spherically converging shock wave generated within the collapsing bubble. Wu and Roberts (*7*) as well as Greenspan and Nadim (*8*) have demonstrated numerically that such an imploding shock should exist in the SBSL bubble and that extremely short pulse durations (0.1 ps) and high temperatures (1000 eV; 1 eV = 11,600 K) should occur. Using a more accurate equation of state, Moss *et al.* (*9*) have confirmed predictions of extreme temperatures and pressures, obtaining values more in line with the (crude, at this time) experimental measurements, namely, pulse durations on the order of 10 ps and peak temperatures on the order of 10 to 100 eV. Furthermore, their computations suggest that at various locations within the imploded core at the center of the bubble, pressures can be as high as 200 Mbar (1 Mbar = 10^{11} Pa), and densities as high as 13.4 g/cm^3 (at these levels, it is possible that the compressed air near the center of the bubble will have properties similar to that of a metal). Note that all the calculated results cited above are based on one-dimensional calculations assuming a perfectly symmetrical bubble collapse and are mitigated by the effects of dissociation and ionization [which are accounted for in the Moss *et al.* (*9*) computations] and by various radiation and mass transport mechanisms (which are not accounted for by Moss *et al.*).

As indicated in figure 2 of Hiller *et al.* (*10*), a small quantity of argon introduced into a pure nitrogen bubble increases the luminosity of SBSL by nearly two orders of magnitude. What effect does argon have on this system? Does it strongly influence the dissociation, ionization, and radiation transport within the core? Does it have a catalytic effect on electronic transitions within the plasma or material composing the core? Does it readily conduct heat from the hot interior of the core to the outer layers and thus increase the radiated energy or the total volume of high-temperature gas? These questions are difficult to answer with the existing data and clearly require additional measurements and computations.

Figure 3 of Hiller *et al.* (*10*) demonstrates that if the bubble contains certain gas species, the spectra show broad peaks near 300 nm, whereas for other species, no peaks exist and the spectrum monotonically increases down to the water cutoff (the transmissivity of the ultraviolet through water is greatly reduced below 200 nm). Why is it that a maximum exists at all? If the gas core is heated and compressed to the degree predicted by recent theories, then only the outer shell should radiate (like the sun). If there is a broad maxima for xenon, then shouldn't there also be one for helium? These again are anomalous results and perhaps have something to do with the heat transport through the compressed gas.

Finally, the data displayed in figure 5 either have a trivial explanation (for example, a periodic detuning of the cell) or they are truly remarkable. These data suggest that some mechanism, possibly gas diffusion across the gas-liquid interface, is causing the luminosity and equilibrium bubble radius to cycle with a period on the order of seconds. It seems remarkable to us that such long-term memory (on the order of 100,000 acoustic cycles) could exist in a mechanical system.

As we currently understand it, single-bubble sonoluminescence may result in temperatures in excess of 10^5 K, pressures in excess of 10^7 bar, light emissions lasting less than 50 ps, and mechanical energy concentrations of up to 12 orders of magnitude; all this from a simple acoustical system costing a few hundred dollars to construct. It is a remarkable laboratory for physics and chemistry.

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Flu Virus Invasion: Halfway There

Chavela M. Carr and Peter S. Kim

The protective barrier provided by biological membranes is exceedingly difficult to breach. The fusion of two distinct lipid bilayers is therefore energetically unfavorable in the absence of specialized proteins. Fertilization of the egg cell with the sperm, perhaps the most dramatic consequence of membrane fusion, requires the sperm protein PH-30α-β (*1*). Enveloped viruses also use membrane fusion: Infection of animal cells by human immunodeficiency virus (HIV), for example, is aided by the HIV envelope protein gp120-gp41 (*2*).

The best characterized membrane fusion protein is the hemagglutinin (HA) protein found on the surface of influenza (flu) virus. In spite of decades of research, the mechanism by which HA induces fusion remains elusive. Recent developments, however, including a report by Shin and co-workers on page 274 of this issue of *Science* (*3*), suggest that we are getting closer to an understanding of this protein-mediated membrane fusion process.

Flu onset begins with the binding of influenza virus to nasal epithelial cells. In a function that is distinct from membrane fusion, HA mediates viral attachment to sugar groups (sialic acid) on the surface of the host cell. Binding does not lead directly to membrane fusion, since HA in its native state is not fusion active. Instead, the cell internalizes the bound virus and surrounds it with an endosome. Only in the mildly acidic conditions of the mature endosome does the HA protein switch from an inactive to a fusion-active (fusogenic) state. In this fusogenic conformation, HA promotes fusion of the viral and cellular membranes, leading to release of the nucleocapsid into the cytoplasm and thereby initiating replication and proliferation of the virus.

