Membrane Fusion and Fission: Enveloped Viruses

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Abstract: Membrane fusion and fission are two key processes that occur during the replication of enveloped viruses, namely access to the interior of the host-cell (entry, which requires fusion of the viral envelope with the target cell envelope) and dissemination of viral progeny after replication (egress, which involves budding and fission). These dynamic processes are mediated by specialized proteins that modify and bend the lipid bilayer transiently and locally. This review focuses on fusion and fission reactions and on the hypothetical shared mechanism that generates their driving force.

Keywords: Membrane fusion, membrane fission, glycoprotein, enveloped virus.

INTRODUCTION

Membranes are universal biological components of many important cellular structures; in particular, the plasma membrane forms the external boundary of a cell, while other membranes enclose compartments within the cell, such as the nucleus and other organelles responsible for essential cellular processes. Living cells are non-equilibrium systems in which all the components are in a state of constant transformation involving deformations of membrane surfaces.

Two biological processes that play a fundamental role in normal and pathological conditions, fusion and fission, are consequences of major rearrangements of membrane structures, which are generated as a result of a complex interplay between proteins, lipids and forces applied to the membrane surface. The concerted action of several specialized proteins, bound to the membrane surface or embedded in the hydrophobic core of the lipid bilayer, is involved in these processes; a single protein molecule can generate membrane curvature only locally, within a region comparable to its own size, while the concerted action of many proteins is also possible when proteins come very close to each other and give rise to functional domains on the membrane surface.

Merging of two membranes into one (fusion) and division of one membrane into two (fission) are ubiquitous processes and represent key stages of protein trafficking, exo- and endocytosis, viral entry and exit, and many other cellular processes [1, 2].

It has been recently proposed that these two membrane rearrangements proceed through similar configurational stages that occur in the opposite order [3]. Fusion starts with the local joining of two membranes and proceeds through the expansion of the fusion pore. Fission starts with membrane budding, constriction of the bud neck, which corresponds to the opposite of the local joining of the two membranes in fusion, severing of the neck and formation of a vesicle from the original membrane (Fig. **1**).

Any deformation of membranes needs to overcome the resistance of the lipid bilayer, and involves some forces, controlling the direction of the process (fusion vs. fission) and producing the energy necessary for all the stages. To understand membrane fusion and fission, Kozlov and Chernomordik [3] highlight the importance of the identification of both the specific pathway of the process and the mechanism by which the specialized proteins induce the driving force of the reaction; they have also proposed that the driving force for fusion and fission derives from a dense self-assembled coat of membrane-bound activated proteins surrounding the site where membrane deformation starts [3]. In particular, the effective shape of the protein molecules constituting the coat determines the shape that the whole protein coat tends to adopt spontaneously in order to relieve the elastic stress of the bilayer. The fundamental difference between the two processes is the direction of the membrane rearrangements which are determined by the curvature assumed spontaneously by the protein coat involved in the process: positive bending for fission and negative bending for fusion.

The current view, therefore, speculates on the model of each process on the basis of what it is known about the other, considering that they proceed through steps that are similar but in the opposite direction. Local changes in the curvature of the membrane will determine whether the fusion or the fission will be spontaneous. There are two ways in which proteins can change membrane curvature, they can form a coat on the surface of one monolayer or can insert into the upper part of the membrane monolayer and result in a local monolayer deformation [4-6]. To understand the general mechanism of fusion and fission of membranes it is instructive to consider enveloped viruses as a model, because of the relatively simple fusion and fission machineries, relying, in many cases, on the activity of a small number of proteins.

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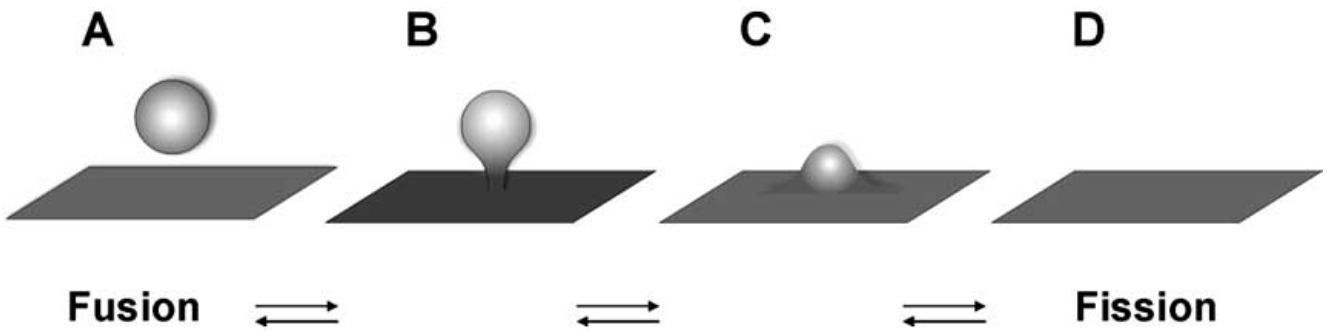

Figure 1. Membrane fusion and fission involve similar steps organised in the opposite order: a→d fusion and d→a fission. b and c represent intermediate stages in both fusion and fission.

