Membrane fusion

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Summary. The factors involved in the regulation of biological membrane fusion and models proposed for the molecular mechanism of biomembrane fusion are reviewed. The results obtained in model systems are critically discussed in the light of the known properties of biomembranes and characteristics of biomembrane fusion. Biological membrane fusion is a local-point event; extremely fast, non-leaky, and under strict control. Fusion follows on a local and most probably protein-modulated destabilization, and a transition of the interacting membranes from a bilayer to a non-bilayer lipid structure. The potential role of type II non-bilayer preferring lipids and of proteins in the local destabilization of the membranes is evaluated. Proteins are not only responsible for the mutual recognition of the fusion partners, but are most likely also to be involved in the initiation of biomembrane fusion, by locally producing or activating fusogens, or by acting as fusogens.

Key words. Local-point fusion; fast-freezing; electron microscopy; fusion proteins; inverted lipid structures.

Introduction

Membrane fusion is a key event in the functioning of a living organism. In fact, life starts as a sperm fuses with the membrane of an egg, leading to its fertilization. The fertilized egg then divides, eventually producing a full-grown multicellular organism, in a process that requires multiple rounds of membrane fusion. Membrane fusion is also required for the formation of the mature muscle fiber (myogenesis), the release of neurotransmitters, hormones and enzymes from their respective storage sites within the cell (examples of exocytosis), the uptake of ligands bound to the cell surface (an example of endocytosis), and intracellular membrane traffic. Finally, membrane fusion is a crucial event in the entry of enveloped viruses into cells during infection.

Many efforts have been made to understand the molecular mechanism and the regulation of membrane fusion[9, 11, 31, 32, 72, 105]. These questions are especially intriguing if one considers the dynamics of intracellular membrane traffic[92]. A fibroblast in culture internalizes half of its membrane surface during each hour of endocytotic activity. A delicate balance between endocytosis and exocytosis assures that the overall dimensions of the cell and vacuolar system remain constant. Taking into account the biosynthetic routes, and the unique structure and composition of the different cellular compartments (nucleus, ER, Golgi, etc.), a picture emerges in which during 'high speed' intracellular membrane traffic both lipids and proteins are constantly sorted and targeted to their specific destinations within the cell; so as to generate and maintain compartmentalization, and to fulfill a number of specialized cellular functions. Clearly, membrane fusion has to occur in a very specific and highly controlled fashion, and therefore specific modulators have to be present determining exactly when and where membrane fusion occurs.

Biological membrane fusion is extremely complex, because of the large number of primary and secondary factors involved in its induction and regulation. To tackle this problem, simplified model systems have been extensively used, ranging from single cells and permeabilized cells to the behavior of two approaching planar lipid bilayers. In fact, most of our current knowledge about the molecular mechanism of membrane fusion is based on results obtained using the simplest model system; the interaction and fusion of pure lipid model membranes.

The aim of this review is not to give an exhaustive overview of the literature on membrane fusion, but to discuss critically the results obtained in model systems and their relevance to biological membrane fusion. First, some of the risks inherent in using model systems to assess the mechanism of biological membrane fusion will be discussed. Subsequently, a morphological description of biological membrane fusion will be given. Morphological techniques have played and still play an important part in providing a conceptual framework for scientists working in the field of biological membrane fusion. Finally, the steps preceding, during and after membrane fusion will be discussed in the light of results obtained in model and biological systems. The role of proteins and peptides in membrane fusion is evaluated; although experimental proof is still scant, proteins are most probably responsible for the control of biomembrane fusion, both in time and space.

I The model system approach

The complexity of membrane fusion and the inaccessibility of the fusion machinery in an intact biological system has encouraged the development of a whole range of different model systems. Some of these are best described as a simplification of the intact biological system, and range from single cells and permeabilized cells to cell-free systems composed of isolated intracellular membrane compartments. These still rather complex model systems

are particularly well suited for the identification of the factors involved in the initiation and regulation of biological membrane fusion. Since membrane fusion ultimately involves the merging of two lipid bilayers, pure lipid model systems have also been studied extensively. These artificial model systems are thought to represent a short cut to the understanding of the molecular mechanism of membrane fusion. Although the use of model systems is unavoidable, every simplification of the intact biological system increases the risk that parts of the fusion machinery may be modified or lost, or additional factors irrelevant to the mechanism of biomembrane fusion may be introduced; some examples are given.

Cell permeabilization may result in the loss of factors involved in the regulation of membrane fusion. This is illustrated by the probable loss of an ATP-sensitive factor on permeabilization of sea urchin eggs; whereas exocytosis in intact eggs is sensitive to metabolic poisoning, ATP-dependence is lost after permeabilization [47]. The risks in using cell-free systems to study the mechanism of biological membrane fusion are clearly illustrated by studies on the behavior of isolated chromaffin granules, which led to the development of the 'chemiosmotic hypothesis of secretion' [75]. Experiments using intact and permeabilized cells have now resulted in the rejection of this hypothesis; the properties of isolated granules were seen to have been quite different from those of granules in (semi)intact cells [13].

The use of the most simple, pure lipid model systems not only carries the risk of 'missing' parts of the fusion machinery, but additional factors irrelevant to the mechanism of biomembrane fusion may be introduced. The universal role of intracellular Ca^{2+} (Ca_i^{2+}) in triggering exocytosis has initiated many studies on divalent cation-induced fusion of acidic phospholipid-containing model membranes [31, 33, 68]. However, the Ca^{2+}-concentration needed to induce fusion in these systems is at least three orders of magnitude higher than the rise in Ca_i^{2+} required for triggering exocytosis, making a direct role of Ca^{2+} unlikely (see section III). Consequently, the molecular mechanism of Ca^{2+}-induced model membrane fusion is not necessarily relevant to the molecular mechanism of exocytotic membrane fusion.

The lipid composition and method of preparation of pure lipid model systems are often selected so as to optimize the fusion process. Influenza virus, for example, fuses most efficiently with vesicles composed of acidic phospholipids [90]. However, in biomembranes studied so far, acidic phospholipids are almost exclusively confined to the cytoplasmic leaflet of the membrane [65], so the interaction of influenza virus with acidic phospholipids during cellular infection seems highly unlikely. Even more seriously, the discrepancy observed between the pH-dependency of activation of the influenza virus fusion protein, and the pH-profile of the fusion activity towards these vesicles, strongly suggests a non-physiological fusion mechanism [91]. A second example is the frequent use of very small (sonicated) vesicles in membrane fusion research; these vesicles are intrinsically unstable [9, 11]. A synthetic polypeptide designed to mimic the behavior of the fusogenic amino acid sequences of viral fusion proteins induces fusion of sonicated vesicles, but fusion is not observed if biologically more relevant larger vesicles are used [69].

In summary, a model system should be carefully chosen in relation to the known properties of biomembranes and biomembrane fusion (lipid composition and asymmetry; local-point fusion, non-leaky, etc.), and the biological problem to be solved. In the end, predictions made based on the results of a model system approach should be tested in the intact biological system. One should always be aware of the possibility that different mechanisms of membrane fusion may be operational, both in biological and in model membrane systems.

II Morphology of biological membrane fusion

In contrast to biochemical techniques, morphological techniques are readily applicable to intact biological systems, and it is not surprising that morphological data have had, and still have, a great impact on the understanding of the molecular mechanism of biomembrane fusion. Even if a putative trigger or modulator of membrane fusion has been identified using biochemical methods, only electron microscopy (EM) offers the possibility of localizing the substance at the site of membrane fusion with sufficient temporal and spatial resolution.