But how does HA induce membrane fusion? Thirteen years ago, the x-ray crystal structure of HA in the native state was reported by Wiley and his co-workers (*4*). Hemagglutinin is a trimeric protein with three identical subunits that span the viral mem-

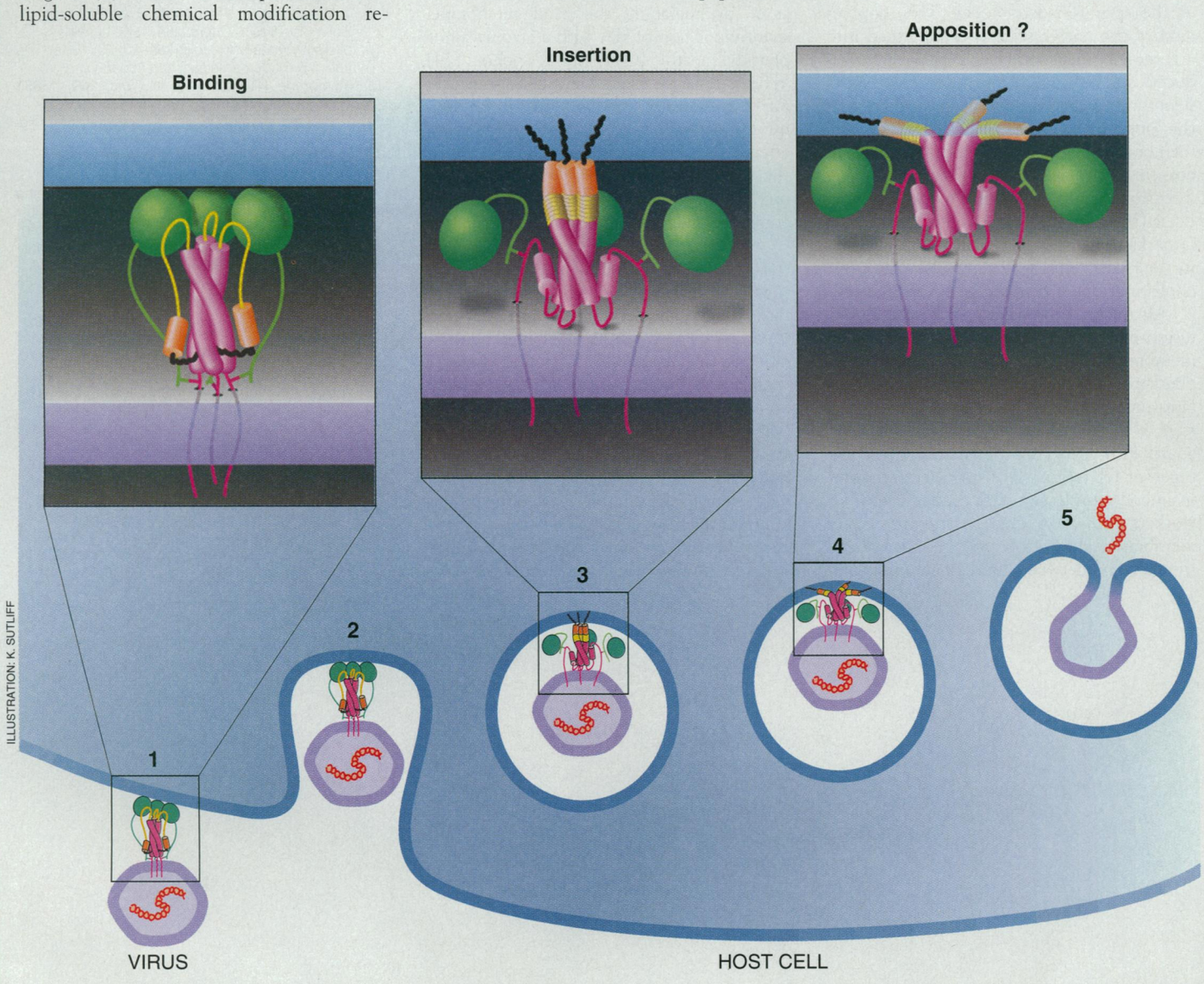
The authors are in the Howard Hughes Medical Institute, Whitehead Institute for Biomedical Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142, USA.

brane (see figure, step 1). Each subunit is synthesized as a precursor that subsequently is proteolytically cleaved (5) in a process required for fusion activity (6). The newly created amino terminus contains a hydrophobic sequence of 25–amino acid residues that are essential for membrane fusion (7); this region is therefore called the "fusion peptide." The baffling aspect of the structure was that the fusion-peptide region is buried deep inside the native HA protein.