MEMBRANE FUSION

Membrane fusion, in addition to its occurrence in normal physiological conditions is essential for the progression of different pathological events such as viral entry into host cells and possibly tumor progression.

Studies on fusion reactions from diverse membrane fusion events [7] revealed that the merging of two biological membranes follows common steps: initially, the two biological membranes are separated by 10-20 nm, their deformation causes a local contact which proceeds through a hemifusion state, in which only the outer leaflets fuse while the inner leaflets remain separated preventing the mixing of aqueous contents; finally, the inner leaflets merge and form the fusion pore allowing contents mixing. The structure and energetic of the membrane intermediates in protein-mediated fusion have been studied for different fusion reactions, but the best-characterized ones are fusion reactions mediated by viral envelope glycoproteins.

The entry of enveloped viruses into host cells relies on the fusion between the viral and the host cell membranes [8]. Viral membrane fusion is mediated by specialized membrane-anchored fusion proteins activated by specific triggers (i.e., low pH or receptor binding). Activated proteins are responsible for both local merging of two membranes into one and expansion of early fusion intermediates to complete fusion. Although membrane intermediates in protein-mediated fusion have been studied in some detail, the global forces driving the reaction still remain unknown.

Three different classes of viral fusion proteins have been identified so far based on their arrangement at the surface of the virion, their three-dimensional structure and the location within the protein of a short stretch of hydrophobic amino acids called the fusion peptide, which is able to induce the initial steps of lipid destabilization in the fusion process [9, 10]. Class I fusion proteins (orthomyxoviruses, paramyxoviruses, retroviruses, filoviruses, coronaviruses) are characterised by trimers of hairpins containing a central α-helical coiled-coil domain. The fusion peptide is located at or near the amino terminus of the fusion protein created by the proteolytic cleavage upon protein maturation. In the fusogenic conformation, the fusion peptide and the transmembrane domain (TM) are located at the same end of the rodlike molecule. Class II fusion proteins (alphaviruses, flaviviruses) are characterised by trimers of hairpins composed of beta-structures and the fusion peptide is in an internal location. Class III fusion proteins (rhabdoviruses, herpesviruses and recently baculoviruses) have only recently been described and combine features of both classes, such as a helix presumably forming a central α-helical trimeric core as in class I proteins and internal fusion peptides as in class II proteins [11]. Depending on the virus family, fusion occurs either at the plasma membrane for pH-independent viruses (e.g., paramyxoviruses, herpesviruses and retroviruses) or in endosomes for pH-dependent viruses (orthomyxoviruses, filoviruses, coronaviruses, arenaviruses, flaviviruses, alphaviruses, rhabdoviruses, some herpesviruses cell types). In both cases a conformational change results in the exposure of hydrophobic peptides, loops or patches; due to the large number of spikes on the viral surface and the oligomeric status of the fusion proteins, multiple fusion peptides might interact with the external leaflet, potentially initiating membrane deformation, and thus initiating the fusion process. All characterised viral fusion proteins are anchored to the envelope by a transmembrane domain that is of importance for its fusogenic activity, and some modifications of this domain blocks the ability of the proteins to form expanding fusion pores.

Recently, several authors have reported the important role played by the preTM domain of several enveloped virus fusion proteins belonging to the three classes, in membrane destabilization and thus in fusion [10].

Recent results obtained from the analysis of the fusion pathway [12-18] seem to be consistent with the hypothesis that fusion of membrane bilayers involves a sequence of local deformations in membrane monolayers. Initially, only locally contacting outer leaflets merge and are referred to as a fusion stalk; only afterwards, the two distal monolayers of the membranes come together (Fig. **2**), forming a small flickering fusion pore, which then expands to complete the fusion reaction. The driving force, directing the fusion reaction through all consecutive stages to the final expansion of the fusion pore, is still unknown [3].

The ”protein coat model” of membrane fusion assumes that activated fusion proteins form a dense interconnected protein coat surrounding the zone of membrane contact; this coat is more rigid than the lipid bilayer and has a strongly curved intrinsic shape, deforming the underlying lipid bilayer and driving its fusion with the target membrane [1]. The effective shape of the coat is determined by the conformations of the protein molecules within the coat and mainly

