Protein-induced Fusion Can Be Modulated by Target Membrane Lipids through a Structural Switch at the Level of the Fusion Peptide*

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Regulatory features of protein-induced membrane fusion are largely unclear, particularly at the level of the fusion peptide. Fusion peptides being part of larger protein complexes, such investigations are met with technical limitations. Here, we show that the fusion activity of influenza virus or Golgi membranes is strongly inhibited by minor amounts of (lyso)lipids when present in the target membrane but not when inserted into the viral or Golgi membrane itself. To investigate the underlying mechanism, we employ a membrane-anchored peptide system and show that fusion is similarly regulated by these lipids when inserted into the target but not when present in the peptide-containing membrane. Peptide-induced fusion is regulated by a reversible switch of secondary structure from a fusion-permissive $\alpha$-helix to a nonfusogenic $\beta$-sheet. The “on/off” activation of this switch is governed by minor amounts of (lyso)phospholipids in targets, causing a drop in $\alpha$-helix and a dramatic increase in $\beta$-sheet contents. Concomitantly, fusion is inhibited, due to impaired peptide insertion into the target membrane. Our observations in biological fusion systems together with the model studies suggest that distinct lipids in target membranes provide a means for regulating membrane fusion by causing a reversible secondary structure switch of the fusion peptides.

Membrane proteins located on the surfaces of viruses, sperm, and intracellular membranes are intimately involved in membrane fusion. The overall fusion machinery, including molecular factors for targeting, attachment, and regulation of these events, can be quite complex. For example, the SNARE (Soluble NSF Attachment Protein REceptors) system, mediating intracellular fusion events, consists of specific membrane proteins, present on vesicle (v-SNARE) and target membrane (t-SNARE), and cytosolic ATPases, such as N-ethylmaleimide-sensitive fusion protein or p97, together with their cofactors $\alpha$-SNAP (Soluble NSF Attachment Protein) and p47, respectively (1, 2). Typical features of the “fusion machinery,” be it a viral or an intracellular system, are careful assembly into distinct complexes, conformational changes and an overall enhanced $\alpha$-helical structure (3–7). But how these overall structural changes are translated into a functional mechanism is unclear. Questions therefore remain as to what mechanisms cause the fusion process to be “switched on,” and once it proceeds, which mechanisms cause fusion to stop.

Because our understanding of mechanisms of biological membrane fusion is still rudimentary, the use of artificial membranes, isolated fusion proteins, or fusogenic peptides is of great value. Synthetic fusion peptides can only partly mimic the overall mechanism by which viral or cellular proteins induce membrane fusion; in particular, the involvement of segments of the proteins other than the fusion sequence cannot be assessed with these peptides. Nevertheless, fusogenic peptides have provided precious information on structural requirements for fusion, e.g. with respect to amino acid specificity (8), secondary structure and orientation in the target membrane (9, 10), and molecular shape (11–13).

Here, the regulation of fusion by target membrane lipids was assessed in two biological systems, the influenza virus and Golgi membranes. Evidence was obtained, indicating that lipids blocking fusion displayed a pronounced inhibitory effect when present into the target membrane and much less when present into the membrane that bears the fusogen. Molecular dissection of these intriguing observations was accomplished by using a simplified membrane system, which relies on a membrane-anchored peptide model that has been shown to trigger fusion with target membranes in a structure-dependent manner (10, 14). When free in solution, the peptide, called WAE, adopts mainly $\beta$-structure, and is fusion-incompetent. However, when covalently attached to liposomes, it folds into an amphipathic $\alpha$-helix. As a consequence, the peptide-coupled liposomes fuse with target membranes. Hence, the coupled peptide represents a convenient model for a fusion peptide, like that located in a membrane-anchored fusion protein. The potential of this model to reveal regulating features of membrane fusion at the molecular level in terms of switching membrane fusion “on” or “off,” was addressed in the present work. By combining structural data obtained by infrared spectroscopy

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1 The abbreviations used are: WAE, N-WAELGEALEC-OH; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; PS, phosphatidyserine; PE, phosphatidylethanolamine; ATR, attenuated total reflection; FTIR, Fourier transform infrared spectroscopy; H/D, proton/deuterium.
and tryptophan fluorescence with functional assays for fusion, we report that a reversible and target membrane lipid-dependent switch from a fusion-permissive α-helix to a fusion-inhibiting β-sheet regulates fusion.