Unfortunately, electron microscopical analysis is only feasible after processing the biological sample [74]. This normally involves chemical fixation, dehydration, plastic embedding and the cutting of thin sections, or chemical fixation, glycerol impregnation and freeze-fracture (referred to as 'conventional' EM techniques throughout the text). The chemical pretreatments have serious implications for the structure of the sample as observed by EM; the observed ultrastructure is correlated with, but certainly not identical to, the native ultrastructure of the sample.

II.1 Conventional EM techniques: IMP clearance and diaphragm formation

All ultrastructural studies on membrane fusion performed before 1979 relied on the use of chemical fixatives to stabilize the structure of a biological specimen. Using conventional thin-section EM, Palade and Bruns [67] studied the fusion of exocytotic vesicles with the plasma membrane of capillary endothelial cells. Based on the staining pattern of the membranes involved in the fusion process, three stages were delineated (fig. 1). First, membranes come into close apposition and a so-called 'pentalaminar structure' is formed (fig. 1, B). This pentalaminar structure converts to a trilaminar structure, or single-bilayer diaphragm [71] (fig. 1, C). Finally, the shared bilayer disintegrates and the vesicle contents are released. Penta-

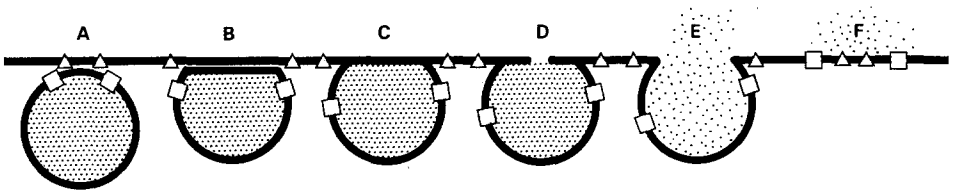

Figure 1. A schematic representation of secretory membrane fusion based on the results of conventional EM techniques. Recognition (A) is followed by clearance of intrinsic (□ and △) and extrinsic membrane proteins, and the formation of a pentalaminar structure (B). This pentalaminar structure converts to a trilaminar structure or single-bilayer diaphragm (C). The shared bilayer disintegrates (D) and the secretory granule contents are released (E).

laminar and trilaminar structures have been found in many cell types after the triggering of membrane fusion, both when (conventional) thin-section EM techniques and freeze-fracture EM were used[16, and references therein]. Freeze-fracture EM showed large areas of the membrane fracture face cleared of intramembrane particles (IMP's). These IMP-free areas were thought to correspond to the sites where penta- and trilaminar contacts had been established. Taken together, these observations led to the assumption that biomembrane fusion is preceded by significant clearance of extrinsic and intrinsic membrane proteins, followed by fusion of the bare lipid patches[66]. Fusion of these bare lipid patches and the formation of the trilaminar structure was thought to be mediated by fusogenic lipids (see V.2). In the final step of membrane fusion, the formation of an aqueous connection, the trilaminar structure 'dissolves'[67, 80], or it simply ruptures as a result of the generation of osmotic forces[55].

II.2 Modern EM techniques: The local-point fusion concept

In order to stabilize the structure of a biological specimen without any chemical pretreatment, modern EM techniques make use of fast-freezing with freezing rates in excess of 10,000 °C/s. Fast-freezing avoids the artifacts induced by chemical pretreatments during conventional chemical fixation, cryoprotection and dehydration procedures[61]. Furthermore, it offers a time resolution better than 1 ms, whereas the chemical fixation even of a single cell takes at least a few seconds[51]; in well-synchronized systems, membrane fusion can be followed over the cause of time, and even short-lived fusion intermediates can be visualized. Both advantages became obvious after the pioneering work of Heuser et al.[42] on synaptic vesicle exocytosis. Exocytosis could be followed on a time-scale of milliseconds, and could even be correlated quantitatively to neurotransmitter release. IMP-clearance, penta- or trilaminar structures, diaphragms, and large vesicles or blebs at the fusion site were not observed. The artificial nature of these phenomena was further confirmed by a direct comparison of fast-freezing with chemical fixation and glycerol impregnation[15, 18]. Chemical fixation increases the number of fusion events observed[43], and the extent to which IMP-clearance and membrane diaphragms are found is determined by the glycerol concentration used after aldehyde fixation[17]. In summary, only pure cryofixation, i.e. fast freezing without chemical pretreatment, is likely to prove reliable in the morphological characterization of membrane fusion[51, 72].

Using pure cryofixation, biomembrane fusion is consistently observed as a local-point event that involves only a very small surface area of the interacting membranes; large single-bilayer diaphragms and, with very few exceptions[36, 89], large-scale IMP clearance are not observed[15–19, 42, 50, 72, 83]. The fusion of liposomes with virus-infected cells[50] (fig. 2a), virus budding[50] (fig. 2b), and exocytosis in chromaffin cells[83] (fig. 2c), all involve a circular membrane area of less than 20 nm in diameter. The local-point fusion concept, shown schematically in figure 3, has important implications for both the molecular mechanism and the regulation of membrane fusion. A local change in the configuration of membrane lipids fits in well with the local-point fusion concept (see V). Moreover, the local-point fusion concept allows for a direct involvement of proteins in the regulation of membrane fusion (compare figs 1 and 3). In this context, it is interesting to mention that during mating of *Chlamydomonas* gametes, IMPs move towards the site where membrane fusion eventually takes place[100]; this is in direct contrast with the concept of IMP-clearance preceding membrane fusion!

In conclusion, biological membrane fusion is a local-point event, extremely fast (ms-range[42]), essentially non-leaky and under strict control. In sections III–VI, the steps preceding, during and following membrane fusion are discussed in more detail.

III Trigger: Factors involved in the initiation of biomembrane fusion

In most biological systems the stimuli eventually leading to biomembrane fusion are known, whilst the components of the fusion machinery and their mode of action have largely remained elusive. Regulated *exocytosis* has been studied most extensively. Both direct measurements of Ca_i^{2+} in intact secretory cells, and the Ca^{2+}-induced secretion in permeabilized cells, have confirmed the central role of Ca_i^{2+} in the regulation of exocytosis in most secretory cells[11, 31, 32, 88]. The increase in Ca_i^{2+} is either the result of an influx of Ca^{2+} from the extracellular fluid, e.g. after activation of voltage-dependent Ca^{2+}-channels in nerve tissue, or results from the release of