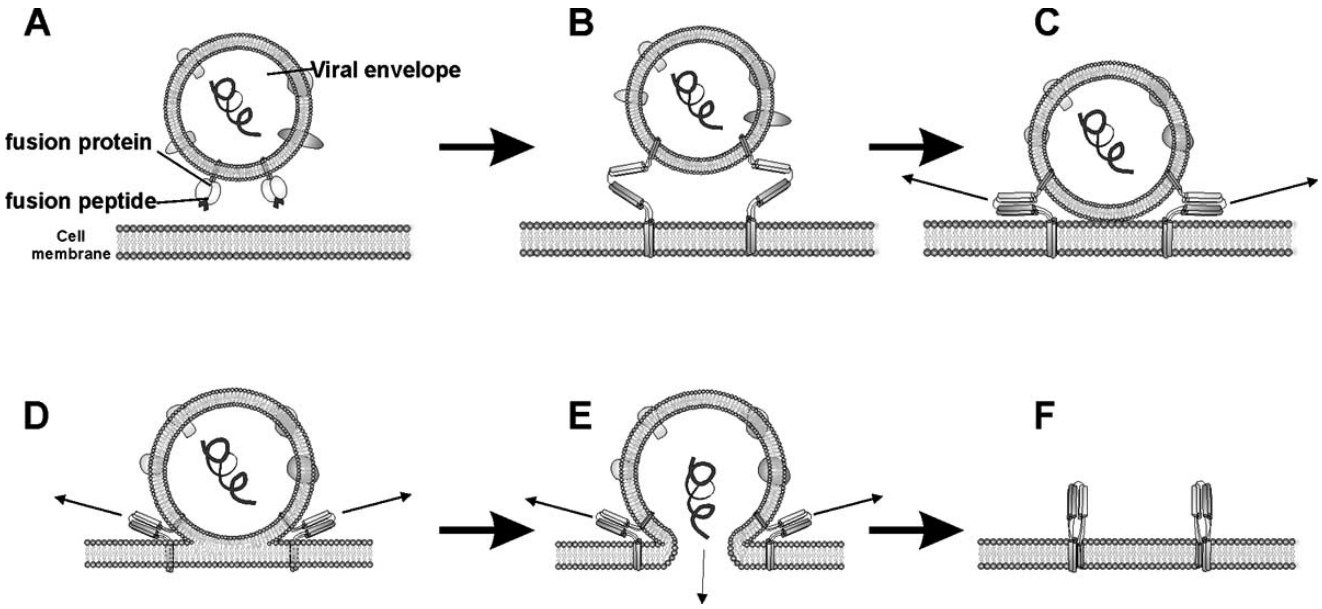

Figure 2. At the initial contact state (a) lipid bilayers of both the virus and the host cell are covered by membrane proteins, including fusion proteins and receptors that mediate membrane binding and fusion. After the interaction of the viral proteins with the host cell receptors (b), fusion proteins undergo a conformational change and expose the fusion peptide. The merger of contacting leaflets (c) is the hemifusion state, which is followed by the opening of the fusion pore (d) , whose expansion completes the fusion process.

by their oligomerization state. The tendency of the protein coat to adopt a negative curvature induces the bending out of the membrane plane into a funnel-like shape which further evolves into a pore and drives the fusion with the target membrane. In particular, curving of the protein coat into a funnel requires the formation of a protein-depleted patch of lipid bilayer and the bending of the underlying lipid bilayer, which has to bulge in the direction of the target membrane. The last step in the process of cell fusion is the expansion of the fusion pore which requires more energy and a higher number of activated fusogens compared to hemifusion. In the pre-fusion state, adjacent proteins do not interact laterally but once these proteins are activated by specific triggers, they undergo conformational changes that lead to their lateral aggregation and formation of a dense interconnected protein coat with negative spontaneous curvature, surrounding the zone of membrane binding.

MEMBRANE FISSION

Virus budding is the process comprising the envelopment of a viral core by a cellular membrane containing viral glycoproteins and subsequent membrane fission to release the particle from the membrane. Budding occurs at either the plasma membrane and virions are directly released into the extracellular space (retroviruses, alphaviruses, rhabdoviruses, ortho and paramyxoviruses) or on intracellular membranes, resulting in accumulation of particles in the lumen of cellular organelles. A wide variety of intracellular membranes such as the nuclear envelope (NE), endoplasmic reticulum (ER) endosomes, intermediate or pre-Golgi compartment (IC) Golgi cisternae and the trans-Golgi-network (TNG) have been proposed to serve as platforms for virus budding. Membrane fission is driven by the layer of proteins assembled at the membrane surface. Intracellular trafficking utilises clathrin, COPI [19] and COPII [20,21] coats. To constrict membranes into tubular shapes, cylinder-forming proteins are required. The first discovered cylinder-forming protein was dynamin, which self-assembles into helices on the membrane surface and constricts flat membranes into collared tubes [22]. *In vitro,* the coats spontaneously acquire a strongly curved shape and form membrane buds with constricted necks. The COP coats and viral matrix proteins are capable of completing the fission reaction without the assistance of any other proteins. The energy needed for membrane budding and for fission of the membrane neck is believed to be produced by the reaction of coat self-assembly and by conformational changes of GTPases (for instance, dynamin) which are involved in the reaction. To induce budding and fission a protein coat has to satisfy two major conditions: the interaction between coat-forming proteins has to be sufficiently strong and the rigidity of the protein coat has to exceed the rigidity of the lipid bilayer (Fig. **3**).

Most DNA viruses replicate in the nucleus and therefore virus egress requires crossing the nuclear envelope and the plasma membrane; for some viruses exit from the nucleus occurs via interaction with nuclear pore complexes followed by budding at a cellular membrane. The nucleocapsids of herpes simplex virus are too large for nuclear export and thus they exit by consecutive envelopment, de-envelopment and re-envelopment processes. In particular, HSV nucleocapsids traverse the nuclear envelope by budding from the inner nuclear membrane into the perinuclear space and subsequently lose their membrane by fusing with the outer nuclear membrane. Naked cytoplasmic nucleocapsids acquire their final envelope by budding at cytoplasmic membranes [23].