EXPERIMENTAL PROCEDURES

Materials—Lipids were purchased from Avanti Polar Lipids (Alabaster, AL). TbCl3, 6H2O was from Alfa. Oleoyl-1-α-lysophosphatidylcholine (oleoyl-1-α-LPC), lysophosphatidylethanolamine (LPE) from egg yolk, acrylamide, and dipicolinic acid were from Sigma.

Fluorescence Assays for Fusion—Large unilamellar WAE-coupled vesicles (LP-WAE) (final peptide/lipid ratio, 1:40), consisting of PC and cholesterol (3.5:1.5) were prepared as described previously (18). Fusion was monitored by lipid mixing, using an assay based upon energy transfer (15). To this end the peptide-coupled vesicles were labeled with 1 μM each of N-NTBD-PE and N-Rh-PE, and at time 0, target vesicles composed of PS/PE (10:3) were added to the WAE-liposome suspension in a 6-to-1 ratio (total lipid concentration, 70 μM), in 10 mM Tris, 150 mM NaCl, pH 7.4, at 37 °C. The initial rates of lipid mixing were determined from the tangents drawn to the steepest parts of the fusion curves. Contents mixing was assayed between LP-WAE loaded with 2.5 mM TbCl3 and target PS/PE vesicles loaded with 50 μM dipicolinic acid, as described earlier (14).

Fluorescence quantum yield is expressed as

\[
\Phi = \frac{F}{F_0}
\]

where \(F\) and \(F_0\) denote the fluorescence intensity at 340 nm after and before the addition of target vesicles, respectively. For acrylamide quenching experiments, emission spectra were recorded at \(\lambda_{ex} = 295\) nm, in the presence of increasing amounts of acrylamide. The quenching data were analyzed by the Stern-Volmer plot using the equation:

\[
F/F_0 = 1 + K_{sv}[Q],
\]

where \(F_0\) and \(F\) are the fluorescence intensities at 340 nm in the absence or presence of a given acrylamide concentration \([Q]\), and \(K_{sv}\) is the Stern-Volmer quenching constant.

Secondary Structure Determination—Oriented multilayers were obtained by slow evaporation of the lipid vesicles under an N2 stream at room temperature on one side of the germanium plate, which is the internal reflection element (50 × 50 × 2 mm, Harrick EJ2121) with an aperture angle of 45°, yielding 25 internal reflections (16). Fourier transform infrared spectroscopy (FTIR) spectra were then recorded after room temperature on a Bruker IFS55 FTIR spectrophotometer equipped with a liquid nitrogen-cooled mercury cadmium telluride detector at a nominal resolution of 4 cm⁻¹ and encoded every 4 cm⁻¹. The conformation analysis was performed on the amide I region of deuterated samples, located in a region of the spectrum often free of other bands, composed of 90% pure C=O vibration (16). To quantify the area of the different components of amide I as revealed by self-deconvolution, a least-squares iterative curve fitting was performed to fit lorentzian line shapes to the spectrum between 1700 and 1600 cm⁻¹. Each band was assigned to a secondary structure according to the frequency of its maximum. The area of all bands assigned to a given secondary structure was then summed up and divided by the total area. This ratio gives the proportion of the polypeptide chain in that conformation (16). The frequency limits for each structure were first assigned to the data determined theoretically or experimentally: 1662–1654 cm⁻¹ (α-helix; 1689–1682 cm⁻¹ and 1637–1613 cm⁻¹, β-sheet; 1644.5–1637 cm⁻¹, random; 1632–1626.5 cm⁻¹, β-turns. These limits have been slightly adjusted to obtain a good agreement between the proportion of each structure determined by attenuated total reflection (ATR)-FTIR and x-ray crystallography. For the deuteration kinetics, samples were spread on the germanium plate, and 10 spectra were recorded by ATR-FTIR to verify the reproducibility of the measurements and stability of the system. At time 0, a D2O-saturated N2 flux was applied to the sample with a flow rate of 100 ml/min, controlled with a Brooks flow meter. The spectrum at each time point is the average of 24 scans, with a resolution of 4 cm⁻¹. The amide I and II band areas were measured between 1700–1600 and 1585–1502 cm⁻¹, respectively. The amide II area was divided by the amide I area for each spectrum to correct for any change in total intensity of the spectra during the deuteration process. The 100% value is defined by the amide II/amide I ratio obtained before deuteration, and the 0% value corresponds to a zero absorption in the amide II region (17).

