It is clear that both viral and intracellular membrane fusion proteins contain a minimal set of domains which must be deployed at the appropriate time during the fusion process. An account of these domains and their functions is given here for the four best-described fusion systems: influenza HA, sendai virus F1, HIV gp120/41 and the neuronal SNARE core composed of synaptobrevin (syn), syntaxin (stx) and the N- and C-termini of SNAP25 (sn25), together with the Ca^{2+} binding protein synaptotagmin (syt). Membrane fusion begins with the binding of the virion or vesicle to the target membrane via receptors. The committed step in influenza HA-mediated fusion begins with an aggregate of HAs (at least eight) with some of their HA2 N-termini, a.k.a. fusion peptides, embedded into the viral bilayer (Bentz, 2000a). The hypothesis presented in Bentz (2000b) is that the conformational change of HA to the extended coiled coil extracts the fusion peptides from the viral bilayer. When this extraction occurs from the center of the site of restricted lipid flow, it exposes acyl chains and parts of the HA transmembrane domains to the aqueous media, i.e. a hydrophobic defect is formed. This is the ‘transition state’ of the committed step of fusion. It is stabilized by a ‘dam’ of HAs, which are inhibited from diffusing away by the rest of the HAs in the aggregate and because that would initially expose more acyl chains to water. Recruitment of lipids from the apposed target membrane can heal this hydrophobic defect, initiating lipid mixing and fusion. The HA transmembrane domains are required to be part of the hydrophobic defect, because the HA aggregate must be closely packed enough to restrict lipid flow. This hypothesis provides a simple and direct coupling between the energy released by the formation of the coiled coil to the energy needed to create and stabilize the high energy intermediates of fusion. Several of these essential domains have been described for the viral fusion proteins SV5 F1 and HIV gp120/41, and for the intracellular SNARE fusion system. By comparing these domains, we have constructed a minimal set which appears to be adequate to explain how the conformational changes can produce a successful fusion event, i.e. communication of aqueous compartments.

INTRODUCTION

Membrane fusion is a crucial event in a multitude of biological processes, and understanding the molecular mechanism is a central goal of biology. The ectodomain of the membrane fusion glycoprotein hemagglutinin (HA) of influenza virus, which requires low pH to initiate fusion, was the first membrane fusion protein whose crystal structure was solved (Wilson et al., 1981) and it remains the prototypical fusion protein (Skehel and Wiley, 1998; Sutton et al., 1998; Baker et al., 1999; Bentz, 2000b). The key differences between the ‘native’ structure of HA and the low pH structure is the formation of an extended coiled coil starting from the N-terminus of the native coiled coil and a helix-turn occurring within the C-terminal end of the native coiled coil, near the

KEYWORDS: enveloped virus; influenza hemagglutinin; Env; HIV; SNARE; hydrophobic; sendai virus.
transmembrane domain (Bullough et al., 1994; Chen et al., 1995).

Recently, ectodomain core complexes of other membrane fusion proteins have shown remarkably similar equilibrium crystal structures with respect to this coiled coil motif (Skehel and Wiley, 1998; Baker et al., 1999; Singh et al., 1999). This has led to speculation that these proteins share a common molecular mechanism for initiating membrane fusion. It has also been speculated that bilayer destabilization is initiated either through a high curvature bending defect (Chernomordik et al., 1998; Kozlov and Chernomordik, 1998; Lentz and Lee, 2000) or a hydrophobic defect Bentz (2000a,b). These two proposals agree on most points, except the direct target of the transduced energy, which is the evolutionary pressure on the conformational change in the first place. So it is actually important to know what is the first step of destabilization.

Most proposed HA-mediated fusion mechanisms contain four distinct intermediates, subsequent to close apposition of the membranes and the low pH-induced exposure of the HA2 N-terminus (Bentz, 1992, 2000a,b; Blumenthal et al., 1996; Chernomordik et al., 1998). Currently, these intermediates are:

1. Aggregates of HA, which are either preformed or form rapidly subsequent to acidification.
2. The first fusion pore defined by the first conductivity (2–5 nS) across the membranes. Additional flickering pores follow which lead to the formation of a terminally open pore.
3. The lipidic channel, which is monitored by lipid dye transfer between membranes.
4. The fusion site, which is monitored by aqueous contents mixing (e.g. fluorophors) and the stable joining of the two membranes and complete aqueous contents mixing.

Dutch et al. (1998) found that fusion of SV5 F-expressing cells with erythrocyte ghosts was faster when the surface density of SV5 F was increased, indicating that aggregation was important for fusion. Fusion of HIV env fusion proteins (the class of homologues to gp120/41) is faster and/or more extensive with higher surface expression (Kozak et al., 1999), likewise suggesting aggregation as an essential step. The other steps are functional steps in membrane destabilization and have been observed, in some fashion, with all fusing systems.

**HA CONFORMATIONS**

For each of these proteins, the ectodomain core complex has been crystallized, a.k.a. the six helix bundle, which corresponds to the final equilibrium structure. We will begin with a brief review of the known conformations of influenza HA, and then develop the analogous knowledge for the other fusion proteins.

During infection, virus bound to the cell surface is endocytosed and exposed to low pH, which produces at least three new conformations in HA as depicted in Figure 1. The native structure of HA (Fig. 1, conformation 1) is based upon the crystal structure of the bromelain-released hemagglutinin ectodomain, BHA (Wilson et al., 1981). HA is a homotrimer and each monomer is composed of two polypeptide segments designated HA1 and HA2. The HA1 segments form sialic acid binding sites, which mediate initial HA attachment to the host cell surface. The HA2 segments form the membrane-spanning anchor, the assembly domain of the homotrimer and its amino-terminal region (Gething et al., 1986).

Upon acidification, exposure of the amino terminus of HA2, known as the fusion peptide, occurs (Fig. 1, conformation 2). This change is rapid compared to fusion and is required to promote fusion between the viral envelope and the target membrane (Skehel et al., 1982; White and Wilson, 1987; Stegmann et al., 1990; Stegmann and Helenius, 1993; Godley et al., 1992; Pak et al., 1994). For reasons alluded to above and described in greater detail in the next section (see Fusion peptide), the HA with its fusion peptide embedded in the viral bilayer is believed by some to be the starting conformation of the fusion pathway defect (Kozlov and Chernomordik, 1998; Bentz, 2000b).