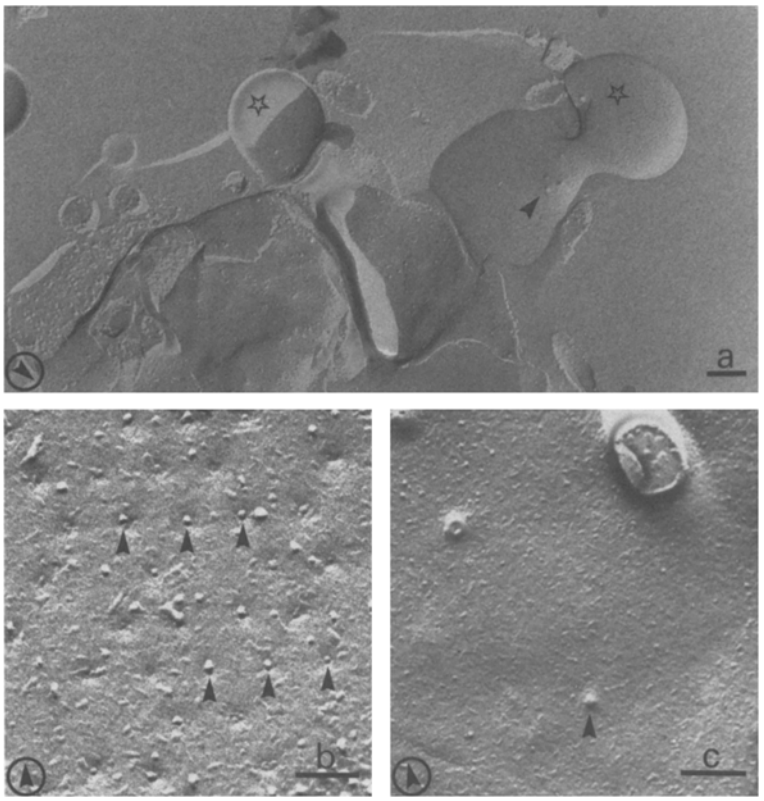

Figure 2. Biological membrane fusion as visualized by fast-freeze freeze-fracture EM. *a* The low-pH induced fusion of liposomes (marked by stars) with influenza virus-infected epithelial cells. *b* Budding of influenza virus at the apical plasma membrane of virus-infected cells. *c* Carbachol-induced exocytosis in chromaffin cells. In each case membrane fusion appears to be a local-point event, and involves a very small surface area of the interacting membranes (marked by arrowheads). For details see text. Direction of Pt/C shadowing indicated by encircled arrowheads. Bars 0.1 μm. *a*[50] and *b*[50]; *c* provided by H. Plattner and reproduced with permission.

Ca^{2+} from intracellular stores, e.g. after ligand-receptor mediated activation of the phosphatidylinositol (PI) cycle[58]. Despite the fact that the similarity in Ca_i^{2+} activation characteristics might suggest a common mechanism in Ca^{2+}-activated secretory exocytosis, the mechanism by which a rise in Ca_i^{2+} triggers membrane fusion is still controversial.

A rise in Ca_i^{2+} may be the crucial signal for exocytosis to occur in vivo, but the low physiological Ca_i^{2+} concentration needed to trigger exocytosis (0.1–1 μM) virtually excludes a direct role of Ca^{2+} in the induction of membrane fusion. In agreement with this, exocytosis in mast cells and permeabilized neutrophils can be triggered in the absence of Ca^{2+}, by increasing the GTP concentration in the presence of high concentrations of a Ca^{2+} chelator[39]. The indirect role of Ca_i^{2+} in the induction of membrane fusion is also supported by various studies that indicate the involvement of high-affinity Ca^{2+}-binding proteins in the regulation of membrane fusion. Ca^{2+}-dependent membrane binding proteins[24] (e.g. synexin and calpactin) and Ca^{2+}-dependent cytoskeleton associated proteins[93] have been proposed to assist membrane fusion in vivo by regulating membrane contact. After permeabilization, exocytosis in most secretory cells requires the presence of MgATP[32, 88], and Ca^{2+}-activated protein kinases and protein phosphorylation could be

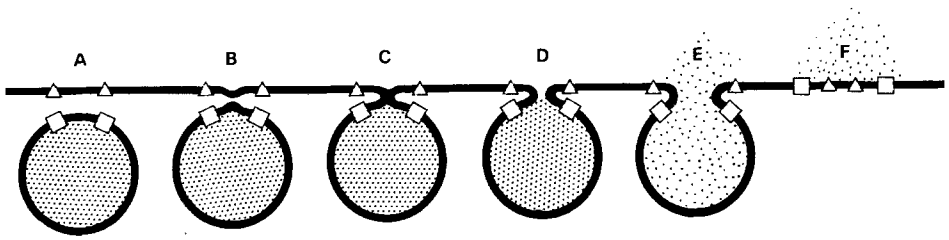

Figure 3. A schematic representation of secretory membrane fusion based on the results of modern EM techniques, i.e. fast-freezing without chemical pretreatment. Recognition (A) is followed by local-point adhesion (B), local-point fusion (C), fission and the formation of a small aqueous pore (D), widening of the pore, decondensation and release of the secretory granule contents (E). The local-point fusion concept allows for a direct role of proteins (e.g. intrinsic membrane proteins □ and △) in the regulation of biomembrane fusion (cf. fig. 1).

involved in the regulation of membrane fusion. On the other hand, protein phosphatases could be activated, and exocytosis in *Paramecium* is accompanied by the dephosphorylation of a phosphoprotein [108]. Some studies indicate the involvement of Ca^{2+}-activated endoproteases [94], which could be required for the local removal of steric barriers to membrane fusion, or for the activation of one or more components of the fusion machinery. It has been proposed that limited proteolysis of cellular proteins may lead to the release of fusogenic peptides capable of a direct induction of membrane fusion [56]. Finally, many secretory processes are accompanied by phospholipase C (PLc) activation and the formation of diacylglycerol (DG) [32, 88]. DG can act in conjunction with Ca_i^{2+} by activating protein kinase C (PKc), and increasing the sensitivity of the exocytotic machinery to Ca_i^{2+} [49]; both DG and phorbol ester (which activates PKc) trigger secretion in blood platelets without raising Ca_i^{2+} [78]. Alternatively, DG may have a more direct role in the induction of membrane fusion by causing a direct destabilization of the membrane (see V.4).

The regulation of *endocytosis and intracellular membrane traffic* appears even more complex than the regulation of exocytosis. The intermingling of biosynthetic, endo- and exocytotic routes, and the constant recycling of various components between the compartments of these routes, vastly complicates any study on the regulation of intracellular membrane traffic in the intact cell. Therefore cell-free systems reconstituting parts of intracellular membrane traffic are essential to the understanding of its regulation. The design of sensitive fusion assays has made it possible to probe specific intracellular membrane fusion events, even in relatively impure cell extracts; early fusion events in the endocytotic pathway [25, 29, 30, 40, 107], and vesicular transport between ER and the Golgi system [7, 8, 62, 64], and within the Golgi stack [5, 38, 99; also see 79], have been reconstituted. In permeabilized cells and cell-free reconstitution systems a number of requirements have to be met in order to obtain efficient fusion of intracellular membranes. Some of these requirements are; ATP or GTP, proteinaceous components present in the cytosol and on the cytoplasmic surface of donor and acceptor membranes, and ions [5, 7, 29, 40, 59, 60, 64, 98]. In general, efficient fusion does not require a rise in Ca^{2+} concentration above the physiological 'resting level' (0.1 μM) [7]. Again, proteins appear to play a crucial role in the regulation of membrane fusion. A potential role for GTP-binding proteins in the regulation of membrane fusion has been put forward [59, 60, 98]. In addition, a protein named NSF (N-ethylmaleimide-sensitive factor) [10], has been identified and shown to be involved in vesicular transport, within the Golgi stack [10], from ER to Golgi [8], and during endocytosis [30]. Although all data indicate that NSF is a general component of an intracellular membrane fusion machinery, the actual role of NSF in the induction of membrane fusion remains to be defined. In this context, it is important to note that NSF does not contain the long stretches of hydrophobic amino acids present in some of the viral fusion proteins [106].