Fusion Assay with Influenza Virus—Recombinant influenza A virus strain X-47 (H3N2) was incubated with LPC (final phospholipid concentration, 5 mol %), for 15 min at room temperature, in 10 mM Tris, 150 mM NaCl, pH 7.4. Unincorporated LPC was eliminated by gel filtration on a Sephadex G-25 column (PD-10, Amersham Pharmacia Biotech) and the virus was subsequently labeled with octadecyl rhodamine B-chloride (R-18; 18 nmol of R-18 per mg of viral protein). Noninserted fluorophore was removed by chromatography on Sephadex G-25. The amount of LPC incorporated into the virus membrane represented 10 mol % with respect to total phospholipid (i.e. 11 μM), as quantitated by densitometric analysis of silica TLC plates, after lipid extraction by the Bligh-Dyer procedure. The amount of R-18 incorporated into the virus membrane was quantitated by fluorescence measurements, as described (18), and represented 7% of the total phospholipids. For the lipid mixing assay influenza virus (20 μg of viral proteins) was prebound for 3 min with washed red blood cells in phosphate-buffered saline, pH 7.4, at 37 °C under mild agitation. Subsequently, fusion was triggered by lowering the pH in the cuvette to 5 by injecting citric acid. The increase in fluorescence (recorded at λem = 580 nm, λex = 590 nm) was normalized to the maximum dequequenching signal obtained at infinite dilution of the probe by lysing the virus with 0.2% (v/v) Triton X-100.

Fusion Assay with Golgi Membranes—Rat liver Golgi membranes were prepared as described earlier (19), and resuspended in 0.25 mM sucrose, 5 mM MgCl2, 100 mM K2HPO4/KH2PO4, pH 6.7. An aliquot (50 μg of membrane proteins) was added to 2 ml of a 0.5% solution of 2-bromophenol blue dye in buffer. The mixture was mixed and allowed to incubate for 3 min with washed red blood cells in phosphate-buffered saline, pH 7.4, at 37 °C under mild agitation. Subsequently, fusion was triggered by lowering the pH in the cuvette to 5 by injecting citric acid. The increase in fluorescence (recorded at λem = 580 nm, λex = 590 nm) was normalized to the maximum dequequenching signal obtained at infinite dilution of the probe by lysing the virus with 0.2% (v/v) Triton X-100.

RESULTS

Protein-induced Fusion in Both Biological and Artificial Membranes Is Similarly Modulated by Lysophospholipids—We monitored the fusion of influenza virus with erythrocytes, after treating either the virus or the red blood cells with LPC. The data revealed that relative to control, the presence of LPC in the donor (viral) membrane caused only a limited inhibition of fusion, whereas a drastic inhibition was seen when the lipid was present in the target membranes at identical concentrations (Fig. 1a). Along the same lines, fusion of isolated Golgi membranes with liposomes was affected and controlled in a corresponding fashion: 40–50% inhibition of fusion was seen when 30 μM LPC was incorporated into Golgi membranes, containing the peripherally associated fusogenic complex p97/p47 (“donor” membranes; see Ref. 19), whereas fusion was almost completely abolished when only 12 μM LPC were included into the target liposomal membranes (Fig. 1b). Recent data obtained on Golgi- and endosome-derived transport vesicles isolated from permeabilized HepG2 cells also showed a prominent inhibition of fusion when LPC was incorporated into the target membrane, and much less so by including LPC in the vesicle membrane.2

Membrane fusion is thought to be driven by the insertion of a specific peptide domain, contained in the fusion protein, into the target membrane. This will lead to bilayer destabilization, and subsequently, membrane merging occurs. Direct evidence for such a penetration has been demonstrated for the N terminus of HA2, subunits in hemagglutinin, and of the F1 subunit of the Sendai virus F protein (20, 21). In the light of these data, our observations could imply that the penetration of such a “fusion-inducing domain,” be it the N terminus of HA2 or a yet-to-be-identified domain of a Golgi- and/or endosome-associated protein, is impaired by the presence of lysophospholipids in the target membranes. If so, the next issue would then be to reveal the mechanism underlying such an impairment.

