Viral fusion: how Flu induces dimples on liposomes

Kay Grünewald

Oxford Particle Imaging Centre, Division of Structural Biology, The Wellcome Trust Centre of Human Genetics, University of Oxford, Oxford, UK
Correspondence to: kay@strubi.ox.ac.uk

The EMBO Journal (2010) 29, 1165–1166. doi:10.1038/emboj.2010.35

Membrane fusion—a basic cellular process enabling crucial cellular functions such as membrane trafficking—is mechanistically only partially understood. Most of the existing knowledge has come from studying host-cell entry of viruses with lipid bilayer envelopes, which proceeds through fusion of viral and host-cell membranes. Crystal structures for a number of viral fusion proteins facilitating this process have contributed substantially to our understanding. One of the next challenges is to merge these high-resolution structures of soluble parts of fusion effectors with native structural information on the proteins in the course of their functional interactions with the target membranes. In this issue of *The EMBO Journal*, Lee (2010) presents an elegant example of such a study, probing the early interaction of the influenza virus with liposomes by cryo-electron tomography (cryoET).

Enveloped virus entry into the host cell typically follows either of two principle routes: direct fusion at the plasma membrane, or viral particle uptake into the endosomal compartment and subsequent fusion of the viral envelope with the endosome membrane. Both cases ultimately result in release of the viral content into the cytosol. Influenza virus follows the latter route, the fusion event being initiated by acidification of the endosomal lumen. This pH change induces a conformational alteration in influenza haemagglutinin, the fusion glycoprotein that projects in hundreds of copies from the virus surface (Harris *et al.*, 2006). As in other fusogens, this conformational change provides the energy needed to overcome the kinetic barrier for drawing the bilayer membranes together.

Membrane fusion promoted by viral surface proteins likely proceeds in a conserved sequence of events (reviewed in Harrison, 2008): (1) The protein ‘opens up’ and forms a bridge between the two bilayers, by inserting its hydrophobic fusion loops or fusion peptides into the target membrane. (2) The bridge collapses, bringing the two membranes into closer apposition and locally distorting the two bilayers into a dimple-like configuration, the exact mechanism of this step remaining incompletely understood. (3) A hemifusion stalk is formed by differential distortion of individual leaflets of the membrane bilayers, and by merger of the outer leaflets. (4) Opening of the hemifusion stalk leads to the formation of a transient fusion pore. (5) This pore is stabilized and expands, often involving a further conformational change of the fusion protein. For some viruses (most notably herpesvirus, as

reviewed in Heldwein and Krummenacher, 2008), these fusion events are the result of a concerted interplay of multiple macromolecular complexes. However, the rule for most viruses surprisingly appears to be that all the required functions are a property of a single—typically trimeric—fusogenic complex, making a mechanistic dissection of the process very challenging.

Although high-resolution crystal structures are available for a number of fusion proteins, functional understanding requires investigating the proteins directly during their interaction with membrane bilayers. CryoET (reviewed in Lucić *et al.*, 2005) offers a dedicated tool for this, at an intermediate resolution of around 4 nm. This technique combines the superior structural preservation of biological specimens with three-dimensional imaging of unique pleomorphic objects and events, without the need for averaging over a number of identical situations. Sample preparation by fast vitrification, i.e. by keeping the water and embedding hydrated specimens in amorphous ice, allows for interpretation of the resulting density maps into the macromolecular range. Thus, cryoET is uniquely positioned at the crossroad between high-resolution structures and live cell imaging, and is hence suited for integrating information from both directions.

A first such study aimed at visualization of fusion processes with macromolecular detail was performed by Maurer *et al.* (2008). In analysing the dynamics of herpesvirus fusion in the most native model system, virus–cell fusion, this pioneering study visually delineated examples of the steps involved. By expanding this study to fusion with neuronal synaptosome preparations, the authors were also able to obtain first glimpses of the shape of molecules next to the fusion site, presumably representing the fusion proteins involved in the process.