Virus-membrane fusion is the only biological membrane fusion event in which the component directly responsible for the induction of membrane fusion has been identified [44, 103]. Enveloped viruses enter and infect their host cell by fusing with either the plasma membrane (best-known example; Sendai virus, a paramyxovirus), or, after cellular uptake by receptor-mediated endocytosis, with the endosomal membrane (e.g., influenza virus, an orthomyxovirus; and vesicular stomatitis virus, VSV, a rhabdovirus). Membrane binding and fusion are accomplished through the specific action of viral glycoproteins or spike proteins, exposed on the outer surface of the virion. Binding and fusion can be separate functions (Sendai virus, HN and F protein, respectively) or both functions may be united in one spike protein (hemagglutinin, HA, in influenza; G protein in VSV). Membrane fusion activity requires a conformational change of the fusion protein; in viruses infecting the host cell via the endocytotic route (influenza, VSV), this conformational change is triggered by the gradual acidification of the endosomal compartment (pH 5–6). In most viruses (Sendai, influenza), this conformational change results in the exposure of a highly conserved and extremely hydrophobic amino acid sequence towards the aqueous medium. This sequence is thought to penetrate the target membrane and induce membrane fusion by some still unknown mechanism. On the other hand, the VSV-G protein does not contain long hydrophobic amino acid stretches in its ectodomain, and the mechanism by which the conformational change of the G protein induced by low pH triggers membrane fusion is even more obscure.

In conclusion, biological membrane fusion appears to be extremely complex; many factors involved in the initiation and regulation of biomembrane fusion have been identified, but the relative importance of each of these factors remains to be defined. Part of the controversy that exists concerning the regulation of biomembrane fusion probably results from the use of ill-synchronized systems, and techniques that offer insufficient temporal resolution. In regulatory exocytosis, membrane fusion is preceded by the transport of vesicles or granules to the plasma membrane and their docking there, and membrane fusion is followed by release of contents and compensatory endocytosis. Many regulatory factors are involved in each of these steps, and the actual rate-limiting step may vary from one biological system to the other. Moreover, many of the factors involved in the regulation of biomembrane fusion will only act locally. Therefore, the use of combined morphological and biochemical techniques offering high resolution, both temporal and spatial, will prove essential in the final analysis of biomembrane fusion [72]. A definite answer concerning the regulation of biomembrane fusion is only possible in systems that can be artificially synchronized (e.g., temperature-blocks of intracellular membrane traffic, and

the low-pH activated fusion activity of certain viruses), or in the small subset of biological systems that already show good synchrony of membrane fusion activity (e.g., *Paramecium*). In *Paramecium*, for example, it was shown that exocytosis is accompanied by dephosphorylation and followed by rephosphorylation of a phosphoprotein, within 5 s after the triggering of exocytosis [108].

Among the many factors involved in the regulation of biomembrane fusion, proteins play a crucial role, most likely both by determining the specificity of biomembrane fusion and by directly inducing membrane fusion.

IV Adhesion: Recognition and molecular contact

Intimate membrane contact is a prerequisite for membrane fusion. Biomembrane adhesion may involve directed movement of one of the fusion partners, removal of steric barriers, specific recognition and aggregation.

IV.1 Movement into close apposition and recognition

The cytoskeleton plays an important part in directing the movement of intracellular organelles; and cytoskeletal elements are very likely to be involved in the directed movement of secretory vesicles to their specific site of exocytosis [28]. Furthermore, cytoskeletal elements and other cytosolic proteins probably play an important role in the spatial organization of the exocytotic machinery. In a number of secretory cells, ill-defined 'connecting material' is found between the secretory vesicle or granule and the plasma membrane [72, 73]; both cytoskeletal elements and this 'connecting material' could be involved in the specific recognition of fusion partners. They could even be indirectly involved in the regulation of membrane fusion, by sterically hindering close apposition of membranes [72]. As discussed earlier in the text (III), cytoskeleton-associated proteins and proteases might affect this barrier and co-regulate membrane fusion activity. In chromaffin cells and pheochromocytoma (PC12) cells, the role of the cytoskeleton in transport, docking and fusion of secretory vesicles has been studied in some detail [20, 70, 81]. These studies indicate the presence of two pools of secretory vesicles, one of immediately releasable vesicles docked at the plasma membrane, and a second pool held some distance away by cytoskeletal elements, and predestined to refill the docking sites as they become available during exocytosis. The fast-reacting pool is bound to the plasma membrane in a manner independent of cytoskeletal elements (actin, tubulin), and Ca^{2+} or Ca^{2+}-binding proteins [81]. However, the second pool does interact with the cytoskeleton, presumably via an actin-binding protein (α-fodrin) [70], and exocytosis depends on a transient depolymerization of the cortical actin network [20]. These data indicate, once more, that biological membrane fusion may involve distinct and separately regulated steps.

Membrane proteins could also sterically (or electrostatically) hinder close apposition of membranes; the Ca^{2+}-mediated fusion of coated vesicles with lysosomes in a cell-free system only occurred after stripping the vesicles of their clathrin coat [3]. However, the results of using modern EM techniques, i.e., local-point fusion without IMP-clearance, virtually exclude any role for large-scale clearance of membrane proteins in the regulation of biological membrane fusion. Of course, a very limited (specific?) clearance, an enzymatic modification, or a change in conformation of an integral or peripheral membrane protein could still be essential in the regulation of membrane fusion, by allowing *local* close apposition and fusion of membranes.

The variety and high specificity of biological membrane fusion events strongly suggests the involvement of proteins in specific recognition of the fusion partners. Despite the obvious need for specific recognition, both in exocytosis, endocytosis and fertilization, only very few proteins have been shown to be involved. Some secretory systems (in protozoa; and the neuromuscular synapse) are characterized by the presence of regular IMP arrays in the plasma membrane; in protozoa, the use of fusion-defective mutants has provided strong evidence for the involvement of some of these IMP's in the attachment of the secretory compartment to the plasma membrane [72, 73]. As discussed earlier (III), the rise in Ca_i^{2+} prior to exocytosis may activate Ca^{2+}-dependent membrane binding proteins. Some of these proteins are capable of cross-linking membranes (e.g. synexin, calelectrin and calpactin), but they seem to do so rather unspecifically [cf. 84]. Recently, a chromaffin granule binding protein (CGBP) has been isolated which selectively binds to chromaffin granules [84]. CGBP is a plasma membrane-associated protein, and granule binding is not Ca^{2+}-dependent. Specific anti-CGBP Ab's inhibit exocytosis in intact chromaffin cells and PC12 cells, which strongly suggests that CGBP is part of an intracellular recognition site for chromaffin granules on the cytoplasmic side of the plasma membrane. With respect to fertilization, a protein present in the head of mouse sperm has been identified and shown to interact specifically with a solubilized protein from the zona pellucida of the egg cell [53]. Similarly, during mating of *Chlamydomonas* gametes the specialized IMP-arrays present in the plasma membrane of both gametes were shown to be essential for proper gamete adhesion [35]. Finally, enveloped viruses interact more or less specifically with their host cell; specific binding to cell-surface receptors, e.g. sialic acid-containing glycoproteins and glycolipids, is mediated by a glycoprotein exposed on the outer surface of the virion [44, 103].