2 O. Maier, E.-I. Pécheur, and D. Hoeckstra, unpublished observations.
A Lipid-induced α/β Switch Regulates Membrane Fusion

Fig. 1. Inhibition of fusion of influenza virus with red blood cells (a) and of rat liver Golgi membranes with liposomes (b) by membrane-incorporated LPC. Panel a, curves a and b show the kinetics of influenza virus, fusing with erythrocytes before (curve a) and after (curve b) treatment of the virus with LPC (see under “Experimental Procedures”). Alternatively, red blood cells were preincubated with 6 μM LPC, for 2 min at 37 °C, in phosphate-buffered saline at pH 7.4. Influenza virus was then added to the LPC-labeled red blood cells, and after a 3-min preincubation, fusion was triggered by injecting citric acid to decrease pH to 5 (curve c). The curves presented are representative of three separate experiments. Panel b, curves a and b correspond to the fusion of rat liver Golgi membranes pretreated (curve b) or not (curve a) by 30 μM LPC for 30 min on ice, with NBD/Rh-labeled liposomes (final lipid concentration, 70 μM; see under “Experimental Procedures”). Curve c represents the fusion of rat liver Golgi membranes with liposomes preincubated with 12 μM LPC for 3 min.

Testing this hypothesis (and its implications) at the level of an entire protein or protein complex is not yet possible, due to technical limitations. However, the WAE peptide model system provides this possibility, in that it closely reproduces the biological context. WAE-coupled vesicles and target liposomes, upon addition of Ca2+ causes close apposition of the membranes, which allows WAE to trigger fusion. Note that the fusion event was essentially nonleaky, as reflected by the remarkable similarity in the kinetics of mixing of membrane lipids and aqueous contents.

How does WAE bring about membrane fusion? Current experimental evidence concerning the mechanism of protein-induced membrane fusion supports a stalk-pore model of fusion intermediates (22, 23). Formation of the stalk is affected by modulating the intrinsic curvature of the membrane. Thus, lipids advancing negative intrinsic curvature, like the cone-shaped HII phase-forming dioleoyl-PE, promote fusion. This can be counteracted by lipids favoring positive curvature, such as inverse-cone shaped, micelle-forming lysolipids. The mechanism by which LP-WAE vesicles fuse with PS/PE target vesicles is entirely consistent with this scenario. Insertion of LPC, accomplished by either exogenous addition to target vesicles (Fig. 2, curve c; Fig. 3, circles) or upon incorporation during their preparation (not shown) inhibited WAE-induced fusion in a dose-dependent manner. Similar alterations in the fusion activity were observed with LPE (not shown, see below), and intriguingly, also when the target membrane contained as little as 2 mol % dipalmitoylphosphatidylcholine (DPPC) (Fig. 2, curve d).

Interestingly, when LPC (or LPE) was incorporated into the LP-WAE donor membrane, its propensity to fuse with target vesicles was impaired in comparison with LPC-devoid donor membranes, but to a much lesser extent than when LPC was present in the target membrane at identical concentrations (Fig. 2, compare curves c and e, respectively). Hence, depending on its localization relative to that of the fusion-inducing peptide, LPC affects fusion differently.

Distinct Lipids Interfere with Peptide Penetration into the
Target Membrane—The effect of lysophospholipids on the capacity of WAE to penetrate into the target membrane was assessed by monitoring changes in intrinsic fluorescence of the N-terminal Trp residue of the peptide.

As shown in Fig. 3 (squares), in parallel to the LPC-dependent inhibition of fusion (circles), the intrinsic Trp fluorescence dropped. This decrease, in conjunction with a red shift of the emission maximum (10–15 nm) is indicative of an LPC-induced shift in the environment of the peptide from a hydrophobic to a more hydrophilic one. It thus appears that LPC impairs the capacity of WAE to engage in hydrophobic interactions with and penetrate into the target membranes.

