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Mechanics of membrane fusion/pore formation

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ABSTRACT

Lipid bilayers play a fundamental role in many biological processes, and a considerable effort has been invested in understanding their behavior and the mechanism of topological changes like fusion and pore formation. Due to the time- and length-scale on which these processes occur, computational methods have proven to be an especially useful tool in their study. With their help, a number of interesting findings about the shape of fusion intermediates could be obtained, and novel hypotheses about the mechanism of topological changes and the involvement of peptides therein were suggested. In this work, we try to present a summary of these developments together with some hitherto unpublished results, featuring, among others, the shape of stalks and fusion pores, possible modes of action of the influenza HA fusion peptide and the SNARE protein complex, the mechanism of supported lipid bilayer formation by vesicle spreading, and the free energy and transition pathway of the fusion process.

1. Introduction

Lipid bilayers are aggregates of large numbers of individual lipid molecules. Simply put, these layers are held together by the lipids' amphiphilic nature in an attempt to isolate their hydrophobic tails from the aqueous environment. Lipid bilayers combine properties of solids and liquids in that they maintain their structure at the macroscopic level, but at the same time allow the lipids to diffuse and deform, changing both their location and conformation within the boundaries dictated by the topology of the aggregate (Singer and Nicolson, 1972; Vereb et al., 2003).

This dual nature is a requirement for their biological function as cell membrane. These membranes are a crucial part of the organization of living organisms and need to reliably maintain the division into cellular and subcellular compartments. However, at the same time they need to be flexible enough to allow (regulated) transport of molecules ranging in size from relatively small water molecules or ions on the one hand to complete strands of mRNA on the other. Also, the cell membrane has to accommodate a large amount of different proteins serving various functions including enzymatic activity, transport, signal transduction, and cell–cell recognition. In fact, with the respiratory chain and photosynthesis, the very heart of the cells' energy metabolism is not only located right inside the cell membrane, but directly requires the membrane for its function (Alberts et al., 2002).

The actual topology of the compartmentalization is not constant, but subject to frequent, though carefully regulated, changes. These changes can be divided into three groups: poration-, fission- and fusion-events. The most straightforward of these changes is the poration, in which a hole in the lipid bilayer is introduced. In fission events, an initially connected region of space surrounded by a continuous lipid bilayer is divided into two disconnected regions of space, each surrounded by its own closed bilayer shell. The reversion of this process is fusion events, in which two initially separate bilayer shells are combined into a single closed lipid bilayer surrounding a continuous volume.

Since the majority of this review will be discussing findings related to fusion, we will describe the fusion process in a bit more detail. Fig. 1 schematically illustrates the terms used in this review and gives a summary of the suggested pathways published in literature. As can be seen, except for the net effect described above, what is going on at the molecular level is not known with certainty at present. However, it is a logical requirement, and therefore generally agreed, that a connection between the two separate bilayers needs to be introduced, and at least one pore has to be opened. The first step of the connection introduced between the two-bilayer shells is usually referred to as stalk. The stalk is understood to be a metastable bridge connecting the hydrophobic tails of the outer (cis) monolayers of the two membranes about to be fused, whereas the inner (trans) monolayers remain at approximately the same distance as before the connection.

The second stage of the fusion process that is a common feature of all suggested pathways is the last step that completes the topological changes involved in the fusion process. This state is conventionally called a fusion pore, and describes an

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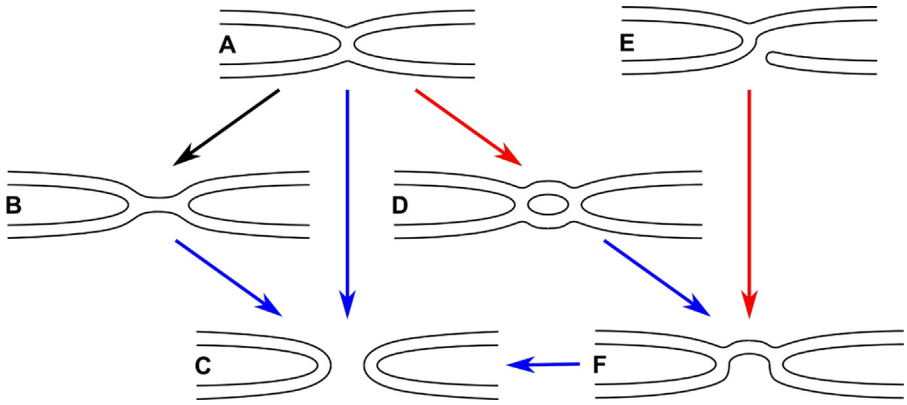

Fig. 1. Schematic illustration of the fusion process, trying to give an overview of the suggested pathways connecting the fusion intermediates stalk (A), hemifusion diaphragm (B), fusion pore (C), circular stalk (D), stalk-pore complex (E), and π-shaped hemifusion diaphragm (F). Black arrows represent radial stalk expansion, red arrows indicate linear stalk elongation, and blue arrows show pore formation. A detailed description is given in the article text. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

hourglass-shaped section of bilayer with a lumen connecting the interiors of the fused bilayer shells. It is therefore not a pore in the sense of a perforation of the bilayer, and the bilayer of the fusion pore is in fact already continuous and can be formed into a closed sphere without necessity of further topological changes.