IV.2 Molecular contact

During membrane fusion the lipid bilayers of the interacting membranes eventually come into very close contact and coalesce. The forces between two approaching pure lipid bilayers have been studied extensively [76, 77]. From these studies it became clear that the net force acting between two lipid bilayers at a distance shorter

than 30 Å is dominated by a repulsive hydration force, and that closer approach of the membranes requires removal of bound water from the headgroup of the lipid molecules. This partially explains why lipids with a low headgroup hydration, like phosphatidylethanolamine (PE), generally enhance membrane fusion in model membrane systems (also see V.3). In biomembrane fusion, lipids like PE may play an important role in determining the overall fusogenicity of the interacting membranes. Stimulus-evoked metabolism of phospholipids could even play a direct role in the regulation of biomembrane fusion. Many biological membrane fusion reactions are accompanied by breakdown of PI resulting in the formation of DG and phosphatidic acid (PA)[32, 88]; the loss of the bulky hydrated inositol headgroup will facilitate close apposition, whereas DG could play a direct role in the induction of membrane fusion[63, 87] (see V.4). Ions, polyamines (spermine, spermidine) chemicals like, e.g., polyethyleneglycol, certain proteins and polypeptides can all affect the repulsive hydration force; a reduction facilitates close apposition and enhances membrane fusion[11, 31]. The properties of fusogenic lipids, such as the tendency to form type II non-bilayer lipid structures, and the molecular mechanism of membrane fusion, will be discussed in the next section.

V Membrane coalescence: Bilayer destabilization and the question of fusion intermediates

During membrane fusion the equilibrium bilayer configuration of the membrane has to be temporarily abandoned. It is essential to realize that most biomembrane fusion reactions will be non-leaky and that fusion normally does not occur via a simple rupture and resealing of the interacting membranes.

V.1 Molecular models: The semi-fusion intermediate of membrane fusion

At some intermediate stage of membrane fusion the two interacting membranes coalesce; the outer lipid monolayers of the two membranes are continuous, whereas the inner lipid monolayers are still separated. The different molecular models that have been proposed for this intermediate or 'semi-fusion' stage of membrane fusion are schematically depicted in figure 4.

The mechanism of biological membrane fusion was originally thought to involve the formation of a large IMP-free bilayer diaphragm (fig. 4, A); membrane fusion is completed by the formation of an aqueous pore after dissolution or osmotic rupture of the shared bilayer. This model is supported by morphological data obtained in

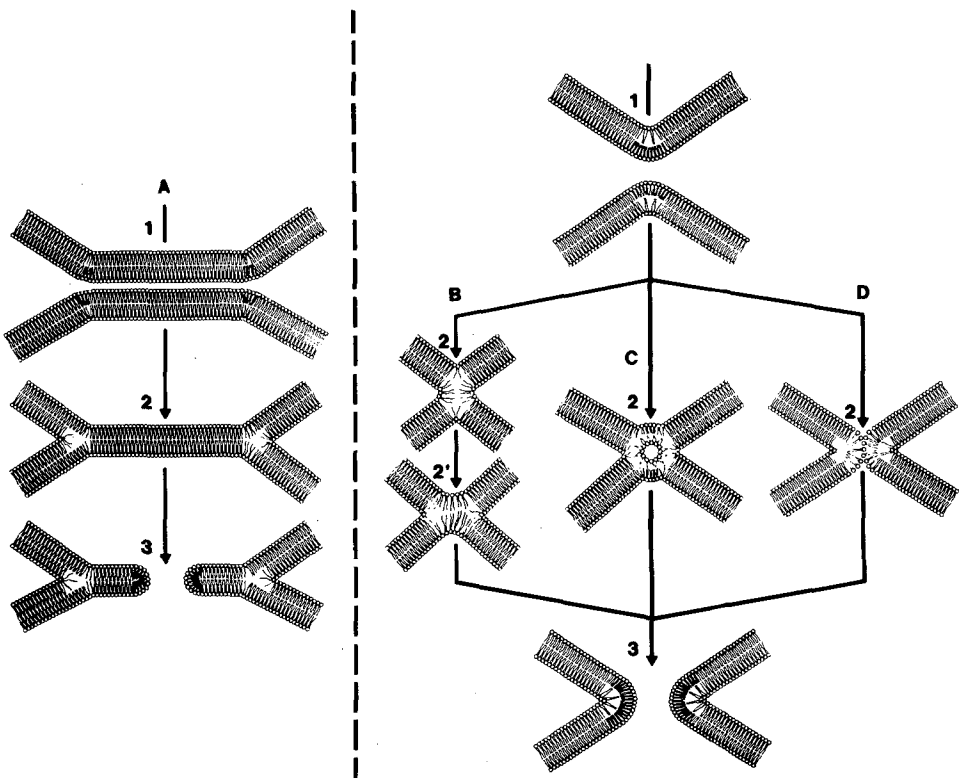

Figure 4. A schematic representation of the molecular models proposed for membrane fusion. Membrane adhesion (1) is followed by the formation of a semi-fusion intermediate (2), and fusion is completed after the formation of an aqueous channel (3). Fusion is thought to involve a large area of the interacting membranes, and a large single-bilayer diaphragm is formed (A.2)[55]. Or fusion is considered a local-point event, leading to the formation of a monolayer stalk (B.2) and a very small single-bilayer diaphragm (B.2')[22], an inverted lipid micelle (C.2)[97], or a local-point defect (D.2)[46]. For details see text.

chemically fixed cells, but the results of modern EM techniques have now clearly proved it to be wrong (see section II).

If biomembrane fusion is a local-point event (which it now appears to be), what is the nature of the semi-fusion intermediate? Different molecular models have been proposed (fig. 4, B–D). First of all, the presence of bilayer diaphragms in chemically fixed and dehydrated cells, clearly demonstrates that under certain (artificial) conditions two membranes can join to form one shared bilayer[16, 66, 67, 71]. Studies on pure lipid bilayers forced into close contact support this notion, and a local-point membrane fusion mechanism has been proposed in which two interacting membranes first join to form a so-called monolayer-stalk, which subsequently develops into a very small bilayer diaphragm (fig. 4, B)[21, 22, 52]. This bilayer diaphragm could be very short-lived, and its rupture might result in the formation of a small aqueous pore[57].

On the other hand, a significant proportion of the lipid present in any biomembrane reveals a tendency to form inverted, or 'type II' non-bilayer lipid structures (typically the inverted hexagonal or H_{II} phase)[26]. These lipids could be essential to biomembrane fusion by allowing the formation of an inverted lipid micelle sandwiched in between the two interacting membranes, at the semi-fusion stage (fig. 4, C)[97]. Morphological evidence in favor of this model has been obtained (see V.3).

A third model is based on the fact that, although EM has revealed the presence of specific structural features in numerous pure lipid model systems undergoing membrane fusion, no direct evidence has been obtained to date, proving the actual involvement of these structures as semi-fusion intermediates in the act of membrane fusion. It has been argued that the observed structural features are in fact equilibrium structures not necessarily identical to the 'real' fusion intermediates; the real fusion intermediate being simply a local disordering of lipid molecules or a fluctuation in lipid packing (a 'local-point defect'[46]; fig. 4, D)[33, 57, 105].