The intermediate structures formed in the process of membrane fission remain much less explored than those in membrane fusion. A topologically equivalent membrane scission event is needed for completion of cytokinesis, which also requires the scission of a thin bridge of membrane con-

necting the daughter cells. Cytokinesis also requires dramatic changes of plasma membranes and several vesicle-trafficking components. Thus, virus budding and cytokinesis use a similar subset of cellular components to carry out topologically similar membrane fission events [24].

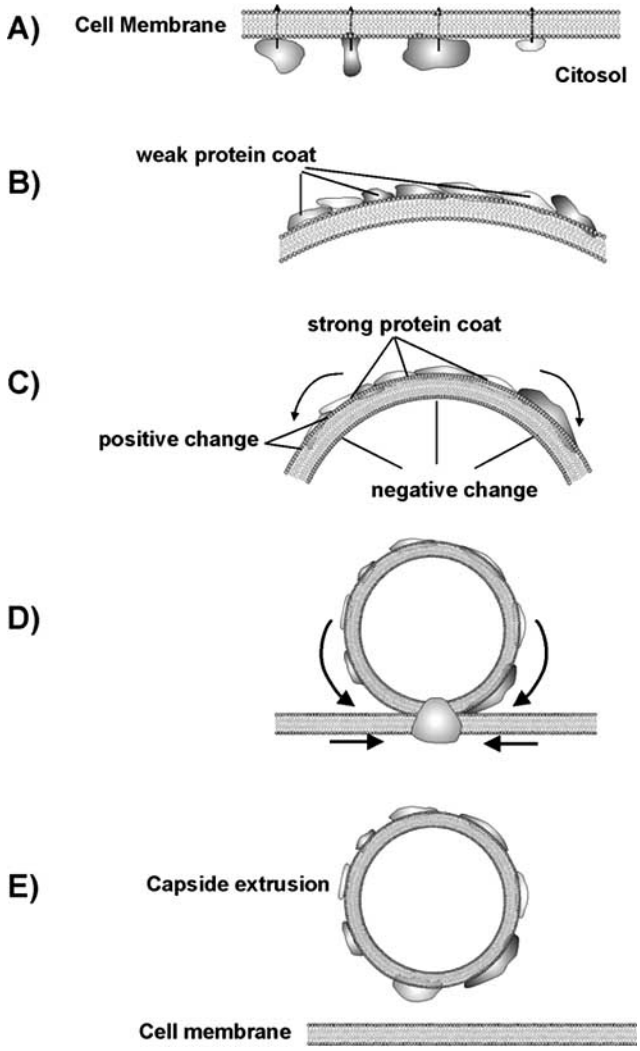

Figure 3. Mechanism by which proteins can generate membrane curvature during fission; b and c formation of the protein coat and deformation of membrane curvature, d budding and e vesicle separation.

ENVELOPED VIRUSES: FUSION AND FISSION

Enveloped viruses can penetrate target cells via fusion with cellular membranes, while they are released via budding at a host cell membrane, obtaining a cell-derived membrane containing viral proteins (Fig. **4**) [25]. The ability of enveloped viruses to cross lipid bilayers during virus entry (fusion) and exit (fission) without compromising membrane integrity increases the efficacy of viral spread and gives directionality to the viral egress-entry process that can be temporally regulated at various stages. The hypothesis that coat proteins generate the driving force for both fusion and fission offers a new interpretation for a number of known features of both processes [3]; the analysis of the prototype fusion protein, influenza hemagglutinin (HA), might bring new insights into the mechanism of other fusion and fission reactions.

HA is synthesized as a single polypeptide, which is later cleaved into its receptor binding (HA1) and fusion (HA2) subunits, which remain linked by a single disulfide bond [10]. At neutral pH, a trimer of globular HA1 subunits is located at the top of the HA molecule; in this state, HA trimers do not interact with each other, and are characterised by a relatively high lateral mobility. The fusion reaction is triggered by acidification of the endosome, which generates a cascade of conformational events which end in the formation of a fusion-competent structure characterised by the extrusion of the fusion peptide and the extension of the triple-stranded α-helical coiled-coil of HA2. The major changes in HA2 comprise the exposure of hydrophobic moieties such as the fusion peptides, the kink region, and other membrane interacting moieties; the interaction between exposed hydrophobic regions which drives the aggregation and formation of very large complexes at the membrane surface. Moreover, the well-defined rod-like spikes of HA on the surface of a native particle in the pre-fusogenic state are no longer visible as separate entities. Thus, it may be assumed that numerous activated HA trimers under fusion conditions aggregate into a dense interconnected protein coat with temperature-dependent properties. Large globular HA1 subunits remain connected to the HA2 ectodomains by the S-S-bonds close to the region of the molecule that is adjacent to the lipid bilayer [26], and moreover HA loses its ability to bind to membrane-anchored receptors at later stages of the fusion process [27], which is consistent with relocation of HA1 subunits towards the viral membrane and each HA molecule becoming thicker in its base than at its top. This change in the shape of the HA trimer is consistent with the protein coat developing a negative spontaneous curvature. At low-pH the fusion peptides are located at the top of the trimer and interactions between fusion peptides of adjacent HA molecules pull the tops of HA molecules closer to each other and thus contribute to the negative spontaneous curvature of the protein coat [3].