The second conformational change leads to the formation of the extended coiled coil of HA2 (Fig. 1, conformation 3), which was predicted by Carr and Kim (1993), proven for the crystallographic structure of a fragment of BHA (TBHA2, residues 38-175 of HA2 and 1-27 of HA1 held together by the disulfide bond) by Bullough et al. (1994) and morphologically observed on the intact virus by Shangguan et al. (1998), as discussed in Bentz (2000a). In addition, Qiao et al. (1998) showed that site-directed point mutations predicted to inhibit the formation of the extended coiled coil did inhibit the fusion of erythrocytes to HA-expressing cells. This conformational change extracts the fusion peptide from the viral bilayer and relocates it more than 10 nm towards the target membrane. New stretches of coiled coil structure are formed, which should release the largest free energy of all the HA conformational changes (Bentz, 2000b).

The crystal structure of TBHA2 (Bullough et al., 1994) also shows that the C-terminal end of HA2,
Fig. 1. The conformations of HA. (1) This is the native conformation of BHA, adapted from Wilson et al. (1981). The globular HA1 headgroups sit on top of the spike-like HA2. Regions of α-helix and coiled coil are shown as cylinders. The bottom aggregate of α-helices is the transmembrane domain, striped and denoted TM.

(2) Low pH causes exposure of HA2 N-terminus or fusion peptide, some of which will be embedded in the viral membrane.

(3) Transition of HA2 from (2) to extended coiled coil requires dissociation of HA1 headgroups and would translocate the fusion peptide toward the target membrane.

(4) The helix-turn transition at the base of one or two of the extended coiled coils would tilt the HA and allow closer membrane apposition. The hydrophobic kink region (HA2 aa 105-113) is shown in the expanded box.
where the native coiled coil flares out to accommodate the fusion peptide in the native state, flips up in helix-turn between residues 106–112 of HA2 and forms an antiparallel α-helical annulus from residues 113–129 of HA2, i.e. at the base of the extended coiled coil (see Fig. 1, conformation 4). This helix-turn transition, as yet unproven for membrane bound HA, has been proposed as the essential conformational change to cause the initial destabilization of the apposed bilayers (Bullough et al., 1994; Chen et al., 1995, 1999a; Hernandez et al., 1996; Carr et al., 1997; Weissenhorn et al., 1997, 1998; Skehel and Wiley, 1998). Bentz (2000b) argued that this transition is not energetic enough to accomplish this task and that the formation of the extended coiled coil should be assigned to form the initial high-energy bilayer defect between the membranes. Kim et al. (1998) have argued that the helix-turn region, which they term the kink region of HA2 (aa 105–113), is important for fusion, while Epand et al. (1999) and Leikina et al. (2000) have found that the FHA2 fragment (the equilibrium structure of aa 1–127 of HA2, which runs from the fusion peptide to the end of the annular α-helix, with the extended coiled coil in place and the kink exposed) mediates membrane destabilization and lipid mixing in a pH-dependent fashion. FHA2 reversibly aggregates at low pH via the kink region (Yu et al., 1994; Kim et al. 1998; Leikina et al., 2000), almost certainly due to hydrophobic amino acids.

**FUNCTION OF MEMBRANE FUSION PROTEIN DOMAINS DURING FUSION**

It is clear that both viral and intracellular membrane fusion proteins contain a minimal set of domains which must be deployed at the appropriate time in order to assure a successful fusion event, i.e. transfer of contents between compartments. Some of these domains are well known, e.g. the fusion peptide or a binding site for something on the target membrane. Others are recent discoveries and will require greater elucidation below. In Table 1, we propose seven essential steps for fusion and cite the domains or conformational changes which accomplish these steps for the four best described fusion systems: influenza virus, HIV, sendai virus and the neuronal SNARE system. Influenza HA is the only member of this group whose native metastable structure is known (Wilson et al., 1981). Thus, only for HA do we know how it looks before and after fusion. While there are many strains and homologues within each of these fusion systems, for the sake of concreteness, we will focus mainly on the versions whose structure is known, i.e. X31 for HA (Bullough et al., 1994), SV5 F1 for sendai virus (Baker et al., 1999), gp120/41 for HIV (Chan et al., 1997; Weissenhorn et al., 1997) and the neuronal SNARE 4 stranded coiled coil core composed of synaptobrevin (syn2), syntaxin (stx1) and the N- and C-termini of SNAP25 (sn25) (Sutton et al., 1998), together with the Ca²⁺ binding protein synaptotagmin (syt) (Shao et al., 1998).

**Binding protein and target receptor**

We will begin by listing the binding protein and target receptor for these systems, which gets the virus or vesicle to an appropriate target membrane. HA contains a sialate binding site within the HA1 subunit which can bind to glycosylated proteins and gangliosides (Martin et al., 1998). This provides a wide range of target receptors. Sendai virus also binds to surface sialates via the HN membrane glycoprotein, which has a neuraminidase activity (Dutch et al., 1998). HIV, on the other hand, is quite specific with gp120 binding first to CD4 on macrophages or T-cells and then to a target cell chemokine receptor (Doms and Peiper, 1998). It is thought that the initial binding of the intracellular vesicles to their target membrane is mediated by Rab proteins, but there are other membrane partners (Grote and Novick, 1999; Price et al., 2000). It has become clear that the SNARE complex, per se, is not responsible for the initial binding of vesicle to target (Yang et al., 1999).