V.2 Specific lipid involvement: 'fusogenic' lipids

A large number of different 'fusogenic' lipids have been proposed to be specifically involved in membrane fusion. First of all, lysolecithin, monoacylglycerides and cis-unsaturated fatty acids were proposed to be involved in the formation and rupture of the bilayer diaphragm (fig. 4, A); lysolecithin by forming micelles and monoacylglycerides, and cis-unsaturated fatty acids by increasing membrane fluidity[54, 55]. The role of these lipids in biomembrane fusion is, however, questionable for several reasons. The presence of lipid micelles in a membrane directly conflicts with one of the most important functions of a biomembrane; that of maintaining a semi-permeability barrier. Moreover, sophisticated lipid analysis techniques which prevent lipid breakdown have shown that (in most biomembranes) the aforementioned fusogenic lipids are either completely absent or, if they are present, then only in very small amounts. Finally, model membrane studies have shown that a simple relationship between increased membrane fluidity and enhancement of membrane fusion does not exist[11]. However, it has been shown that cis-unsaturated fatty acids do induce fusion of aggregated chromaffin granules in vitro[23]. The relatively large amounts of arachidonic acid released after activation of chromaffin cells, blood platelets, mast cells or pituitary cells[37] could play a role in enhancing membrane fusion, possibly by promoting the formation of type II non-bilayer lipid structures[45].

Acidic phospholipids, especially PA and PS, have been proposed to play a crucial role in the induction of biomembrane fusion in conjunction with Ca^{2+}[31, 33, 68]. A general fusion mechanism was proposed, involving Ca^{2+}-induced lateral phase separation of acidic phospholipids[68]. According to this model, Ca^{2+}-induced fusion occurs through defects in lipid packing at the boundaries of gel-phase domains of acidic phospholipids within the fluid mixed-lipid membrane. Extensive model membrane studies on divalent cation-induced membrane fusion have shown, however, that an isothermal phase transition from a fluid to a gel state, and macroscopic lateral phase separation, are not essential prerequisites for divalent cation-induced membrane fusion[31, 33, 105]. Instead divalent cations may induce membrane fusion of acidic phospholipid-containing membranes by a combination of charge neutralization, cross-linking of membranes and local dehydration. A disordering of lipid molecules in the membrane contact area may subsequently result in a local-point defect and eventually in membrane fusion (fig. 4, D)[31, 33, 105]. However, as mentioned earlier (sections I and III), a direct role for acidic phospholipids and Ca^{2+} in the induction of biological membrane fusion seems unlikely, and the molecular mechanism of Ca^{2+}-induced model membrane fusion may even be of little relevance to the molecular mechanism of biological membrane fusion.

In contrast to the fusogenic lipids mentioned so far, type II non-bilayer preferring lipids are present in almost every biological membrane; in relatively high amounts in both membrane leaflets[26, 97]. Furthermore, their phase preference can be triggered and membrane fusion induced by a large number of biologically relevant factors including certain proteins and polypeptides[9, 27]. At this stage it is important to note that, although the role of type II non-bilayer preferring lipids in (bio)membrane fusion is rather well established, the molecular nature of the semi-fusion intermediate is still controversial owing to a lack of direct experimental data; in principle, the fusogenic properties of type II non-bilayer preferring lipids can be explained using any of the three models mentioned for local-point membrane fusion[cf. 21, 26, 105] (fig. 4, B–D). Recent theoretical models of bilayer to type II non-bilayer lipid phase transitions and membrane fusion favor the involvement of an inverted lipid micelle

in membrane fusion[85,86] (fig. 4, C), but local-point defects (fig. 4, D) are not easily 'caught' in a theoretical model, and therefore cannot be excluded on theoretical grounds.

V.3 Type II non-bilayer lipid structures: The inverted micellar intermediate

One of the non-bilayer lipid phases formed by type II non-bilayer preferring lipids is the inverted hexagonal or 'H_{II}' phase[9,26,27,85,86,97]. The lipid molecules in the H_{II} phase are organized in hexagonally arranged cylinders, with the polar headgroups lining a central aqueous channel (fig. 5a, b). Almost any biological membrane contains significant amounts of lipids that, upon isolation, will form an H_{II} phase under physiological conditions of ionic strength, pH and temperature; examples are the unsaturated PEs present in all eukaryotic membranes, monogalactosyldiacylglycerides present in chloroplasts, and monoglucosyldiacylglycerides present in certain prokaryotes. The formation of an H_{II} phase by other naturally occurring lipids like cardiolipin and PA depends on the pH and the presence of divalent cations. Beside divalent cations, pH and temperature, a bilayer to H_{II} phase transition can be triggered by DG and a number of proteins and polypeptides (see V.4), and can be modulated by the presence of e.g. cholesterol (-derivatives), dolichol (-derivatives), glycerolmonooleate, retinol and cis-unsaturated fatty acids[9,27,45].

The transition from a bilayer to an H_{II} phase, which occurs through membrane fusion, is therefore absolutely dependent on membrane contact[9,34,85,86,97], and is accompanied by only a very small change in enthalpy. Inverted lipid micelles are thought to be involved both in membrane fusion and in the formation of the H_{II} phase.

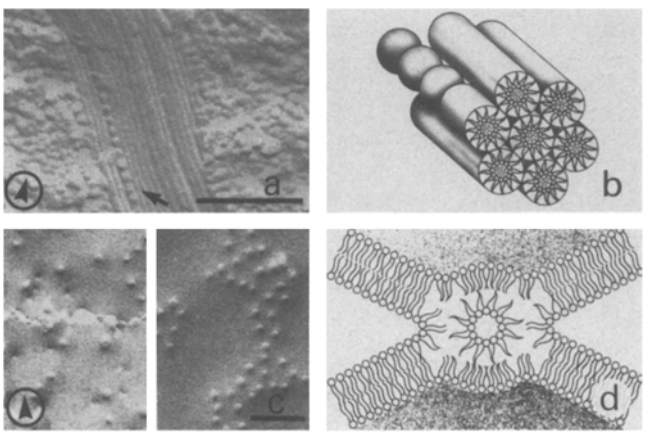

Figure 5. Type II non-bilayer lipid structures and membrane fusion. Dioleoyl-PE fast-frozen from 20 °C, lipidic particles are observed in line with an H_{II} tube (marked by arrow in (*a*)). Similar lipidic particles are observed, together with their complementary pits, in a sample of dioleoyl-PE/dioleoyl-PC/cholesterol (molar ratio 3:1:2) heated to 60 °C for 10 min and fast-frozen from 4 °C (*c*). The transition from the bilayer to the hexagonal H_{II} phase (*a*) and membrane fusion (*b*) are thought to share a common intermediate, the inverted lipid micelle (cf. (*b*) and (*d*)). For details see text. Direction of Pt/C shadowing indicated by encircled arrowhead. Bars. 0.1 μm. (*a*)[96], (*c* and *d*)[97], reproduced with permission.

Experimental evidence in favor of this hypothesis has been obtained in single-lipid systems, and in mixed-lipid systems in which one of the lipids prefers the H_{II} phase; under conditions revealing the H_{II} preference of (part of) the lipids, membrane fusion is observed and often well-defined particles (referred to as 'lipidic particles') are detected on the fracture faces of the interacting membranes (fig. 5c). Occasionally, lipidic particles are observed in line with an H_{II} tube[96,97] (fig. 5a); this observation suggests a direct structural relationship between these lipidic particles and the H_{II} phase (fig. 5b), and corroborates the interpretation of the well-defined lipidic particles as being inverted lipid micelles (fig. 5d). Furthermore, the involvement of inverted lipid micelles in local-point fusion of membranes containing type II-preferring lipids is supported by recent theoretical models[85,86].

