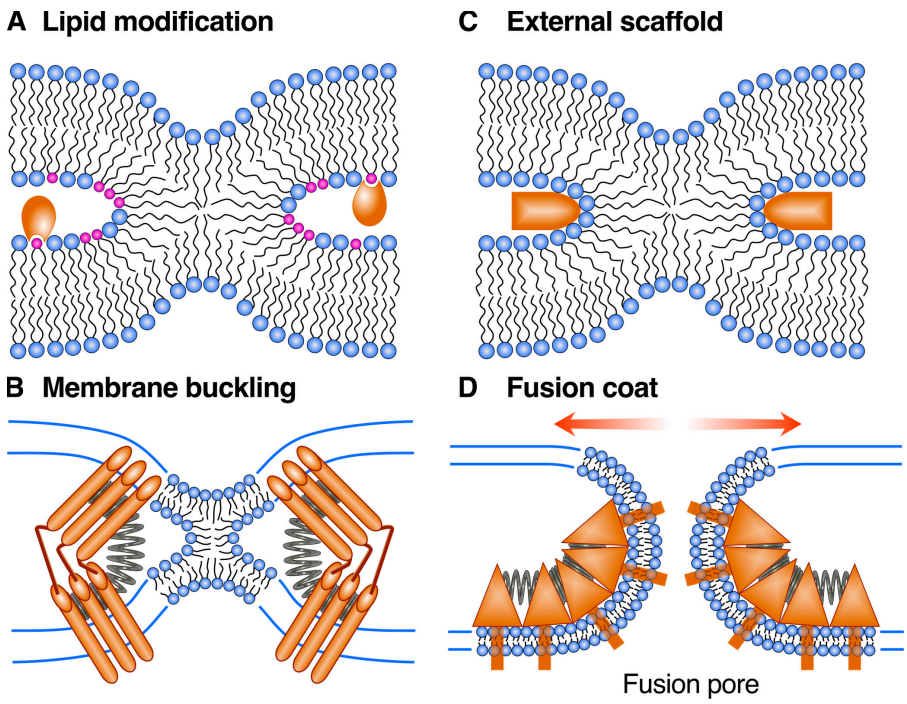

Figure 2. **Mechanisms by which fusion proteins might promote hemifusion and fusion pore development.** (A) Fusion proteins (shown as orange shapes) might change local lipid composition by generating fusogenic lipids (shown in pink) of contacting leaflets of the fusing membranes to that promoting hemifusion. Lipid-modifying enzymes might also change the composition of distal leaflets to that promoting pore development (not shown). (B) Folding of fusion proteins (shown as the release of an extended spring) might drive hemifusion and fusion by producing bending stresses in bilayers bulged toward each other. (C) Lipids might be scaffolded onto surfaces of fusion proteins. For instance, protein scaffold located outside the hemifusion connection might present a positively charged electrostatic surface that would bind negatively charged lipids, and facilitate hemifusion and provide a handle for "pulling" the stalk open. (D) Proteins might develop a dense interconnected protein coat around the fusion site. Because of protein–protein interactions and protein shape, the protein coat has an intrinsic curvature (shown here as springs). This coat "wants" to deform the underlying lipid bilayers, thus, producing the lateral tension that drives the transition from hemifusion to opening an expanding fusion pore.

characteristic area is ~100 nm^2 (Kozlovsky and Kozlov, 2002). Hence, the stress (energy per unit area) needed to drive this fusion stage should be at least ~0.1 kcal/mol · nm^2 ~0.7 mJ/m^2.

Assuming that the fusion proteins form a ring around the fusion site, and that the diameter of one fusion protein in the membrane plane is ~5 nm, the energy required from one fusion protein is only a few kilocalories per mole. Both fusion protein refolding and FP–membrane interactions might readily provide the required energy (Kozlov and Chernomordik, 1998). Sufficient energy for hemifusion can be also released by moderately strong protein–protein interactions based on electrostatic and hydrophobic forces or hydrogen bonds, such as the interactions in actin assembly and antibody–antigen binding (dissociation constant in micromolar range). In contrast, interactions mediated by membrane elasticity, such as the aggregation of membrane proteins based on a mismatch between the length of their TMD and membrane thickness, release <1 kcal/mol (Weikl et al., 1998) and, thus, are too weak to drive hemifusion. Expansion of a stalk into HD requires much more energy than stalk formation. The fusion proteins have to produce ~100 pN force acting on the HD rim (Kozlovsky et al., 2002). Generation of such force requires the energy released per protein to reach the value of a few tens of kilocalories per mole, which may be provided by fusion protein refolding (Kozlov and Chernomordik, 1998).

