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Complementary Approaches to Dissecting Mechanisms of Protein-mediated Membrane Fusion

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Enveloped viruses use specialized protein machinery to attach to host cells and fuse their membrane with the cellular membrane in order to deliver their genetic material for replication. In influenza virus, the trimeric hemagglutinin (HA) glycoprotein spike is responsible for attachment and mediating membrane fusion following endocytosis. While structures of a subset of HA conformations and parts of the fusion machinery have been characterized, the states that drive the fusion process have proven to be refractory to classical structure determination. In addition, the nature of membrane deformations during fusion has eluded characterization. We employ a combination of structural and biophysical methods to study the process of HA-mediated membrane fusion. Electron tomography is ideally suited for characterizing enveloped virus ultrastructure and the interaction of virus with membranes during fusion. At a resolution of ~2 nm, cryo-electron tomography (cryo-ET) provides the ability to image individual viral glycoprotein spikes and the leaflets of lipid bilayers as well as details of virus ultrastructure. In order to characterize structural changes in the fusion protein itself in more detail, hydrogen/deuterium-exchange mass spectroscopy (HDX-MS) is used to monitor the structural dynamic changes in the HA complex during acid-induced fusion activation.

Previous cryo-ET studies demonstrated that during early stages of fusion between influenza virions and liposomal target membranes, membrane remodeling is generally focused on the target membrane while the virus envelope remains unaltered (1). With pure phosphatidylcholine (PC) liposomes, in the early stages of fusion, HA induces highly localized target membrane deformations and open-mouthed dimples consistent with a high degree of content leakage, Cryo-ET and fluorescence spectroscopy experiments are now being used to test the effect of two additional lipid components, cholesterol and lyso-bisphosphatidic acid (LBPA), on the fusion process. Cholesterol is a major constituent of plasma membranes while LBPA is enriched in late endosomes. Addition of either of these components to PC-based liposomes makes the lipid mixing stage of membrane fusion more efficient as monitored by fluorescence spectroscopy; cholesterol also reduces content leakage, while LBPA does not have a major effect on the degree of liposomal content leakage. 3-D imaging by cryo-ET reveals that the liposomes with 25-50% cholesterol or LBPA exhibit a greater proportion of closely apposed, extended contacts between virus and target membranes, in striking contrast to the more localized, punctate deformations observed with pure PC liposomes (Figure 1). We hypothesize that the closely apposed, extended contacts represent a key intermediate leading to efficient membrane merging.

In order to characterize the nature of conformational changes in HA during fusion-activation, in a recent study, HDX-MS was also used to examine the HA ectodomain at pH values approaching fusion-triggering endosomal conditions (pH 6.0-5.5) (2). HA was found to exhibit increased dynamics at the fusion peptide and associated regions, while the interface between receptor-binding subunits (HA1) becomes bolstered (Figure 2). In contrast to many activation models, the HDX-MS data suggest that HA responds to endosomal acidification by releasing the fusion peptide prior to HA1 uncaging and spring-loaded refolding of HA2. A similar model has been proposed by Fontana *et al*., based upon cryo-ET data, although in that case, the sub-tomogram averaged models were not of sufficient resolution to identify the protein segments involved in the rearrangement (3).

Taken together, these complementary techniques are starting to reveal the staged, sequential nature of fusion protein activation and the resulting remodeling of target and viral membranes that take place during influenza HA-mediated fusion.

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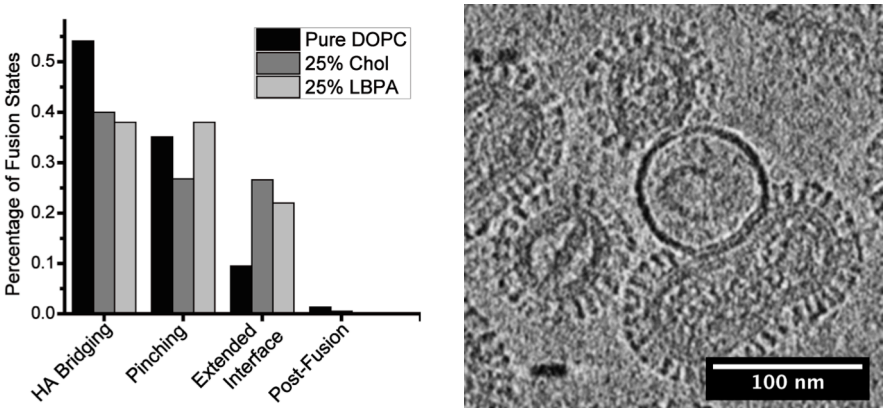

FIGURE 1. Population distribution of virus-target membrane contacts observed by cryo-ET (left). Addition of cholesterol or LBPA enhances fusion efficiency and is associated with increased formation of extended interfaces (right). This may be due to the lower cost of dehydrating the target leaflet with Chol or LBPA present. We propose that the extended, closely apposed contacts are key intermediates leading to fusion pore formation.

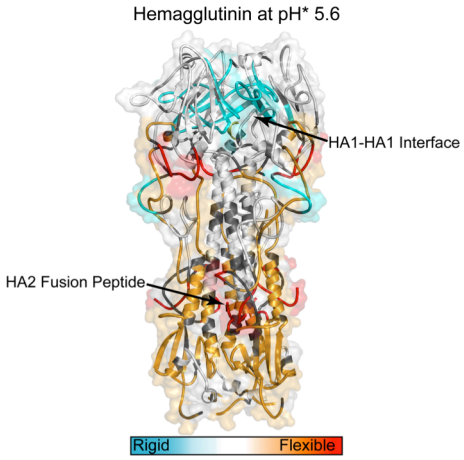

FIGURE 2. HDX-MS analysis of influenza HA activation reveals the dynamic profile of a prefusion intermediate. The heat map shows the regions including fusion peptide that become increasingly dynamic as activation pH is approached. We hypothesize that this intermediate allows the fusion peptides to be deployed and presented by the intact trimer cage prior to HA2 hairpin formation; such staging of conformational changes allows for the efficient target membrane engagement followed by fusion. Garcia et al. (2015).