It is important to note that H_{II} phase formation is accompanied by leakage, and since biomembrane fusion is essentially non-leaky, it is not the H_{II} phase itself but the precursors of the H_{II} phase, i.e. inverted lipid micelles, which may be relevant to biological membrane fusion. Indeed, in PE-containing model membranes it has been shown that non-leaky fusion only occurs at a temperature slightly below the H_{II} phase transition temperature (T_H). In contrast, induction of fusion at $T > T_H$ always resulted in leaky fusion and the formation of an H_{II} phase[34].

Biological membrane fusion is a local-point event, non-leaky, and also extremely fast (ms-range). The short lifetime predicted for the inverted lipid micelle by theoretical modelling, which is on the ms-range, agrees well with the fusion rate observed in biological membrane fusion[85,86]. On the other hand, the rapidity of the process probably also explains why lipidic particles have never been observed during the initial rounds of model membrane fusion. The lipidic particles seen at later stages in multilamellar lipid aggregates apparently have a longer lifetime. Again on the basis of theoretical calculations, Siegel has in fact suggested that the lipidic particles represent a stage already beyond the formation of an inverted lipid micelle, namely post-fusion structures with a minute (often not detectable) aqueous channel already formed ('interlamellar attachment sites', or ILAs); these ILAs are predicted to have a longer lifetime than the inverted lipid micelles[85,86]. In conclusion, type II-preferring lipids and a local bilayer-to-non-bilayer lipid structure transition are attractive candidates for a direct involvement in biomembrane fusion.

V.4 Biomembrane fusion and type II non-bilayer lipid structures

The probable involvement of type II non-bilayer preferring lipids in biomembrane fusion is substantiated in particular by studies on the lipid dependency of virus-model membrane fusion; virus-model membrane fusion is greatly enhanced by the presence of unsaturated PEs in

the target membrane[82, 90, 95, 102]. In (semi)intact biological systems, a direct role of type II preferring lipids in membrane fusion has not been demonstrated. However, recent studies have shown that the amount of type II preferring lipids present in the membrane of certain prokaryotes is under strict metabolic control, suggesting at least a general importance of these lipids in the intact biological system[104].

Is there any direct evidence that indicates the presence of type II non-bilayer lipid structures during biomembrane fusion? The 'capturing' of fusion intermediates during biomembrane fusion is greatly complicated by their short lifetime, the low density of fusion events and mostly (but not always, see section III) by a lack in synchrony[51]. In addition, thermodynamically unstable intermediates such as inverted lipid micelles might be lost, even during fast-freezing[86]. Nonetheless, 'fusion-correlated particles' have been observed by freeze-fracture EM after synchronized fast-freezing, during influenza HA-induced fusion of liposomes with virus-infected cells[50] (fig. 2a), and during exocytosis in chromaffin cells[83] (fig. 2c).

In the case of HA-induced membrane fusion, arguments can be presented in favor of a lipidic nature of the observed fusion-correlated particles[14, 50]. The exclusive presence of these particles under fusion conditions strongly suggests that they either represent (a part of) the HA glycoprotein or a trapped (lipidic) fusion intermediate. The size of the fusion sequence of HA, thought to penetrate the target membrane[41] (the liposomal membrane in this system) and initiate membrane fusion, is far too small for it to be the origin of visible (protein) particles on the fracture faces of the liposomal membrane; it is likely that these particles (fig. 2a) are of a lipidic nature and probably represent a trapped fusion intermediate. In fact, the fusion-correlated particles are very similar in size and shape to the well-defined spherical particles often observed in pure lipid systems (fig. 5a)[97], and may therefore represent an inverted lipid micelle at the semi-fusion stage of membrane fusion, or a post-fusion structure with a minute aqueous channel already formed (ILA, see section V.3).

The presence of 'fusion pores' has been observed by capacitance measurements during exocytotic membrane fusion[109, 110], and these have been tentatively interpreted as ILAs[101]. Interestingly, capacitance measurements have also shown that the fusion pore in a biological system is much more stable than the pore formed during Ca^{2+}-induced fusion of acidic phospholipid-containing vesicles with a planar lipid bilayer[109]. This increase in lifetime of the (post-)fusion intermediate may explain why fusion-correlated (lipidic) particles could be detected in the first round of fusion during biomembrane fusion (fig. 2), but not during model membrane fusion (see V.3). The reason for a more stable *biological* fusion intermediate is not clear, but may be related to the steric constraints imposed on biomembrane fusion by membrane-cytoskeleton interactions. The data presented so far support a role of type II non-bilayer preferring lipids in determining the overall fusogenicity of a biomembrane, and a possible involvement of type II non-bilayer lipid structures in biomembrane fusion.

Factors capable of inducing type II non-bilayer lipid structures could be directly involved in the initiation of biomembrane fusion. Experimental evidence to support this notion has, so far, only been obtained in model membrane studies. Melittin, a protein from bee venom, triggers a bilayer to H_{II} phase transition in cardiolipin model membranes; and at a lower peptide to lipid molar ratio, melittin induces non-leaky fusion of large unilamellar cardiolipin vesicles[6]. Gramicidin, a bacterial polypeptide antibiotic, is capable of inducing the H_{II} phase, even in the bilayer preferring phospholipid PC[27]. At a sufficiently low peptide-to-lipid molar ratio, gramicidin induces a fast and efficient non-leaky fusion of dioleoyl-PC large unilamellar vesicles (fig. 6; Tournois, Fabrie, Burger, Mandersloot, Hilgers, Van Dalen, De Gier and De Kruijff, unpublished observations). A relatively mild chemical modification of gramicidin by formylation of the four tryptophan residues results in a complete loss of its H_{II} phase inducing capacity[48]. Although membrane insertion is essentially unaltered, the loss of H_{II} phase inducing capacity is parallelled by an almost complete loss of the capacity to induce membrane fusion (fig. 6). These data strongly suggest a direct relation between the ability of a peptide to induce type II non-bilayer lipid structures and the ability to induce membrane fusion. Similarly, a protein involved in the regulation of biomembrane fusion may directly initiate membrane fusion by locally inducing a bilayer to non-bilayer lipid structure transition.

The PI-cycle and the formation of DG seem to play a primary role in the regulation of membrane fusion in many biological systems (see section III). Physiological levels of DG can substantially lower the temperature at which a PE-containing model system converts to the H_{II}

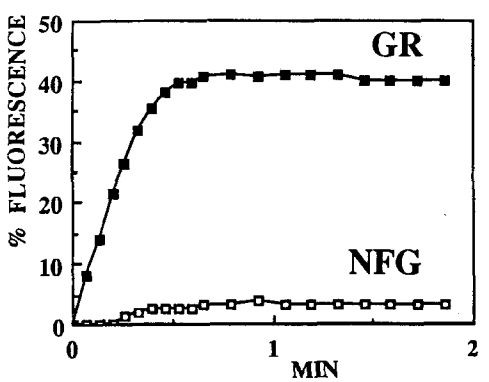

Figure 6. Gramicidin (analog)-induced fusion of large unilamellar dioleoyl-PC vesicles. Vesicles were prepared in the presence of a high concentration of a fluorescently labeled dextran. Gramicidin (GR) or its formylated analog (NFG) were added from dimethylsulfoxide to yield a peptide-to-lipid molar ratio of 0.07, and the time-course of aqueous contents mixing was measured by monitoring the dequenching of dextran fluorescence. For details see text.

phase[87]; and DG produced by the local action of PLc added to mixed-lipid large unilamellar vesicles induces fast and efficient non-leaky membrane fusion[63]. Therefore, in addition to an indirect regulation of membrane fusion, e.g., by activating PKc (see III), DG could also play a direct role in the induction of biomembrane fusion, again by locally inducing a bilayer to non-bilayer lipid structure transition.