Proteins must be sufficiently rigid to effectively transmit the released energy into bilayer stress, i.e., the effective bending rigidity of fusion protein domains or multiprotein structures has to exceed the bending rigidity of a lipid bilayer. For example, zippering of fusion proteins into hairpin conformations will bulge the bilayers toward each other (Weissenhorn et al., 1997; Kielian and Rey, 2006; Roche et al., 2006) only if the protein domains that connect the hairpins with the bilayer matrix are more difficult to bend than the lipid bilayer. Whereas the bending rigidity of lipid bilayer is ~12 kcal/mole, rigidities of relevant protein domains are unknown. Molecular dynamic simulations performed for SNAREs (Knecht and Grubmuller, 2003) suggest that the protein domain that links the helical bundles formed by these proteins with the bilayer is rigid enough to transfer required mechanical energy from proteins to membranes.

The force that the protein machine can apply to the membrane, and, consequently, the strength of the resulting bilayer stress, is also limited by how tightly this machine is membrane anchored. Although TMDs of integral proteins anchor well, FPs are less reliable. The force needed to detach the FP of HA from a bilayer is estimated to be ~20 pN (Kozlov and Chernomordik, 1998), somewhat exceeding the force needed to bend a lipid bilayer into a fusogenic bulge. In addition, all three FPs of the HA trimer may be engaged in membrane attachment. For other fusion proteins, including those with shorter or less hydrophobic membrane-interacting sequences, the protein-generated force is likely delivered to membranes via the concerted action of multiple anchors (Kozlov and Chernomordik, 1998; Kweon et al., 2003).

Proteins could induce hemifusion by bringing bilayers together within 1 nm. For such bulging to proceed against hydration repulsion, a strong bending moment has to be applied by proteins to the edges of the lipid bilayer patches. Estimations using the conventional model for hydration repulsion and the hydration parameters of PC show that to decrease the intramembrane distance to 1 nm requires bending moments corresponding to unrealistically large curvatures of the protein-depleted lipid patches (radius of <3 nm). Hence, such mechanisms appear unlikely.

Hemifusion might be promoted by protein assemblies at the fusion site that scaffold lipids onto protein surfaces.

Perhaps conformational changes in the proteins that form a hypothetical protein scaffold between the membranes raise hydrophobicity of the surface of the scaffold. Hydrocarbon tails of the lipids of the contacting membrane leaflets would cover this scaffold and, thus, merge the membranes (Jackson and Chapman, 2006). However, the subsequent stages of fusion would require a radical transformation of the protein properties to release the lipid–scaffold interactions. The energy of such a hemifusion connection would likely be dominated by the lipid–scaffold interactions and, thus, is expected to be rather insensitive to the spontaneous curvature of the lipid monolayers. Thus, the similarity between the effects of nonbilayer lipids on protein-mediated hemifusion and hemifusion of protein-free bilayers indirectly argues against this mechanism.

A more likely role for protein scaffolds is to function after stalk formation to increase the radius of the stalk, using electrostatics, which is a weaker force than the hydrophobic effect. This putative protein scaffold can be located outside rather than inside the hemifusion connection (Fig. 2 C). For instance, the C2b domain of synaptotagmin, one of the key components of the intracellular fusion machinery, may arrange around the fusion site and present an electrostatic surface sufficiently positive to strongly bind negatively charged lipids (Rizo et al., 2006). Indeed, synaptotagmin has a large positive charge when its ligand Ca^{2+} is bound. Further, the quenching of fluorescence upon membrane binding suggests that membrane curves around the globular C2b domain. Hence, the geometry of poststalk stages may be impacted upon by such protein–lipid interactions to promote expansion of the stalk (Zimmerberg et al., 2006).

Conversely, proteins may act to specifically prevent the widening of a ring of proteins surrounding the stalk. These would act as brakes, or clamps, to the fusion process. This may be the mode of action of complexin, a molecule that binds to SNAREs and is important for exocytosis (Giraudo et al., 2006). Indeed, in reconstituted systems of complexin plus SNAREs, only hemifusion results (Schaub et al., 2006). Effective transition from hemifusion to complete fusion upon reversing complexin inhibition by synaptotagmin and calcium suggests that complexin prevents the SNARE ring from widening the stalk radius. An intermediate fusion stage that is set for rapid fusion completion upon a final triggering event might be important for the fastest fusion reactions, such as neurotransmitter release (Jahn et al., 2003; Zimmerberg and Chernomordik, 2005).

Opening and expansion of a fusion pore

Fusion pores in protein-free membranes. To complete fusion, the hemifusion intermediate must transition to a fusion pore. The pore might open directly from a fusion stalk (Siegel, 1993; Kuzmin et al., 2001) or within the HD formed upon expansion of the stalk (Chernomordik et al., 1987; Kozlovsky et al., 2002). The dependence of fusion pore opening on the composition of distal membrane leaflets that form HD is consistent with the latter pathway. Because the curvature of the distal lipid monolayer forming the edge of the pore in the HD is opposite to that in a fusion stalk, lipids that inhibit hemifusion (e.g., LPC) are expected and, indeed, promote pore formation (Chernomordik and Kozlov, 2003).