**Signal for fusion**

Once bound to the target cell, the influenza virion is endocytosed. Acidification of the endosome protonates HA, which is the signal for fusion. This initiates the cascade of conformational changes leading to merger of the viral and endosomal membranes. Sendai virus fuses at neutral pH and most strains require about 1:1 mol ratios of the homotypic HN for maximal fusion or infection (Dutch et al., 1998). It appears that the binding of sialates to HN, which activates its neuraminidase, alters its conformation so that it can activate the F1 protein to start the fusion process. Oddly, SV5 F does not appear to require its homotypic HN for fusion, although the rate is much faster with about 1:1 mol ratios of its homotypic HN (Dutch et al., 1998). HIV is activated when the binding of gp120 to target cell CD4 which exposes an epitope on gp120 which allows it to bind to a chemokine receptor, either CXCR4 or CCR5 (Feng et al.,
<table>
<thead>
<tr>
<th>Function</th>
<th>Influenza HA</th>
<th>Sendai</th>
<th>HIV gp120/41</th>
<th>SNARE syn/stx/sn25 (neuronal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus/vesicle</td>
<td>HA1</td>
<td>HN</td>
<td>gp120</td>
<td>Rabs+others?</td>
</tr>
<tr>
<td>Target Membrane</td>
<td>Sialosides</td>
<td>Sialosides</td>
<td>CD4</td>
<td>Rabs+others?</td>
</tr>
<tr>
<td>Signal for fusion</td>
<td>H^+ binding to several sites on HA</td>
<td>Could be HN binding to and activating F protein, perhaps due to neuraminidase activity</td>
<td>CD4 binding exposes a chemokine receptor binding site for gp120, activating gp41</td>
<td>Ca^{2+} binding to C2 domains of synaptotagmin</td>
</tr>
<tr>
<td>Fusion peptide</td>
<td>N-terminus of HA2</td>
<td>N-terminus of F1</td>
<td>N-terminus of gp41</td>
<td>Could be aa 38-55 of synaptobrevin</td>
</tr>
<tr>
<td>Aggregation: (proposed mechanism)</td>
<td>Tension on membrane from fusion peptide embedded in viral envelope</td>
<td>Could be similar to HA mechanism</td>
<td>Could be like HA or could be multiple interactions between gp120, CD4 and CCR5 or CXCR4</td>
<td>C2B domain of synaptotagmin bound to ternary SNARE complex</td>
</tr>
<tr>
<td>High energy conformational change: Extraction of fusion peptide</td>
<td>Formation of extended coiled coil</td>
<td>Expected to be formation of extended coiled coil</td>
<td>Expected to be formation of extended coiled coil</td>
<td>Formation of ternary SNARE complex with synaptobrevin</td>
</tr>
<tr>
<td>Hydrophobic Kink: Stabilizing lipids enroute to the hydrophobic defect</td>
<td>Helix turn or ‘kink’ region of low pH structure</td>
<td>Should be near either the C-terminal end of the N-heptad repeat or the N-terminal end of the C-heptad repeat</td>
<td>Should be near either the C-terminal end of the N-heptad repeat or the N-terminal end of the C-heptad repeat</td>
<td>C2A loop region of synaptotagmin bound to SNARE ternary complex after Ca^{2+} binding</td>
</tr>
<tr>
<td>Low energy conformational change: Formation of hydrophobic kink or loop</td>
<td>Helix turn of C-terminus of native coiled coil</td>
<td>?</td>
<td>?</td>
<td>The conformational change of the C2A domain of synaptotagmin which exposes the hydrophobic loops</td>
</tr>
</tbody>
</table>
This ternary structure then signals gp41 to start the fusion process. Hoffman et al. (1999) have isolated a mutant HIV strain which binds directly to either chemokine receptor and mediates fusion, something like SV5 F. For the HIV env mutant, the direct binding to the cytokine receptor is the signal. For SV5 F, the signal is not known in the absence of HN, suggesting that SV5 F must bind directly to an (as yet) unknown target membrane receptor to signal that fusion should start. For the neuronal SNARE system, the signal appears to be Ca2+ binding to synaptotagmin, which then interacts with the ternary SNARE complex of synaptobrevin/syntaxin/SNAP25 (Shao et al., 1998; Chen et al., 1999b; Davis et al., 1999; Sugita and Südhof, 2000; Leveque et al., 2000).

**Fusion peptide**

After the signal for fusion, at least for HA2, sendai F1 and HIV gp41, the fusion peptide is exposed on the N-terminus of these proteins (Hernández et al., 1996; Durell et al., 1997; Pecheur et al., 1999). While there has been a long literature proposing that the exposed N-terminal of HA next inserts into the target membrane to start fusion, it is more accurate to say that HAs can have their fusion peptides either suspended between the membranes or embedded in the target bilayer or in their own bilayer (Bentz et al., 1999; Gaudin et al., 1995; Shangguan et al., 1998; Kozlov and Chernomordik, 1998). The fraction in each state probably depends upon time and the real question is: which state or sequence of states is on the fusion pathway? Kozlov and Chernomordik (1998) and Bentz (2000) argue that those HAs whose fusion peptides embed initially into the viral/HA-expressing cell envelope are on the fusion pathway. Reaching the target membrane represents a later step in the process. Thus, in Figure 1 (panel 2) the fusion peptide is shown here to be embedded in the viral envelope and this is the population we follow.

No fusion peptide has been proven for the SNARE complex, but it is possible that the v-SNAREs do contain a ‘fusion peptide’ within the core complex (Jahn and Südhof, 1994). Before binding to the binary syntaxin/SNAP25 complex, aa 38–55 of synaptobrevin, syb2, could embed into the vesicle membrane as an amphipathic a-helix (Bentz, 2000b), similarly to the viral fusion proteins. Figure 2 shows a helical wheel representation of the fusion peptide of HA and of this 18 amino acid sequence of synaptobrevin 2 which is the v-SNARE protein associated with the t-SNAREs syntaxin and SNAP 25 (Weber et al., 1998). The black band highlights the hydrophobic face of both peptides and it is clear that syb2 shows a ‘fusion peptide’ profile. This stretch (amino acids 38–55) occupies the center of the syb2 coiled coil domain in the crystallized 7S core complex, just N-terminal to the central arginine (R56) of the 7S complex (Sutton et al., 1998). Figure 2 also shows analogous sequences for the other v-SNAREs (Weimbs et al., 1998) on helical wheel representations, e.g. regions 35–52 of sncl, 172–189 of Nyv1, 137–154 of sar1 and 138–155 of sec22. Thus this region of sncl could act as a fusion peptide before forming the four-stranded coiled coil with the binary sso1/sec9 complex (Fiebig et al., 1999). The syntaxin family (Weimbs et al., 1998) do not show the same extensive hydrophobic face on the helical wheel (data not shown).