VI Fission: Completion of fusion and the release of contents

The preceding section focussed in particular on the molecular mechanism that underlies the formation of a semi-fusion intermediate during (bio)membrane fusion. However, fusion is not completed before a pore is formed connecting two originally separated aqueous compartments (of course membrane fusion can also result in the formation of two compartments out of one, e.g. during endocytosis and virus budding). What is the driving force behind the disruption of the semi-fusion intermediate and the formation of an aqueous pore?

Osmotic forces have been proposed to be involved[57]; exocytosis is often accompanied by swelling of secretory granules, and can be inhibited by hyperosmotic solutions in a number of intact secretory cells[13,47,101]. The suggested role of osmotic swelling as the driving force in membrane fusion and fission was supported by a model-system approach to exocytosis, which involved monitoring the fusion of negatively charged vesicles with a planar lipid bilayer; addition of Ca^{2+} results in vesicle binding, but fusion only occurs if the vesicles are allowed to swell[2]. However, by monitoring granule swelling and membrane fusion simultaneously in real time, in intact beige mouse mast cells, secretory granule swelling was shown to follow and not precede membrane fusion[110]; this virtually ruled out a direct role for an osmotic force in membrane fusion and fission (of course, a highly localized osmotic swelling cannot be excluded[1]). Part of the controversy about the role of osmotic forces arises from a discrepancy between membrane fusion and the formation of a small (not readily detectable) exocytotic pore on the one hand, and enlargement of this pore and release of granule contents on the other hand. If, for example, exocytosis in *Paramecium* is triggered in the absence of extracellular Ca^{2+}, fusion is not followed by a release of contents[73]; and in sea urchin eggs it was shown that Ca^{2+}-induced granule swelling is absolutely required for dispersal of the granule contents[101]. Taken together, these data suggest that during exocytosis a small exocytotic pore forms spontaneously, and that in some cell types an influx of ions (Ca^{2+}) or water (swelling) through this pore is required for a decondensation of the granule core and release of contents into the extracellular medium[4,19,101]. The possibility of a spontaneous formation of fusion pores is supported by the theoretical models of Siegel, which predict an extremely unstable inverted lipid micelle as the semi-fusion intermediate[85,86]. This inverted micelle either collapses, and the original situation of two separate bilayers is restored, or spontaneously converts into a post-fusion structure or fusion pore (ILA) with a small aqueous channel. Of course, an osmotic gradient and influx of water could play a role in stabilizing or enlarging the fusion pore, and increase the efficiency of biomembrane fusion in some cell systems[4,101].

Conclusions and prospects

Even a rather selective compilation of data, as presented in the current review, clearly demonstrates the enormous complexity of biological membrane fusion. The regulation of biomembrane fusion is particularly complex because the merging of two membranes and the formation of an aqueous pore is only one event in a long series of events that precedes and follows membrane fusion. During secretory exocytosis, for example, membrane fusion is preceded by the transport of secretory granules to the plasma membrane, removal of steric barriers, recognition and docking, whereas fusion is followed by widening of the aqueous pore, decondensation of the granule core, release of granule contents, and compensatory endocytosis. Proteins appear to play a key role in many of these steps.

Proteins are involved in the directed movement of secretory vesicles, and in the spatial organization of the fusion machinery. Proteins may co-regulate membrane fusion by sterically hindering close apposition of membranes, and proteins are involved in the mutual recognition of fusion partners. Finally, proteins are very likely to be directly involved in the initiation of membrane fusion, by locally producing or activating a fusogen, or by acting as a fusogen.

Biomembrane fusion is a local-point event and requires a local and transient loss of the equilibrium bilayer configuration. The presence of significant amounts of type II non-bilayer preferring lipids in any biomembrane, and the fact that their phase-preference can be triggered and membrane fusion induced by biologically relevant fusion factors, strongly suggests a central role for type II non-bilayer preferring lipids in biomembrane fusion. The willingness of a biomembrane to fuse is largely determined by a delicate balance between bilayer and non-bilayer preferring lipids present in the membrane, a balance which may be under strict metabolic control. During biomembrane fusion this balance is locally disturbed, which leads to a local bilayer to non-bilayer lipid structure transition and membrane fusion. Proteins are likely to play a key role in the local destabilization of two interacting membranes. First of all, PLc activation accompanies many biomembrane fusion reactions and results in a local production of DG; DG is a potent inducer of type II non-bilayer lipid structures and membrane fusion. Secondly, a number of proteins and peptides can directly trigger a bilayer to type II non-bilayer phase

transition and induce efficient and non-leaky membrane fusion.

In conclusion, the regulation of biomembrane fusion involves specific recognition of the fusion partners, removal of steric or electrostatic barriers that prevent close apposition of membranes, followed by a local, and most likely protein-modulated, destabilization and bilayer to non-bilayer lipid structure transition of the interacting membranes.

The component directly responsible for the induction of biomembrane fusion has only been identified in the case of enveloped viruses; and viral fusion proteins will probably be the first biologically relevant fusogenic proteins for which the fusion mechanism will be unravelled. Since viral fusion proteins have presumably evolved from a preexisting intracellular fusion protein[103], their fusion mechanism may turn out to be very similar to that of other biological fusion proteins. Based on the encouraging results recently obtained in cell-free reconstitution systems of biomembrane fusion, one may predict that many factors involved in the regulation of biomembrane fusion will be identified in the near future. Each of the identified fusion factors should be studied in the intact biological system using a combined biochemical and morphological approach and techniques that offer sufficient temporal and spatial resolution. In the end, a fusogen has to be localized at the fusion site and in relation to the fusion process. A study of the behavior of the isolated and purified fusogen in a carefully chosen model system may subsequently answer many of the questions with respect to the mechanism, the specificity and the regulation of biomembrane fusion.

Abbreviations. Ca_i^{2+}, intracellular calcium; DG, diacylglycerol; EM, electron microscopy; ER, endoplasmic reticulum; HA, influenza virus hemagglutinin; H_{II}, inverted hexagonal; ILA, interlamellar attachment site; IMP, intramembrane particle; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PKc, protein kinase C; PLc, phospholipase C; PS, phosphatidylserine; type II, inverted lipid.

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Transmembrane movements of lipids

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Summary. Membranes allow the rapid passage of uncharged lipids. Phospholipids on the other hand diffuse very slowly from one monolayer to another with a half-time of several hours. This slow spontaneous movement in a pure lipid bilayer can be selectively modulated in biological membranes by intrinsic proteins. In microsomes, and probably in bacterial membranes, non-specific phospholipid flippases allow the rapid redistribution of newly synthesized phospholipids. In eukaryotic plasma membranes, aminophospholipid translocase selectively pumps phosphatidylserine (PS) and phosphatidylethanolamine (PE) from the outer to the inner leaflet and establishes a permanent lipid asymmetry. The discovery of an aminophospholipid translocase in chromaffin granules proves that eukaryotic organelles may also contain lipid translocators.
Key words. Flippase; aminophospholipid translocase; flip-flop; lipid asymmetry.