The new study by Lee (2010), in this issue of *The EMBO Journal*, now takes this a step further, by probing particularly the early influenza virus–liposomes interaction using cryoET. This system provides two advantages compared with the herpesvirus study: the involvement of just one, distinctly shaped fusion protein, haemagglutinin (as compared with the multiple players involved in herpesvirus fusion); and the smaller size of the specimens, enabling for better beam penetration, increased signal-to-noise ratio in the resulting tomograms, and consequently higher-resolution imaging. In addition, the influenza system allows to experimentally

trigger the process by pH changes: lowering the pH to only 5.5 results in incomplete fusion arrested at the second intermediate step in the fusion sequence. The three-dimensional cryoET analysis of influenza virus–liposome complexes at pH 5.5 thus offers novel insights into how haemagglutinin modulates viral and target membranes to mediate their fusion: Early remodelling upon contact between liposomes and virus particles induced a striking deformation of the target membrane, progressing from minute dimples towards the formation of a membrane-disrupting ‘pinched liposomal funnel’, while the viral envelope remained apparently still unperturbed. This unequal effect on the two fusing membranes suggests that the viral matrix layer underlying the inner side of the viral envelope may serve as a ‘cortical endoskeleton’ for the virus, forming a firm foundation for haemagglutinin and preserving the virus shape. Consequently, the force generated by the conformational changes would be fully exerted onto the target membrane of the liposome. Although this effect may be less drastic in the scenario of viral entry into a cell, wherein the actin cytoskeleton should provide some cytosolic counter-foundation, it may still have a role given that the cytosolic counterforce is probably not as strong as the viral matrix layer. Whether the surrounding haemagglutinin molecules—perhaps being in the pre-hairpin intermediate conformation, with their thin flexible fusion loops inserted in the liposome—may act to stabilize the open-appearing membrane ends of the liposome side funnel cannot be revealed at the obtained resolution, but the

relatively frequent observation of this state is interpreted by the author to argue against it being just a short-lived, transient intermediate. Moreover, fluorescence spectroscopy monitoring of fusion between liposomes and virions revealed the ‘leakage’ of liposome contents before lipid mixing, consistent with this interpretation. Further experiments lowering the pH to 5.0 showed that the fusion process in this system could indeed continue beyond that step, involving dissociation of the matrix layer barrier and resulting in completion of fusion. The observed prefusion funnel structure therefore appears to be a *bona fide* functional fusion intermediate.

Overall, the striking images and impressive tomographic reconstructions present valuable novel insights into this crucial intermediate step in membrane fusion. The obvious next set of experiments will provide new challenges, involving searching for conditions that trap other intermediates and imaging the system with optimized data acquisition parameters. Ultimately, combining this with subtomogram averaging and classification of individual haemagglutinin complexes is expected to improve the resolution substantially, and might allow for docking of high-resolution structures, yielding even closer insights into the fusion mechanism.

Conflict of interest

The author declares that he has no conflict of interest.

References

- Harris A, Cardone G, Winkler DC, Heymann JB, Brecher M, White JM, Steven AC (2006) Influenza virus pleiomorphy characterized by cryoelectron tomography. *Proc Natl Acad Sci USA* **103**: 19123–19127
- Harrison SC (2008) Viral membrane fusion. *Nat Struct Mol Biol* **15**: 690–698
- Heldwein EE, Krummenacher C (2008) Entry of herpesviruses into mammalian cells. *Cell Mol Life Sci* **65**: 1653–1668
- Lee KK (2010) Architecture of a nascent viral fusion pore. *EMBO J* **29**: 1299–1311
- Lucić V, Förster F, Baumeister W (2005) Structural studies by electron tomography: from cells to molecules. *Annu Rev Biochem* **74**: 833–865
- Maurer UE, Sodeik B, Grünewald K (2008) Native 3D intermediates of membrane fusion in herpes simplex virus 1 entry. *Proc Natl Acad Sci USA* **105**: 10559–10564