**Aggregation**

The next step is aggregation of the fusion proteins. Bentz (2000a) showed that rapid aggregation of eight or more HAs followed by a slow ‘essential’ conformational change of two or three of the HAs within the aggregate fitted the kinetics of first fusion pore formation between HA expressing cells and planar bilayers. The cause of this rapid HA aggregation is not known. It could be adhesion of the fusion peptides within the aqueous space (Ruigrok et al., 1988), although this would render the fusion peptides useless for other functions. Kozlov and Chernomordik (1998) have proposed a mechanism based on membrane curvature mini-
coiled coil another 10 Å, or so, and shortens the line to the fusion peptide by as much as 14 Å, or so.

This could be accomplished by a bobbing action of the HA in the bilayer, as illustrated by Figure 3, where HA1 has been deleted for clarity. When HA bobs down, relative to the bilayer, this would allow enough slack in all three chains of the nascent extended coiled coil domain to form at the same time, as shown in Panel B. According to Kozlov and Chernomordik (1998), the heptad repeat of a single HA has insufficient binding energy to hold up the bilayer against the curvature energy. However, when several HAs are close, the net curvature to the bilayer needed for all of them to sustain one (or more) heptad repeat binding reactions is sufficiently small to permit the action. Once a cluster of HAs have formed their first heptad repeats of the extended coiled coil, this holds the bilayer curvature in place and the aggregate is formed, as shown in Panel C. Other HAs can diffuse into the aggregate and form their next heptad repeat, thereby increasing the bilayer curvature. This process would repeat, with HAs in the aggregate forming additional heptad repeats, gradually creating a dimple of bilayer in the center of the HA aggregate.

This same mechanism for fusion protein aggregation could work for sendai virus F1 and HIV gp120/41. However, for HIV gp120/41, it is also possible that the multivalent interactions between gp120, CD4 and the cytokine receptor could yield stable aggregates. For the SNARE complex, such a mechanism seems less likely, due to the geometry of the proteins and a better mechanism suggests itself. The binding of Ca²⁺ to the C2B domain of synaptotagmin induces self aggregation, as well as its binding to the syntaxin within the SNARE core (Davis et al., 1999; Sugita and Südhof, 2000). Thus, synaptotagmin would cause the aggregation of the SNARE cores to form the nascent fusion site.

Beginning with the model of Kozlov and Chernomordik (1998), there is an appealing segue with the model proposed in Bentz (2000b). With the fusion peptides inserted into the viral membrane, the HAs will aggregate and begin to form the dimple toward the target membrane. The collar of HAs will tighten until the site becomes lipid flow-restricted. Then the dimple can grow no further.
The tension on the fusion peptides will cause some of them to pull out the viral envelope (presumably one hydrophobic residue at a time, which would require less energy or force than removal of the whole peptide at once) and move to the target membrane following the formation of the extended coiled coil. Figure 4, panel A, shows an aggregate of HA2s (without HA1s for clarity) with fusion peptides embedded in the viral envelope, which is the aggregation step of the model of Kozlov and Chernomordik (1998) and the starting point for of the model of Bentz (2000) for HA aggregation and fusion. HA1s are present but are deleted in the figure for clarity. The curvature of the bilayer is also omitted for clarity.

Recently, Günther-Ausborn et al. (2000) have claimed that reconstituted HA virosomes do not show evidence of 'cooperativity', since virosomes with perhaps as few as one or two active HAs show some lipid mixing capacity. While the disagreement could reside in the difference between cell expressed HA and detergent reconstituted HA (and both could differ from viral HA), it could well be that all the studies are compatible. The analysis in Bentz (2000a) showed that at very high surface densities of HA, such as those found on the influenza virion, the fusion competent aggregates would probably be preformed. The detergent reconstituted virions have native HA surface densities (Günther-Ausborn et al., 2000). The finding that only two or
three HAs within the fusion competent aggregate need to undergo the essential conformational change (Bentz, 2000a), presumably to the extended coiled coil, would explain the observations of Günther-Ausborn et al. (2000). Perhaps on the reconstituted virosomes only two or one HA within the fusion competent aggregate needs to undergo the essential conformational change to initiate the bilayer destabilization leading to lipid mixing.

High energy conformational change

The idea that membrane fusion proteins could share a common mechanism became reasonable when it was discovered that so many systems have ectodomain core complexes composed of a six-helix bundle (Skehel and Wiley, 1998; Baker et al., 1999; Singh et al., 1999). In particular, the energy of the conformational changes could be transduced to create fusion. The initial defect has been proposed to be high curvature (Chernomordik et al., 1998; Kozlov and Chernomordik, 1998) or hydrophobic (Bentz, 2000b). The largest bolus of free energy should be used to create the initial defect at the nascent fusion site, so that the elaboration of the fusion site could proceed down the free energy pathway, following the paradigm used by enzymes. This is especially important for the known fusion proteins wherein the only source of energy to transduce to the formation of bilayer defects is within the conformational change. ATP is used only to lock the fusion proteins into a high energy metastable state, including the SNARE system. For HA, it seems clear that the formation of the extended coiled coil (Fig. 1, panel 3) would release the greater bolus of free energy, relative to the helix-turn (Fig. 1, panel 4) (Bentz, 2000b). Since some viral fusion proteins show no evidence of coiled coils, e.g. the E glycoprotein of tick-borne encephalitis virus, TBE-Ê (Corver et al., 2000), it is worth emphasizing here that coiled coils are not the sole providers of high energy conformational changes. Two β-sheets coming together could provide the high energy conformational change needed for fusion.

As shown in Figure 3, each ratchet of the heptad repeat increases the tension on the fusion peptide embedded in the viral bilayer as the curvature of the bilayer increases. The question is whether the peptide can remain embedded until the dimple reaches the target membrane. Kozlov and Chernomordik (1998) estimated that the energy it takes to remove an alpha helical hydrophobic peptide from a bilayer was too great and hypothesized that this membrane curvature increases until a dimple from the viral bilayer reaches the target membrane. However, as argued in Bentz (2000b), there seems to be enough energy in the formation of the extended coiled coil to extract the HA fusion peptide from the viral envelope. For example, within the HA aggregate the surface density of fusion peptides is very large and aggregates of fusion peptides could form (Ulrich et al., 1999), substantially reducing the energy needed to extract them from the bilayer. The release of the fusion peptide from the viral bilayer would likely precipitate the rapid completion of the extended coiled coil for that HA and the translocation of that trio of fusion peptides to the target membrane.