To more accurately define the molecular environment of the peptide upon fusion, in either the presence or absence of fusion-inhibiting lipid species in the target membrane, we monitored the accessibility of the Trp residue to an aqueous quencher, acrylamide. Thus the more hydrophilic the environment, the reduced accessibility of the Trp residue to the aqueous quencher, acrylamide. Hence, at fusogenic conditions, the peptide is (at least partly) buried into the target membrane. Surprisingly, at conditions at which fusion is strongly inhibited, i.e. in the presence of LPC, a further decrease in the accessibility of the Trp residue to the aqueous quencher was seen. To clarify this point, the accessibility of WAE to deuterium oxide (D$_2$O) was monitored as a function of the exposure time to D$_2$O-saturated nitrogen, by determining the H/D exchange of the amide group. As shown in Fig. 4b, prior to fusion, WAE was fully accessible to H/D exchange, which was completed after approximately 10–15 min. After fusion with target vesicles in the absence of LPC, this exchange amounted to approximately 50–60%. Hence, in accordance with the Trp fluorescence quenching experiments, the reduced accessibility of the peptide was due to its penetration into the target membrane. Intriguingly, this exchange is severely diminished when the target membranes contained LPC, consistent with the Trp data. Again, a very similar feature was seen when the target membrane contained only a few mol % of DPPC (Fig. 4), which, like LPC and LPE, strongly inhibits WAE-induced fusion (Fig. 2, curve d). However, the degree of exchange is such (approximately 20%), compared with approximately 50% at control conditions) that these data are unlikely explained by an enhanced insertion of WAE at notably nonfusogenic conditions. Rather, a potential explanation could be that the peptide becomes clustered, thereby shielding the Trp residues and, similarly, protons for deuterium exchange. The data thus dictate that structural features of the peptide should be taken into account.

A Lipid-dependent Conformational Switch Regulates Peptide-induced Fusion—Secondary structure determination in a lipidic environment at the various experimental conditions was carried out by FTIR (16). Prior to fusion, the coupled peptide showed an α-helix content of approx. 55% and little if any β-sheet structure (Fig. 5a, upper panel). A virtually identical
spectrum was obtained in the presence of PS/PE target vesicles with Ca\(^{2+}\) (Fig. 5b, spectrum 1) or when LPC or LPE were inserted into the peptide-coupled vesicles by exogenous addition in the absence (Fig. 5a, lower panel, and Table I) or presence of target vesicles (Table I). These data thus indicate that under fusogenic conditions, the secondary structure of the peptide per se was affected neither by target vesicles nor by LPC or LPE (see also legend to Table I). Indeed, LPC-containing LP-WAE vesicles still fuse, although less avidly than LPC-devoid vesicles, because the effect on donor membrane curvature obviously interferes with their fusion susceptibility (Fig. 2, curve c, and see above). However, when LPC was incorporated into the target vesicles, followed by addition of the peptide-coupled vesicles, a dramatic shift in the structure of the peptide from an \(\alpha\)-helix to a \(\beta\)-sheet was observed (Fig. 5b, spectrum 2). Under these conditions the \(\alpha\)-helix content decreases from 55 to 25\%, whereas the \(\beta\)-sheet content increases from 3 to 63–75\% (depending on the topology of the LPC; Table I). Consistently, a very similar increase in the \(\beta\)-sheet content (up to 90\%) was observed when the target membrane contained LPE (Fig. 5b, spectrum 2; Table I) or 2 mol \% DPPC (Fig. 5b, spectrum 3; Table I), accompanied by a decrease in the \(\alpha\)-helix content.

Thus, the presence of relatively minor amounts of distinct lipids in the target membrane provoked a switch in the secondary structure of the (coupled) fusion peptide from a fusion-permissive \(\alpha\)-helix to a fusion-inhibiting \(\beta\)-sheet. Furthermore, the maximum absorbance of the \(\beta\)-sheet was observed at approximately 1625 cm\(^{-1}\) (Fig. 5). This low frequency suggests that the \(\beta\)-strands formed strong hydrogen bonds arising from intermolecular bonds between aggregated or oligomerized peptides (25, 26). Obviously, WAE aggregates will be less accessible to acrylamide quenching and H/D exchange, as was observed when LPC and DPPC were present in the target membranes (Fig. 4). It is also apparent that such clustering would preclude a facile insertion of the peptide into the bilayer, thereby rationalizing the inhibition of fusion. A reversal toward the fusion-permissive \(\alpha\)-helical conformation should then give rise to WAE resuming its ability to trigger fusion. Indeed, fusion proceeded upon removal of LPC, as accomplished by washing with bovine serum albumin (Fig. 1, inset; see also Ref. 14), or upon reversal of the curvature effect of LPC by adding cone-shaped monoolein (not shown) (27). Consistently, under such conditions, WAE refolded into an amphipathic \(\alpha\)-helix, as demonstrated by ATR-FTIR (Fig. 5b, spectrum 4; Table I). These data imply that the \(\alpha\)/\(\beta\) switch that controls WAE-induced fusion is a genuine switch, i.e. it does not involve an LPC- or LPE-induced “denaturation” of WAE due to clustering.