The elastic energy of the bent lipid monolayer at the edge of a lipidic pore is rather high (~12 kcal/mol for a 1-nm radius pore in a PC bilayer; Chernomordik and Kozlov, 2003). Thus, until the fusion pore expands beyond the HD and the area of a tight membrane contact, pore development remains very energy intensive. Further expansion likely proceeds spontaneously (Chizmadzhev et al., 1995). In many cases both HD expansion and the opening and expansion of a fusion pore in a lipid bilayer are driven by lateral tension generated in a membrane monolayer. The effects of tension on hemifusion and fusion have been observed experimentally (Cohen et al., 1980; Chernomordik et al., 1987; Ohki, 1988) and confirmed in numerical simulations of the fusion process (Shillcock and Lipowsky, 2005). Theory shows that effective formation of a pore in HD requires tension to reach values of at least a few milliNewtons/meter (Kozlovsky et al., 2002) that is significantly higher than the estimate of the apparent plasma membrane tension for fibroblasts (0.03 mN/m; Raucher and Sheetz, 2000), but within the range of tensions described for biological membranes (Morris and Homann, 2001).

Fusion pores in biological membranes. Fusion pores in biological membranes resemble those in protein-free bilayers in their electrophysiological characteristics (Zimmerberg et al., 1987; Melikyan et al., 1993; Chanturiya et al., 1997) and in their dependence on lipids in the distal membrane leaflets for pore formation (Chernomordik et al., 1998). The contrasting dependence of hemifusion and fusion pore development on the composition of different leaflets of the fusing membranes may explain the promotion of neurotransmitter release by snake venom phospholipase A2 (Rigoni et al., 2005; Zimmerberg and Chernomordik, 2005). Phospholipase A2 hydrolysis produces LPC and OA, and whereas OA quickly partitions into the inner leaflet of plasma membrane and promotes hemifusion between this membrane and synaptic vesicle, LPC stays in the outer leaflet of the plasma membrane and promotes fusion pore opening.

In viral and intracellular fusion, pores can close and reopen with the final outcome of the process dependent on both the proteins involved and the membrane lipids (Melikyan et al., 1993; Razinkov and Cohen, 2000). The transition from a small flickering pore to a larger expanding pore likely represents the most energy-demanding fusion stage (Chernomordik and Kozlov, 2003; Cohen and Melikyan, 2004; Reese and Mayer, 2005; Xu et al., 2005). For intracellular fusion, the decision between closing a fusion pore or complete fusion is sometimes referred to as "kiss-and-run versus complete fusion." Indeed, small fusion pores in mast cell exocytosis are stabilized in hyperosmotic solutions that delay the hydration of exocytotic vesicle contents (unpublished data). This suggests that lateral tension developed by swelling of the vesicle helps to expand the fusion pore until the vesicle contents are fully released and it flattens into the plasma membrane, completing fusion. In contrast to these final fusion stages, opening and moderate widening of a fusion pore proceed in flaccid vesicles (Zimmerberg et al., 1987), indicating that tension that drives these stages is generated by fusion proteins rather than by the swelling of the entire vesicle.

Stalk–pore transition may involve the aforementioned electrostatic attraction of biological membranes to ringlike scaffolds of protein (Zimmerberg et al., 2006) that drives stalk expansion toward the point of fusion pore formation. Because such an electrostatic switch can operate extremely quickly, it helps to explain the extremely rapid fusion pore opening that characterizes synaptic release in the nervous system.

Another mechanism by which proteins might generate lateral tension that drives opening and expansion of a fusion pore is suggested by the fusion coat hypothesis (Kozlov and Chernomordik, 2002). Activated fusion proteins interconnect into a membrane coat that bends the membrane out of its initial shape and expands the fusion site (Fig. 2 D). A requirement for this mechanism is that the bending rigidity of the protein coat greatly exceeds that of a lipid bilayer. Indeed, estimates show that the coat must be 50–100 times more rigid than the lipid bilayer (Kozlov and Chernomordik, 2002). Accordingly, the formation of large fusion pores requires the participation of a considerably larger number of activated fusion proteins than that needed for all the previous fusion stages (Leikina and Chernomordik, 2000; Zaitseva et al., 2005).

Conclusion

Evolution has had many millions of years to design membrane fusion reactions, and we are just scratching the surface in our understanding of their complexity. The coupling between proteins and membranes that is at the heart of fusion is likely to be particular to each system. However, similar effects of membrane lipids on fusion between protein-free bilayers and on biological fusion, along with recent findings that diverse fusion proteins form hemifusion intermediates, substantiate the hypothesis that proteins drive membrane rearrangement through a conserved pathway defined by the properties of lipid bilayers. Acceptance of this paradigm will hopefully accelerate the ongoing exploration of the specific mechanisms by which proteins catalyze and direct distinct stages of this lipidic pathway.

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