Bentz (2000a) showed that only two or three of the HAs within the aggregate need to undergo an essential conformational change for the first fusion pore to form, i.e. the first flickering of conductivity between the membranes. The model of Kozlov and Chernomordik (1998) appears to require the concerted bending of six or more HAs, so there is no obvious role for only two to three HAs undergoing a slow conformational change. Bentz (2000b) provided a detailed model of how the hydrophobic defect left behind by this extraction could be the key transduction of energy from the protein’s conformational change to the formation of the initial high energy fusion intermediate.

The formation of the extended coiled coil of HA2 must extract the fusion peptides from the outer monolayer of the viral envelope and send them toward the target membrane. For those HA in isolation or in aggregates not in the restricted flow state, the evacuated space in the viral outer monolayer will be quickly filled by lipid diffusion and little or nothing significant will happen on the viral envelope. Presumably the fusion peptides of all the HAs undergoing the formation of the extended coiled coil would embed in the target membrane, which would explain the hydrophobic binding of virions to target membranes, e.g. liposomes, which occurs before fusion (Stegmann and Helenius, 1993; Brunner and Tseredome, 1993; Chernomordik et al., 1998; Bentz, 2000a, b). However, for those fusion peptides embedded initially within the site of restricted lipid flow, the evacuated space cannot be refilled instantly, since the aggregated HA transmembrane domains and the remaining embedded fusion peptides would block the flow. Thus, a hydrophobic defect would be created, stabilized by the large aggregate of HAs, like a dam (Bentz, 2000b). Figure 4, panel B, shows the aggregate of HA2s surrounding the hydrophobic defect. In fact, taking all of the data together, we believe that the fusion peptide must
(A) Fusion peptide exposed by low pH and embedded in viral envelope.

(B) Transition to extended coiled coil removes fusion peptides from the viral envelope.

(C) Helix-turn allows close apposition of the membranes and hydrophobic kink to face the hydrophobic defect.

(D) Formation of a stalk intermediate stabilizes close approach.

(E) Breakage of the aperture leads to contents mixing.

Viral bilayer from the top:
- Phospholipid Headgroup
- Transmembrane domain of HA trimer
- 3 embedded loops of a fusion peptide displaces 2 - 3 lipids

Hydrophobic defect within fusion site is stabilized by restricted lipid flow through HA aggregate.
fail to hold in order for fusion (contents mixing) to occur (Bentz, 2000b). The HA transmembrane domains are required to be part of the hydrophobic defect, because the HA aggregate must be closely packed enough to restrict lipid flow (see Fig. 4B and Bentz (2000b)). Having the transmembrane domains as part of the hydrophobic defect also lowered the free energy required for its creation. This observation alone could explain the reported importance of transmembrane domain flexibility on viral fusion protein activity (Cleverley and Lenard, 1998). A rigid transmembrane domain should not participate in the defect surface as well, i.e. to lower its free energy of formation.

Turning to the SNARE system, before the docking of the v-SNARE syb-2 with the t-SNARE complex, syb2 is relatively unstructured (Fasshauer et al., 1997; Fiebig et al., 1999; Xu et al., 2000). Thus, it could be that the stretch of amino acids shown in Figure 2 as a putative fusion peptide could play two roles in its life cycle: (1) As a 'fusion peptide' embedded in the secretory vesicle's outer membrane until docking with the target membrane; and (2) as a piece of the new four-stranded coiled coil formed by syb2, syntaxin and the N- and C-termini of SNAP25 after docking. The high energy conformational change for SNARE protein complex mediated fusion is depicted in Figure 5, analogously to panels 3A and 3B for HA. The binary complex of syntaxin and both the N- and C-heptad repeats of SNAP25 are shown on the target membrane prior to binding of syb2 from the vesicle membrane (adapted from Fiebig et al., 1999). Synaptotagmin is also shown, with its C2A and C2B domains. The formation of the new coiled coil would pull the putative fusion peptide of syb2 out of the vesicle membrane and, if vesicular membrane proteins restrict lipid flow, then this eukaryotic fusion system could work the same way as HA (Bentz, 2000b).

**Low energy conformational change**

As discussed above and shown in Figure 4, panel C, the helix-turn of HA2 permits close approach of the membranes and forms a hydrophobic collar over the hydrophobic defect. Bentz (2000b) argued that for HA this conformational change is likely to release much less free energy than the formation of the extended coiled coils. The core complexes of SV5 F1 and HIV gp41 show longer annular helices around the N-heptad repeat core than does HA2, suggesting these bind more stably. Such an extension has been achieved for HA2 using an E. coli expression system (Chen et al. 1999a). For HA2, the helix-turn could be quite reversible, with much bending up and down. In fact, the formation of the first stable intermediate between the bilayers, presumably the lipidic stalk (Chernomordik et al., 1999), might be stabilize the intermediate shown in Figure 4, panel D. While peptides homologous to C-terminus heptad repeats of SV5 F1 and gp41 will inhibit syncitia formation and membrane destabilization, as discussed in detail in Bentz (2000b), no such reports have been published for HA2. If the helix-turn transition of HA is weaker than those for SV5 F1 and gp41, suggested by the length of the helix contacts to the extended coiled coil, then the C-peptides for HA may not inhibit syncitia formation, due to weak binding. Without the native structures of SV5 F1 and gp41, little can be known about the low energy conformational change. For the SNARE system, it seems likely that it is synaptotagmin which performs this function, by analogy to HA.
Hydrophobic kink

It has recently been shown that the kink formed by the helix-turn of HA mediates the low-pH-dependent aggregation of the HA fragment known as FHA2, which is aa 1–127, i.e. the fusion peptide through the C-terminus of the six-helix bundle at equilibrium (Yu et al., 1994; Kim et al., 1998). This aggregation appears to be hydrophobically driven, perhaps following the protonation of aspartates within the kink region. This same fragment induces lipid mixing between liposomes (Epand et al., 1999) and between cells (Leikina et al., 2000). Mutants in the kink region or lacking the fusion peptide do not induce lipid mixing. We will discuss membrane destabilization by soluble domains below, but suffice it to say that this fragment is unusual in its activity.