Depending on the number of available activated HA molecules, the fusion reaction reaches different stages [14, 28]; when a high number of activated molecules is available, it is possible to have a fusion pore with mixing of the lipids of the two fusing membranes, and merging of their aqueous contents; when a lower number of molecules is available, only small non-expanding fusion pores form (unrestricted hemifusion), i.e. lipid mixing with no fusion pore, and thus no contents mixing. If there are not enough molecules to establish hemifusion, they can still form fusion intermediates (restricted hemifusion), in which neither lipid mixing nor contents mixing can be detected but there is a very tight membrane contact or, probably, local hemifusion. These intermediates can be transformed into complete fusion (lipid- and contents- mixing) with treatments known to destabilize the hemifusion state. Thus, only a fraction of all the HA molecules needed for fusion is required in order to locally merge the outer leaflets of two membranes; additional molecules stabilize the hemifusion connection and then expand it to allow lipid redistribution through it. Additional molecules advance the reaction beyond hemifusion to an opening and then expansion of a fusion pore to complete fusion.

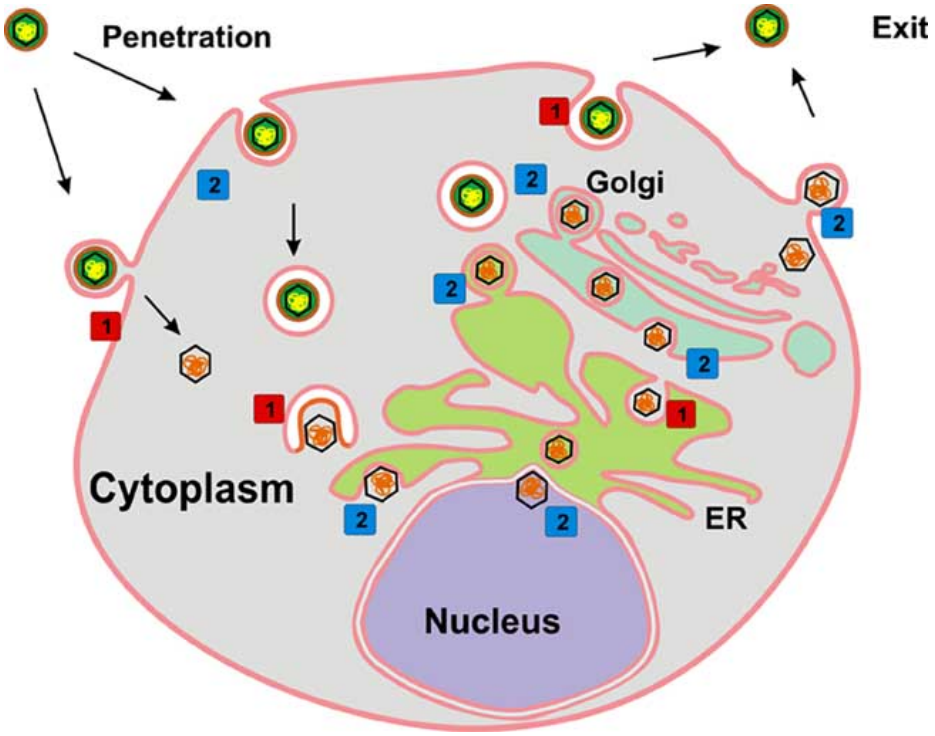

Figure 4. Schematic representation of the possible fusion (1) and fission (2) events during the mechanisms of virus entry and exit from the cell. Enveloped viruses enter the cell through direct fusion at the plasma membrane or by receptor mediated endocytosis followed by fusion between the endocytotic vesicle and the viral envelope. During the exit from the cell, many enveloped viruses (i.e. Retroviruses, paramyxoviruses, orthomyxoviruses, arenaviruses, etc.) bud from the plasma membrane of the infected cell where they become enveloped by fission, while others (i.e. herpesviruses, flaviviruses, etc.) acquire their envelope from the membranes of the intracellular compartment and are transported outside by different fission and fusion events.

Some mutations in the fusion peptide are reported to affect fusion either by blocking the opening of a fusion pore or by influencing the rate of its expansion [29, 30]. The protein coat model for fusion may explain the pivotal role played by the HA fusion peptide in fusion pore forming and expansion. The efficiency of the HA-mediated opening of an expanding fusion pore depends on the correct anchoring of proteins in the membrane. The lipid-anchored ectodomain of HA (GPI-HA) although capable of mediating lipid mixing between the membranes and of forming small fusion pores, is incapable of forming an expanding fusion pore; probably, a protein layer anchored only in the outer membrane monolayers does not restrict redistribution of the lipids of the inner monolayers of viral membrane to the region of the growing hemifusion state, thus weakening lateral tension and inhibiting the opening and expansion of a fusion pore [3]. These considerations may also explain why the transmembrane domains of fusion proteins are important at later stages of the fusion reaction [3]. Moreover, fusion proteins outside the contact zone may influence fusion through a mechanism that has not yet been understood but may be similar to that of the proteins mediating membrane budding from the outside of the final fission site [31].