**DISCUSSION**

The \(\alpha\)/\(\beta\) Switch Regulates Membrane Fusion—Increasing evidence indicates a direct involvement of distinct peptides, contained in viral or cellular fusion proteins, in the destabilization of the target bilayer, presumably resulting from peptide penetration, that initiates the fusion reaction (20, 21, 28). However, the influence of the lipid composition of the target membranes and the exact translation of the structural features of
the peptide into a functional mechanism remain to be established. Here, we report in a simplified fusion model system, consisting of a membrane-coupled fusogenic peptide and target vesicles, the existence of a lipid-dependent on/off structural switch for membrane fusion. The on position corresponds to the peptide adopting an α-helical structure that faces and penetrates into a fusion-competent target bilayer, whereas the off position corresponds to a β-sheet structure of the peptide that locates roughly parallel to the surface of a fusion-nonpermissive membrane, as imposed by small amounts of fusion-inhibiting lipids. Moreover, the α/β switch is reversible, at conditions that are consistent with the stalk-pore model for membrane fusion (22). Interestingly, in a functional context, very similar features could be simulated in fusion events involving biological membranes.

Formation of a stalk of negative curvature between two apposed membranes, and its further expansion into a fusion pore, can be counteracted by lipids possessing a spontaneous positive curvature, such as lysosphospholipids (fusion inhibitors), or promoted by lipids of negative curvature (e.g. unsaturated PE, cis-unsaturated fatty acids, or monoolein (fusion promoters)). Removal of lysolipids from the contacting leaflets should reverse the inhibition, whereas the simultaneous presence of fusion inhibitors and promoters should compensate their separate membrane effect. Consistently, our data indicate that extracting LPC from bovine serum albumin fully restores their separate membrane effect. Consistently, our data indicate that extracting LPC with bovine serum albumin fully restores their separate membrane effect.

Mechanism of the Modulation—The inhibition of fusion by lysolipids is considered to be due to their shape-related curvature effects on membranes (36). Here, we demonstrate that target membrane-incorporated lysolipids also display an effect on the secondary structure of a fusion peptide, in addition to their direct effect on membrane curvature. The latter is clearly revealed when the lipid is incorporated into the peptide-containing donor membrane, when there is no interference with the secondary structure of the peptide (Fig. 5). Intriguingly, an α/β switch was also triggered by DPPC, a cylinder-shaped phospholipid. This leads us to conclude that although lipid shape-dependent factors clearly modulate fusion, the regulation of this α/β switch does not solely rely on such a propensity.

Because both LPC and LPE gave comparable results (Fig. 5), phospholipids are not crucial for the switch. LPC and LPE are also present at similar concentrations in the plasma membrane, indicating that the lipid composition of the plasma membrane does not alter the switch. Moreover, our data suggest that the switch does not solely rely on such a propensity. Because both LPC and LPE gave comparable results (Fig. 5), phospholipids are not crucial for the switch. LPC and LPE are also present at similar concentrations in the plasma membrane, indicating that the lipid composition of the plasma membrane does not alter the switch. Moreover, our data suggest that the switch does not solely rely on such a propensity.
attributed to disaturated phosphatidylcholine. It is possible, therefore, that in the narrow fluid spacing between the donor and the target membranes, the peptide could sense such subtle variations, induced by incorporating into the target membrane tiny amounts of lipids unrelated in terms of molecular shape, which in turn could affect the structure of the peptide toward its off β position.

Whether lysolipids or DPPC (and perhaps other lipids as well) act as modulators of the α/β switch at physiological conditions remains to be determined. Thus far, fusion peptides have been identified in viral and sperm fusion proteins (6, 39), but their localization in other fusion machineries has yet to be elucidated. Also, direct structural support will await technical advances that allow the discrimination of structural changes in a fusion peptide at the level of the entire protein. However, functionally, the data obtained in two relevant biological systems were entirely consistent with a mechanistic feature involving an α/β switch, as revealed in the WAE model system. This notion lends support to the concept of a switch, as revealed in the WAE model system.

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