The function of such a hydrophobic kink is readily apparent in the model of fusion shown in Figure 4B, panel C. The formation of one or two helix-turns would bend HA, allowing the close approach of the membranes. Just as importantly, the hydrophobic kink would now form a hydrophobic collar just above the hydrophobic defect. Phospholipids making an excursion from the target membrane would be stabilized by this hydrophobic collar and thereby have a substantially greater chance of reaching the hydrophobic defect, i.e. begin lipid mixing. Like in a proper enzyme, the hydrophobic collar would stabilize the transition state of fusion.

Chanturiya et al. (1999) found that short chain alcohols (ca. 0.5 M) promote an early stage of hemi-fusion with HA-expressing cells and labelled RBC, and with liposomes and planar bilayers. Since the effect was observed with and without a fusion protein, and the alcohols did not appreciably alter the normal constituents of membrane curvature energy, Chanturiya et al. (1999) suggested that the alcohols favored the formation of semistalks, i.e. dimples with hydrophobic defects at their tips which must join to form an interbilayer stalk. However, even if the lipidic stalk is one of the relatively stable resting points for both liposome fusion and HA-mediated fusion (Chernomordik et al., 1999), the molecular pathways leading to
that morphology can be quite different. The same result would be predicted by the model proposed here, in that the lipids making excursions could more easily reach the hydrophobic kink and, then, the hydrophobic defect, as compared with making the trip in just water. At present, it is difficult to imagine an experiment which could clearly distinguish between these two possibilities. Functionally, the hydrophobic defects at the end of the semistalks proposed in Chanturiya et al. (1999) could be formed by the extraction of the fusion peptides. Nevertheless, there is no obvious role for the hydrophobic kink in the model of Kozlov and Chernomordik (1998).

For the viral fusion proteins using coiled coils and folding something like HA (Skehel and Wiley, 1998; Baker et al., 1999), this hydrophobic kink should be near the C-terminus of the N-heptad repeat region or near the N-terminus of the C-heptad repeat region. Interestingly, Peisajovich et al. (2000b) claim to have found a ‘second fusion peptide’ in the sendai virus F1 protein near the C-terminus of the N-heptad repeat domain.

The SNARE system also has a hydrophobic domain exposed by the signal for fusion. Davis et al. (1999) found that the C2A domain of synaptotagmin exposes two hydrophobic domains near its transmembrane domain upon the binding of Ca\(^{2+}\). The exposure of the hydrophobic loops was rapid, reversible and under Ca\(^{2+}\) binding control. The hydrophobicity of the kink in HA2 is known to be reversible and under H\(^+\) binding control. It is likely to be rapid also. The binding of Ca\(^{2+}\) to soluble synaptotagmin C2A fragments doesn’t cause much of a conformational change, as measured by NMR (Davis et al., 1999), but the dramatic functional change is clearly similar to the kink of HA. Thus, synaptotagmin could mediate two key functions during fusion: aggregation of the proteins into the fusion site via C2B and formation of a hydrophobic collar via C2A over the hydrophobic defect in the vesicle bilayer.

In order to obtain a sense of size for these protein complexes, another crude representation of synaptotagmin and the SNARE proteins is shown in Figure 6A. The shape of the C2A domain is approximately correct (Shao et al., 1998), while the volumes of the C2B domain and the intervening sequence is approximately correct. Finally, the synaptotagmin/SNARE complex is depicted.

Fig. 6. Panel A shows space filling depictions of the SNARE protein complex, based upon their crystal structure (Sutton et al., 1998) and the NMR-derived structure of their yeast homologues (Fiebig et al., 1999). A space-filling depiction of synaptotagmin is shown, although a structure is known only for the C2A domain (Shao et al., 1998). The shape of the C2A domain is approximately correct, while the volumes of the C2B domain and the intervening sequence is approximately correct. Finally, the synaptotagmin/SNARE complex is depicted, in order to obtain a sense of size for these protein complexes. Panel B shows the size of an aggregate of eight of these synaptotagmin/SNARE complexes on a 4 nm thick bilayer, which is 20 nm on each side. The C2A hydrophobic loops are displayed above the center of aggregate, just like for HA in Figure 4B. While the number of proteins shown is arbitrary, it is likely to be roughly correct, clearly showing the very large size of the proposed eukaryotic fusion structure.

NMR-derived structure of their yeast homologues (Fiebig et al., 1999). Finally, the synaptotagmin/SNARE complex is depicted.

Figure 6B shows the size of an aggregate of eight of these synaptotagmin/SNARE complexes on a 4 nm thick bilayer, which is 20 nm on each side. The C2A hydrophobic loops are displayed above the center of aggregate, just like for HA in Figure 4B. Several considerations guided these depictions. The volume of protein is approximately correct, which is essential to understanding how difficult it is to gain lipidic communication between the
b canadian domain, in order that the separation between the bilayers not be greater than 4 nm, synaptotagmin cannot be too thick. The C2B domain would need to be rather elongated, if it is to bind to adjacent syntaxins. Such a complex would have a diameter of roughly 20 nm, which would make it a target for imaging using current technologies.

It is worth noting that the various domains discussed here as putative hydrophobic kink formers do not show any obvious sequence homology (data not shown). Tertiary structure could be important. It is also interesting that the fusion protein of the tick-borne encephalitis virus, TBE-E (Rey et al., 1995), not only has two putative amphipathic α-helices just N-terminal to its transmembrane domain, starting at L399 and A426 (Stasny et al., 1996), which could fusion as a hydrophobic collar, but there is a putative internal fusion peptide about 150 aa upstream, starting near L244 (data not shown).