Virus budding can take place at the plasma membrane or on various intracellular membranes of the secretory system. The viral proteins involved may include transmembrane proteins, capsid proteins and matrix-type proteins that associate with the inner face of the budding membrane [32]. The membrane bends and after fission the completed virus particle is released. The topology of virus budding is similar to that of vesicle budding into the lumen of the late endosomal multivesicular body (MVB) compartment and a number of enveloped viruses exploit the MVB machinery of mammalian cells for their budding reactions [33]. MVB formation requires the activity of a network of cytoplasmic protein complexes, known as ESCRT (endosomal sorting complex required for transport) complexes I and II that are recruited to the endosomal membrane to sequester cargo proteins and drive vesicularization into the endosome [34]. ESCRT proteins present binding pockets for the so called late domains motifs of viral proteins, which are short peptide motifs identified in most enveloped viruses, mutation of which leads to an arrest of virus replication at the late stage of budding [33,35,36]. These data supported the view that these interactions redirect the ESCRT machinery to the site of virus budding where viral particle vesicularization is required and are thought to constitute a pushing force for virus budding [25]. The final step of budding seems to involve the protein Vps4 (a cellular AAA+ ATPase) that is thought to catalitically remove the ESCRT complex from the endosomal membrane resulting in contraction of the membrane surrounding the nascent vesicle and leading to membrane fission. This mechanism does not apply universally and often it is not sufficient for budding of all enveloped viruses as the budding of enveloped viruses can be more complex than simply redirecting the ESCRT components. In particular there are some viruses that also require additional viral proteins for budding.

The protein machinery involved in budding and fission of influenza virus has been studied extensively and there are several discrepancies between different studies. Initially it was reported that expression of just one viral matrix protein M1, which assembles under the membrane, is sufficient to bend membranes into buds and release viruslike particles; M1 seemed to have all the structural information needed for self-assembly, interaction with cell membranes and accomplishment of the budding process and this conclusion was consistent with the notion that internal viral structural proteins are sufficient to drive virus budding due to the recruitment of cellular factors [37]. Numerous studies have examined the contribution of the HA and neuramidase (NA) cytoplasmic domains to influenza virus assembly and budding [38, 39]. Authentic virion assembly probably involves a series of protein-protein interactions between the glycoproteins and the internal virion components (ribonucleoprotein complexes); it is now believed that M1 coordinates assembly between the surface glycoproteins through interactions with their cytoplasmic tails, concomitantly forming a bridge to the internal ribonucleoprotein complexes. These studies suggest that the secondary structure or orientation of the glycoprotein cytoplasmic domains could be important for proper assembly of the virus, and thus serve for virus budding. Although using the cellular machinery is an attractive mechanism for virus budding, there is evidence that virus budding can also proceed spontaneously without the need for host factors. In particular, the intrinsic association of viral proteins into microdomains on the plasma membrane may be sufficient to drive virus budding by inducing membrane curvature and supplying a pulling force for virus formation [40]. The lipid raft association of many enveloped virus proteins has suggested that these microdomains serve to concentrate viral proteins and facilitate efficient virus budding [41, 42]. For influenza virus, lipid raft association is required for efficient virus replication; HA and NA cytoplasmic tails, together with the M2 cytoplasmic tail recruit M1 and contribute to packaging of the viral RNA-containing ribonucleoprotein complex. Lipid microdomain association may play an additional role in budding; in particular it may promote independently of host budding machinery the clustering of a critical mass of viral proteins to nucleate membrane vesicularitation.

MEMBRANE DEFORMATION BY PEPTIDES

A relative increase in surface area of one leaflet of a closed bilayer increases the spontaneous curvature of the bilayer; to minimise its energy state and maintain hydrophobic and van der Waals interactions between the leaflets, an opposed bilayer will conform to its spontaneous curvature. Membrane curvature is a fundamental modulator of both fusion and fission; the curvature stress of a highly curved membrane can provide the driving force that reduces the energetic barrier needed for the two processes. Membrane curvature can be produced by proteins that bind to the membrane surface [5] and/or by the insertion of small hydrophobic or amphipathic moieties inside the lipid bilayer [43, 44].

The amphipathic α-helix is a membrane binding motif found in many proteins and peptides which may function as promoters of membrane curvature in protein coats [45-48]. The segregation of hydrophobic and polar residues on two opposite faces of the helix matches well with the chemistry of the membrane interface. The shallow insertion of an amphipathic helix inside the upper part of a membrane monolayer perturbs the packing of the lipid polar headgroups and results in local monolayer deformations [6]. In particular, a shallow penetration of the amphipathic domain expands the upper part of one lipid monolayer and produces a positive curvature of the upper monolayer leading to positive curvature of the whole membrane. Campelo *et al.* [6] report that biologically relevant numbers of insertions of amphipathic helices are sufficient to create membrane curvatures necessary for fusion. Shallow insertion of an amphipathic helix, occurs in endophilin [47,48] where the N terminal amphipathic helix (N-BAR domain) induces local bending stresses that do not depend on protein shape but rather rely on perturbation of the target lipid monolayer into which it is inserted [5]. The amphipathic helices interact with phospholipid headgroups via the hydrophilic face and then partially embed into the bilayer via the hydrophobic patch in a manner that favours membrane deformation (Fig. **5**).