Apart from these two elements, however, the exact pathway and sequence of events are not known with certainty, and it is not unlikely that the fusion process follows different pathways under different conditions. A commonly accepted option is the stalk-hemifusion route (Chernomordik and Kozlov, 2005), in which the stalk radially expands in area until the trans monolayers meet and form a bilayer that separates the two volumes as a so-called hemifusion diaphragm. Subsequent rupture of this diaphragm forms the fusion pore and thereby completes the fusion process. This corresponds to the sequence ABC in Fig. 1.

An alternative to this route has been reported as the linear stalk elongation (Müller et al., 2002; Marrink et al., 2008; Risselada et al., 2012) in which the stalk does not perform a radial expansion, but elongates linearly along a circular path. In this way the trans monolayers do not make direct contact, but the creation of a closed, circular stalk forms a small third compartment in-between the bilayers. In this scenario, two pores need to open to connect all compartments: one to form a topology similar to the hemifused state (except that the diaphragm originates from different membrane leaflets), and a second to form the final fusion pore. This is illustrated by the sequence ADFC in Fig. 1. However, a predicted mutual facilitation between stalks and pores supports such mechanism, and a sequence in which the first pore opens before the stalk elongation has been argued to be an energetically feasible, leaky fusion pathway (Katsov et al., 2006), corresponding to the sequence EFC in Fig. 1.

A third option completely foregoes the hemifusion diaphragm as a metastable state and finds rapid fusion directly after stalk formation without further interruption (Kasson et al., 2006), which is shown in Fig. 1 as the sequence AC.

Due to the microscopic level on which these events occur (nm) and the very short time scale (μs) over which they persist, it is near impossible to gain direct experimental insight into the mechanism of the pathway and the nature of the intermediate states. However, spectroscopic methods have been employed to study the interesting phase behavior of lipid mixtures as reviewed by Seddon (1990), Seddon and Templer (1995). Since some of these lyotropic phases share structural key elements with fusion intermediates, it is possible to make predictions on the fusion mechanism based on changes in the phase diagram. An example for this would be the induction of inverted phases by certain fusion peptides (Yeagle et al., 1991; Epand and Epand, 1994; Epand et al., 1994; Davies et al., 1998; Peisajovich et al., 2000; Aranda et al., 2003), which has been interpreted as an indication of the fusogenicity of these peptides. Another experimental method is to use fluorescent labels on the different lipid vesicles and their cargo, which makes it possible to trace events like stalk formation via lipid mixing, and pore formation or completion of the fusion process via content leakage or mixing, respectively. A good overview of these findings is given in a recent review by Jahn et al. (2003).

If the goal is to gather information on the exact shape of a fusion intermediate or on the mechanism of a certain step of the process, it is therefore necessary to employ theoretical calculations and/or simulations. A common strategy for calculations is to consider the lipid bilayer as an elastic sheet and minimize the free energy to find the optimal shape for a given topology (Markin and Albanesi, 2002; Kozlovsky and Kozlov, 2002). This approach tries to relate the membrane behavior to experimentally known physical properties of lipid bilayers like, *e.g.*, bending stiffness and compressibility. In addition, such calculations require comparatively little computational resources and are therefore widely available. In many cases, however, a particle based description of the lipid bilayer is necessary to capture the complex interactions of the individual lipids, especially if additional, non-lipid, agents like, *e.g.*, peptides are also involved. On top of that, only in simulations it is possible to directly observe topological changes as they happen, and to follow them at molecular resolution. Thanks to the universality of the behavior of lipid bilayers, a wide range of models using different levels of detail is able to correctly reproduce the experimentally known phenomena (Müller et al., 2006), and atomistic (Berger et al., 1997), as well as a large number of coarse-grained and even solvent-free models are available, as reviewed by Venturoli et al. (2006). The choice of model should reflect the properties believed to play a role in the process under observation. It should be noted, however, that due to the collective nature of lipid aggregates, it is often necessary to simulate large numbers of molecules for a considerable time to realistically represent most processes. As every particle and interaction included in the simulation increases the computational costs, coarse-graining is a necessity, but has been shown to be successful in studying many different aspects of lipid behavior, as recently reviewed in Müller et al. (2006), Venturoli et al. (2006), Marrink et al. (2009).

The aim of this review is to give a perspective onto recent findings in the field of membrane fusion and poration, with a focus on the different pathways along which these topological changes occur. Due to this focus, the presented findings are based on theoretical calculations and simulation which, at the moment,