**MEMBRANE DESTABILIZATION BY SOLUBLE PROTEIN FRAGMENTS**

The model for fusion proposed in Bentz (2000a,b) covers only up to the initial intermediate between membranes. The elaboration of this fusion site clearly depends on many factors (Chernomordik et al., 1999; Melikyan et al., 1999). One crucial question is what determines whether the destabilization leads to fusion or simply deadends in hemifusion, i.e. lipid mixing of the outer monolayers (Chernomordik et al., 1998). It seems likely that the lifetime of the hydrophobic defect will depend critically on the size of the fusion protein aggregate. Likewise, this lifetime should have a major role in determining the fate of nascent fusion site. It is known that with higher pH or fewer HAs expressed on the cell surfaces, contents mixing decreases or is abolished (Chernomordik et al., 1998). Both of these conditions are expected to diminish the size of the HA aggregate (Bentz, 2000a).

To date, soluble fragments of membrane fusion proteins have been able to induce lysis and lipid mixing between liposomes and other membranes. Most of the studies done with soluble fragments are inspired by the in vivo capabilities and/or disabilities of mutated fusion proteins, and involve experimentation on fragments whose mutations are known to affect fusion (see reviews: Pecheur et al., 1999; Peuvot et al., 1999; Durell et al., 1997; Brasseur et al., 1997). While it has been shown that some amino acids within these fragments are essential for fusion in vivo, the geometric constraints placed on these amino acids by the rest of the protein are just as important. That is, the soluble factors have a membrane destabilizing function needed to disrupt the lipid bilayer. However, the fusion protein arranges these destabilizing domains so as to cause the destabilization and to contain the damage so that a productive fusion event occurs. Hemifusion with or without lysis is rarely biologically desirable.

Table 2 shows some recent studies on fragments of some fusion proteins, mainly the fusion peptides or the heptad repeat regions. We have included only those studies that have used two or more assays, i.e. lipid mixing along with leakage or contents mixing. A single assay rarely suggests clear answers to fundamental questions like: does leakage come from single vesicles or cells, or is interbilayer destabilization required, which appears more relevant to fusion protein function? In fact, even in the studies listed, it is not possible to know the answers to these questions.

Studies done with fusion peptide sequences show that these fragments either lower the T1 of membranes or facilitate transition to HII lipidic phases (Pereira et al., 1999; Epand et al., 1992, 1994a and b; Peisajovich, 2000a), although influenza virus fusion with glycoporphin-containing liposomes was not sensitive to this phase boundary (Alford et al., 1994). Further, α-helicity along with a specific tilt is a highly associated with the ‘fusogenic’ ability for most of the fusion peptide fragments (Murata et al., 1993; Pecheur et al., 1999; Peuvot et al., 1999). However, synthetic fusion peptides from HIV and sperm have been found to adopt β-structure in membranes (Nieva et al., 1994; Pereira et al., 1995, 1997; Pritsker et al., 1998; Muga et al., 1994). Nieva et al. (1994) found different structural requirements for lipid mixing and leakage for HIV gp41 fusion peptide, suggesting that leakage and lipid mixing experiments do not combine to yield clear criteria of ‘fusogenicity’. Kliger et al. (1997) concluded that larger the peptide, the more ‘fusogenic’ it is, in contrast to findings of other investigators (see “Length of peptides”, Pecheur et al., 1999).

This leads us to a key aspect of interpreting results obtained from studying interaction of soluble fragments with membranes. How is ‘fusogenicity’ defined? We have tried to summarize the criteria used for defining ‘fusogenicity’ by different investigators in Table 2. It is clear that kinetic data obtained from these studies does not provide adequate information regarding the fusion mechanism(s) via these fragments. Lipid mixing, leakage and/or contents mixing curves obtained using
<table>
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<th>Fragment(s)</th>
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<tr>
<td>HIV gp41 N-terminal (23aa, HIVarg) from strain LAV1a</td>
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<td>Lipid mixing: NBD/Rh Leakage: ANTS/DPX Contents mixing: FITC-Dextran</td>
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<td>HIV gp41 HIVarg and HIV-E2 (V2-&gt;E)</td>
<td>LUVs (POPG)+free peptide</td>
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<td>Extents of lipid mixing and leakage up to 2 min</td>
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<td>HIV gp41 wt and mutants</td>
<td>LUVs (Egg PE/PC)+free peptide</td>
<td>Lipid mixing: NBD/Rh Leakage: Calcein</td>
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<tr>
<td>HIV gp41 N-terminal (33aa) from strain LAV1a</td>
<td>Cell–cell fusion VESICLES (PC:PS:Ch)+free peptide</td>
<td>Lipid mixing: DiI Leakage: diS-C&lt;sub&gt;2&lt;/sub&gt;-5 and Valinomycin</td>
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<td>SIV gp32 wt and mutants</td>
<td>LUVs (DOPE/DOPC/Chol)+free peptide</td>
<td>Lipid mixing: NBD/Rh Leakage: ANTS/DPX</td>
<td>Extents of lipid mixing and leakage up to 5 min</td>
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<tr>
<td>Sendai virus F1 segment heptad repeats SV-150(aa150–186), SV-473(aa473–495)</td>
<td>Virions+erythrocytes+free peptide, SUVs(PC/PS/Chol, PC/Chol)+free peptide</td>
<td>Lipid mixing: NBD/Rh Leakage: ANTS/DPX</td>
<td>Extents of lipid mixing and leakage up to 15 min</td>
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<tr>
<td>Influenza HA2 FHA2, FHA2-90 (90aa N-terminal)</td>
<td>LUVs (PC/PE/Sm/Chol)+free peptide</td>
<td>Lipid mixing: NBD/Rh Leakage: ANTS/DPX</td>
<td>Extents of lipid mixing and leakage for 5–6 min, insignificant contents mixing observed</td>
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<td>Influenza BHA and HA rosettes</td>
<td>LUVs (PC/PE/gangliosides)+free peptide/protein at 0°C</td>
<td>Pore formation and leakage: Calcein, TMRD-3K or TMRD-10K, NBD fluorescence</td>
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<td>WAE 11aa synthetic amphipathic peptide</td>
<td>LUVs (Zwitterionic PC/Chol vesicles; PC/Chol)/PElys, PC/Chol/PE-PDP)</td>
<td>Lipid mixing: NBD/Rh Contents mixing: TbCl3/DPA Leakage (in positively charged liposomes): Tb/DPA</td>
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<td>Pecheur et al., 1997</td>
</tr>
<tr>
<td>WAE</td>
<td>LUVs (PC/Chol/PE-PDP+PS/PE)+Ca&lt;sup&gt;2+&lt;/sup&gt; for aggregation</td>
<td>Lipid mixing: NBD/Rh Content mixing: TbCl3/DPA</td>
<td>Extents of lipid mixing and contents mixing up to 2 min</td>
<td>Extents of lipid mixing and contents mixing &lt;20%</td>
<td>Martin et al., 1999</td>
</tr>
<tr>
<td>WAE</td>
<td>LUVs (PC/Chol/PE-PDP[LP-WAE]+PS/PE)+Ca&lt;sup&gt;2+&lt;/sup&gt; for aggregation</td>
<td>Lipid mixing: NBD/Rh Content mixing: TbCl3/DPA</td>
<td>Extents of lipid mixing and contents mixing up to 2 min</td>
<td>Extents of lipid mixing and contents mixing &lt;15%</td>
<td>Pecheur et al., 2000</td>
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soluble fragments consist of spikes (in fluorescence signals) within the first few seconds of addition of the soluble fragments and ‘fusogenicity’ is judged in terms of extent of the signals reached within the first few seconds. Not only is it impossible to confirm whether leakage happens before or after lipid mixing, these spikes suggest intra-membrane disruptions rather than inter-membrane destabilization. Factors like peptide-induced aggregation followed by hemifusion and/or interaction of the fluorescent probes with free or membrane bound or coupled peptide fragments have to be considered before any conclusions about mechanisms can be reached.