Apart from amphiphatic helices, also tilted peptides are characterised by an asymmetric distribution of their hydrophobic residues when helical, which induces a tilted orientation towards the membrane and thus they are able to disturb the organization of the membrane into which they insert [49, 50]. The relationship between tiltedness and fusion or more generally lipid destabilization has been highlighted experimentally for fusion peptides of different glycoproteins from enveloped viruses.

The Wimley–White membrane-water partitioning free energies of amino acids has been used as an algorithm for predicting fusion domains in viral fusion proteins. The average interfacial hydrophobicity directly reflects the tendency of any sequence to partition from water into membrane interfaces and thus to promote the initial steps of the protein membrane interaction. In particular, being membrane interfaces regions of the bilayer characterised by chemical heterogeneity and sharp polarity changes with distance, hydrophobic sequences rich in aromatic residues (such as tryptophans) show the greatest tendency to partition spontaneously from the aqueous phase into the membrane. More generally the Wimley-White scale for calculating interfacial hydrophobicity could be used to detect functional domains that may transmit protein conformational energy into membranes and thus be involved both in fusion and fission. Fusion peptides represent a force transmitting domain that is able to start the process; and all fusion peptides appear to interact exclusively with the outer leaflet of the target membranes, at least during the initial interaction stage further supporting their role in the modification of the target lipid monolayer. The current view is that the fusion peptide is assisted in this role by several additional elements present in the ectodomain of the fusion protein, commonly called membrane-interactive regions, and these include the pre-transmembrane domain [10]; altogether these domains may be involved in the modification of the membrane curvature. Similarly the use of interfacial hydrophobicity algorithms may lead to the detection of membrane interactive regions of proteins involved in the fission process and thus the protein coat may contain domains able to interact with membranes and induce the initial steps of budding. In general, interfacial sequences might be

required to disrupt interactions between lipid molecules and thus be directly involved in the destabilization of membrane integrity; the differential surface area increase of the monolayers thus contributes to membrane deformation and bending (positive for budding and negative for fusion).

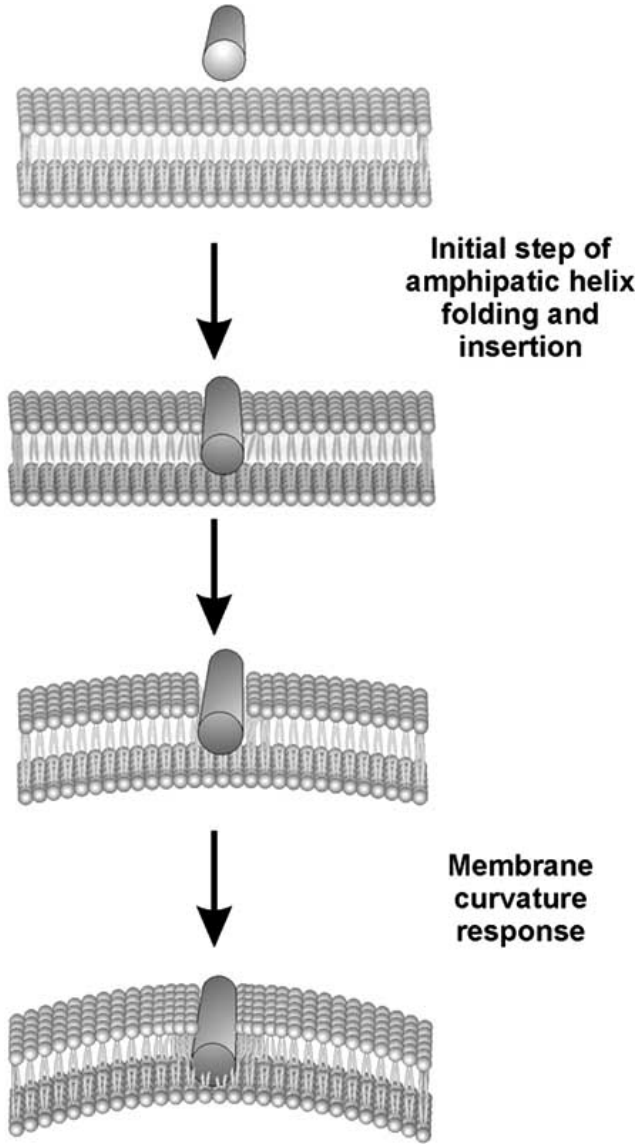

Figure 5. The phospholipids bilayer can be deformed by the insertion of amphipathic helices into one leaflet of the bilayer.

Lorizate *et al*. recently reported that the pre-TM domains of enveloped virus fusion glycoproteins play a fundamental role in fusion and fission, either by transmitting protein conformational energy into membranes and or by perturbing lipid bilayer integrity [51].

Involvement of the VSV G PreTM domain in fission was studied using glycoproteins with truncated stem sequences [52]. Recombinant viruses were generated and those containing chimeras with 12 or more PreTM residues produced near- wild-type levels of particles while viruses encoding chimeras with shorter or no PreTM sequences produced ca. 10- to 20-fold less. The VSV G PreTM might promote virus release by inducing positive membrane curvature at sites where virus budding occurs. The authors suggest that, for VSV G protein and possibly HIV gp41, the membrane proximal domain may be the critical link between two opposing but related activities of virus entry and virus budding.