It seemed that the fusion-peptide regions must be exposed in the fusogenic state. Indeed, when HA is exposed to acid, the fusion-peptide region becomes accessible to antibodies, proteases, and chemical reagents (8). In addition, experiments with lipid-soluble chemical modification reagents showed that the fusion-peptide region of HA inserts into the target membrane before fusion (9, 10). Thus, a key intermediate in the fusion process consists of HA in two different membranes at the same time: The transmembrane region of HA spans the viral membrane and the fusion-peptide region inserts into the target membrane.

Nonetheless, the structural constraints associated with formation of this crucial fusion intermediate appeared formidable. A conformational change that releases the fusion-peptide region from the native HA core would still leave these exposed regions more than 100 Å away from the target membrane. How do the fusion-peptide regions reach the cellular membrane? Imaginative models were proposed to answer this question. For example, one model (11) proposed that HA tilts 90°, lying against the viral membrane to facilitate interaction between the fusion-peptide region and the target membrane.

A different model (12) emerged from the identification within HA of a 28–amino acid sequence with a marked propensity for forming a coiled coil, a structure of interwound α helices. Surprisingly, this sequence does not form a coiled coil in the native conformation of HA, but instead forms an extended "loop" structure. This loop region joins the fusion-peptide region



Flu virus invasion. Step **1.** Hemagglutinin in the native state mediates binding of flu virus to the host cell. The domains at the top of HA (green balls) bind to sialic acid residues on the cell surface. Step **2.** The virus is internalized in an endosome. Step **3.** In the acidic conditions of the mature endosome, HA switches to a fusion-active state. The loop region forms a coiled coil (yellow), which includes the α helix (red). As a result, the fusion-peptide regions (black) insert into the cellular membrane at the top of the molecule. At the opposite end, each long α helix (pink) bends up to pack against the core of the trimer. Hemagglutinin is now attached to both the viral and the cellular membranes. Step **4.** In a model for membrane apposition, the long coiled coil separates and the top of the coiled coil (red and yellow) "melts" into the cellular membrane bringing the two membranes closer together. Step **5**. Ultimately, in a process that is poorly understood, the bilayers fuse, releasing the nucleocapsid into the cell.

to the three-stranded, coiled-coil core of the native state (see figure, step 1). A synthetic peptide corresponding to this loop region forms a trimeric coiled coil at the pH of membrane fusion (*12*). The fact that this loop region could form two completely different structures suggested a "spring-loaded" mechanism for the conformational change of HA (see figure, step 3): At acidic pH the loop region forms a coiled coil, projecting the fusion-peptide regions to the top of the molecule where they can then interact easily with the target membrane.

Last month, Wiley and co-workers reported the crystal structure of a large fragment of the acid-induced state of HA (*13*). The structure confirms the salient feature of the spring-loaded model: The loop region of the native state is transformed into a three-stranded coiled coil in the acid-induced state. In addition, the new crystal structure reveals a second major change at the opposite end of HA, near the viral membrane: The long α helix of the coiled-coil core stops near the bottom and reverses directions, bending upward to pack against itself (see figure, step 3). The transition is remarkable: Fewer than half of the residues in the new crystal structure are in the same conformation as in the native state.

Although the mechanism by which the fusion-peptide region reaches the target membrane may now be understood, this structure presents a new problem. Hemagglutinin in this conformation (see figure, step 3) holds the two membranes 100 Å apart! How then do the membranes come together for fusion? By using electron paramagnetic resonance methods, Shin and co-workers provide a clue to the next step: membrane apposition (3). Acid-induced interactions between lipid membranes and a peptide that corresponds to the loop region of native HA were detected by monitoring changes in the spectral properties of a spin-labeled probe attached at different positions in the peptide. In their model, Shin and co-workers propose that the top of the acid-induced coiled coil splays apart and "melts" into the lipid bilayer of the cellular membrane, thereby bringing the viral and cellular membranes closer together (see figure, step 4). Although verification with intact HA protein is required, this model provides a tantalizing solution to the problem of how the two membranes may be juxtaposed.

Even so, apposition of the two membranes is not the only requirement for fusion. By replacing the viral membrane–spanning region of HA with a glycosyl phosphatidylinositol (GPI) lipid anchor (*14*), White and co-workers identified a new, putative fusion intermediate, in which lipid mixing occurs between cells expressing the altered HA and target red blood cells, but the internal contents of these cells do not mix. Thus, lipid mixing can occur without complete membrane fusion.

Although questions remain, any solution to the membrane fusion problem must now contend with an acid-induced structure of HA that is remarkably different from the native state, biochemical evidence that more than just the fusion peptide can interact with the target membrane, and the observation that lipid mixing induced by GPI-anchored HA is not sufficient for membrane fusion. A major challenge will be to reveal the architecture of the fusion pore that forms as the viral and cellular membranes fuse (*15*). The puzzle is not complete, but we may be halfway to understanding how the flu virus invades our cells.

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