The importance of heptad-repeat segments forming coiled coil moieties in fusion proteins is clear (Skehel and Wiley, 1998). Soluble fragments corresponding to these parts of fusion proteins have also been investigated in an attempt to provide an insight into their role in membrane fusion (Yu et al., 1999; Rabenstein and Shin, 1995; Epand et al., 1999; Ghosh and Shai, 1999; Ben-Efraim et al., 1999). The data from Ghosh and Shai (1999) on the heptad repeat from sendai virus shows a remarkable result in terms of peptide fragment SV-117 (comprising of the fusion peptide and heptad repeat from Sendai virus) inducing lipid mixing at much larger extents as compared to only the fusion peptide or the fragment SV-150 (only the heptad repeat). This showed the importance of heptad-repeat and fusion peptide combined in destabilization, as had been shown by Epand et al. (1999) for the HA equivalent system, i.e. FHA2.

Several studies have found that soluble fragments of the heptad repeat domains of HA2, gp41 and sendai F1 protein appear to melt into the liposomal bilayers (Yu et al., 1994; Rabenstein and Shin, 1995; Ben-Ephraim et al., 1999). The models derived from these studies have the coiled coil domains melting into one bilayer, guiding the lipids down the opening trimeric core to the other bilayer, reminiscent of older models for fusion. Since the hydrophobic effect dominates both the formation of these coiled coils and the binding of amphipathic peptides to bilayers, it is not surprising that these soluble fragments would be found in both environments. Likewise, mutations which weakened the coiled coil would diminish binding to bilayers. The question is whether such an event is kinetically relevant to fusion with the entire protein, and there is no data on this. A mechanism based on melting the coiled coil has no obvious role for an aggregate of fusion proteins (Ellens et al., 1990; Danieli et al., 1996; Blumenthal et al., 1996; Bentz, 2000a), nor for an essential conformational change in two to three of these HAs (Bentz, 2000a), since only one needs to succeed to join the membranes.

Another limitation of these studies is that the lipid mixing assay, even combined with a leakage assay, does not distinguish between hemifusion, wherein only the outer monolayers mix (Ellens et al., 1985) from the expected event of complete bilayer mixing and leakage arising from this destabilization. Bonnafous and Stegmann (2000) have partially overcome this limitation by using a lipid mixing assay which monitors only inner monolayer mixing, thereby ignoring outer monolayer hemifusion. They mixed liposomes with BHA and with HA rosettes and found long-lived pores were created. They also confirmed the result of Shangguan et al. (1996) that influenza virus fusion with liposomes is essentially lytic, even to large molecular weight dextrans (<10K). Virosomes also form leaky pores on their own, presumably from defects formed their fusion peptides. As discussed in Bentz (2000b), the evolution of the hydrophobic defect in the viral bilayer could easily lead to leakage, so these results are consistent with the model for fusion shown here (see Figure 4B). However, the residual detergent in the virosome bilayer (Stegmann et al., 1987) could accelerate a pore formation at the hydrophobic defect relative to the intact viral bilayer.

While not a soluble peptide, an 11 residue synthetic amphipathic peptide coupled to a lipid denoted WAE is included (see Table 2, Pecheur et al., 1997, 2000; Martin et al., 1999). With this system limited contents mixing (<20%) without leakage was found following limited lipid mixing (<20%). The other distinction of WAE is that it is claimed to insert into the bilayer parallel to the acyl chains and it is too short to span the bilayer. Given that it has a free N-terminus and three glutamates, such an orientation would be very high energy. Preaggregation of the liposomes was found necessary for any fusion, possibly because the peptide inserts into its own bilayer unless the target membrane is very close. After insertion into the target bilayer, this coupled peptide may cause molecular contact of the bilayers, because it is too short to span the entire target bilayer. These features make the peptide’s mechanism of destabilization rather different than that proposed for HA.

CONCLUSION

Overall, the model proposed in Bentz (2000b) and extended here provides physical reasons for the need of each of the domains listed in Table 1 to
perform the essential acts of fusion. The focus is on getting the lipids from one membrane to the other, which requires breaking the process down into bite-size activation energies that the different domains of the fusion proteins can stabilize, i.e. speed-up. Fusion proteins are not enzymes, but is seems likely that both must use similar strategies to succeed at their respective jobs. While all of these domains have not yet been found within each fusion protein, it must be noted that the best analogy is between HA and the SNARE/synaptotagmin system, which are quite separate evolutionarily.

REFERENCES


Synaptotagmin I: Does Ca$^{2+}$ induce a conformational change? Biochemistry at the level of the fusion peptide.

The envelope glycoprotein from tick-borne encephalitis virus mediates.

The envelope glycoprotein from influenza virus envelopes.