Alphavirus 6K protein provides further insight into the analysis of interfacial domain activity in fission: this small protein sequence contains a single PreTM-TMD motif [53], and 6K is not required for alphavirus budding, but catalyzes the process [54], which allows recovery of budding defective, but still infectious particles, upon cell transfection with alphaviral replicon.

The budding of alphavirus particles is essentially promoted by interaction of assembled nucleocapsids with the carboxy terminal domain of envelope glycoprotein E2 [32, 55]. Despite the association of the 6K protein with the plasma membrane and its interaction with E1–E2 glycoproteins, very little 6K is incorporated into the budded virus particles. The N-terminal ectodomain of 6K comprises two hydrophobic-at-interface segments that can mediate association of this sequence with the external membrane monolayer [53]. Conservation of the interfacial 6K segments among divergent members of the Alphavirus genus suggests a functional role for these motifs. Sindbis virus (SV) 6K variants containing substitutions interfering with the capacity of the N-terminus to partition into membranes without affecting hydrophobicity resulted in impaired virus buddings.

Differential surface area increase of the external membrane monolayer upon insertion might provide a common molecular mechanism to explain the involvement of membrane interacting regions in fusion and fission [1, 2]. Monolayer surface area imbalance might contribute to membrane deformation and bending (positive curvature) at the points of virus budding [56]. Of particular interest is their interaction with cholesterol in a raft environment. Such interactions could facilitate the enrichment of glycoprotein within plasma membrane raft-domains through which the virus might bud [57]. Thus, membrane interacting domains might function as a raft-sensor to direct glycoproteins to budding sites during viral assembly. The role of lipids in enabling or generating membrane curvature has been extensively studied and certain lipids have been shown to favour bilayer curvature owing to their physico-chemical properties and their relative geometries. Cholesterol, for example, is thought to selectively intercalate into the budding leaflet of the bilayer to enable bud formation without producing unfavourable hydrophobic-hydrophilic interactions as the bilayer is distorted. Selective enrichment of cholesterol into one leaflet of the membrane might alter the relative bilayer surface areas to favour budding and through differential partitioning might preserve hydrophobic and van der Waals forces between the leaflets as the bilayer deforms [57].

The conformational plasticity of membrane interacting domains seems to be fundamental for fusion; and a negative curvature is probably induced during the early stages of the fusion process [58]. Fission presumably requires a significant modification of the lipid environment at the site of assembly, supporting the membrane curvature induced by the protein coat associated with the inner leaflet. Thus several membrane interacting regions may be necessary to facilitate

lipid reorganization, either before or during the budding event.

The involvement of membrane interacting domain of viral glycoproteins in the fusion process may thus be common also to fission processes. In this context, it is of interest to consider recent studies of Herpes simplex virus type 1 whose entry and egress are very complex processes. Glycoproteins gB and gH/gL are known to be intimately involved in fusion and several domains of both proteins have membrane fusion capabilities [59-61]. It was recently reported [62] that gB and gH, acting in a largely redundant fashion, can mediate nuclear egress, functioning directly in the fusion that occurs at the outer NM delivering capsids into the cytoplasm. The use of hydrophobicity at the interface may be used to further determine domains of the two glycoproteins involved in the fission process.

CONCLUDING REMARKS

Membrane fusion and fission can be considered two opposite processes in the entry and egress stages of the lifecycle of enveloped viruses. These processes are mediated by specialized proteins that disrupt and bend the lipid bilayer organization transiently and locally. This review concentrates on fusion and fission reactions and the hypothetical shared mechanism which could provide the driving force for the two processes. The driving forces operating in opposite directions (positive bending for fission and negative bending for fusion), determine the fundamental differences between the two processes, and thus the direction of the membrane rearrangement. If fusion also is driven by the protein coat, it may be speculated that bidirectional membrane recycling during exocytosis vs. endocytosis or during enveloped virus entry vs. virus egress can involve transitions between coats with different spontaneous curvatures. Different proteins interacting with the same or different surfaces of a membrane can form coats with opposite spontaneous curvatures and, hence, drive opposite membrane rearrangements. The hypothesis that membrane fusion and fission are driven by forces which are similar but operate in opposite directions has not yet been proved and future work will be needed to understand the most general features of the ubiquitous process of membrane remodelling. The analysis of disparate fusion reactions induces the search for general motifs in the proteins involved, and in membrane intermediates of the fusion process. This search is indirectly supported by the similarities between the physical mechanisms of diverse fission reactions, that occur during the assembly of enveloped viruses as well as during protein trafficking. Therefore, it is important to analyze protein-mediated fusion and fission reactions in general terms. The protein coat hypothesis is independent of the specific features of the proteins involved in fusion or fission, and can be fairly general. Moreover, although the general mechanism of the protein coat as a driving force may be shared by diverse fusion and fission reactions, specific mechanisms will surely vary for different systems.

The understanding of fusion and fission will not only lead to a better understanding of fundamental cellular processes but may also offer new therapeutic approaches to the control of viral infections, providing key insights into viral persistence under therapy and paving the way for therapy optimization.

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