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Review

Mechanisms of influenza viral membrane fusion

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ABSTRACT

Influenza viral particles are enveloped by a lipid bilayer. A major step in infection is fusion of the viral and host cellular membranes, a process with large kinetic barriers. Influenza membrane fusion is catalyzed by hemagglutinin (HA), a class I viral fusion protein activated by low pH. The exact nature of the HA conformational changes that deliver the energy required for fusion remains poorly understood. This review summarizes our current knowledge of HA structure and dynamics, describes recent single-particle experiments and modeling studies, and discusses their role in understanding how multiple HAs mediate fusion. These approaches provide a mechanistic picture in which HAs independently and stochastically insert into the target membrane, forming a cluster of HAs that is collectively able to overcome the barrier to membrane fusion. The new experimental and modeling approaches described in this review hold promise for a more complete understanding of other viral fusion systems and the protein systems responsible for cellular fusion.

Contents

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1. Introduction

Membrane fusion is a key step in many biological processes. Processes such as intracellular compartmentalization and trafficking, neuronal signaling, entry of enveloped viruses, exocytosis, muscle repair, and cell-to-cell fusion in development all depend on enzymes that catalyze the merging of two lipid bilayers [1–8]. In cellular infection by enveloped viruses, membrane fusion represents the final step before the viral genome is released into the cytosol of the target cell. The key molecular step underlying fusion involves viral proteins that insert hydrophobic sequences into the target membrane and refold to drive merging of the lipid bilayers. So far, three major classes of viral fusion proteins have been characterized [4,5]. The first class comprises the fusion proteins of viruses such as HIV-1, ebola, and influenza. Class I fusion proteins are trimeric proteins with central coiled coil motifs as the key structural scaffold that enables the conformational changes needed for fusion. Class II fusion proteins, found in viruses such as dengue, zika and chikungunya, generally possess extended beta-sheet structures and rearrange from a dimeric geometry in the prefusion state into a trimer in the postfusion form. Class I and II proteins need to undergo a proteolytic priming and triggering event. Class III fusion proteins, for example from vesicular stomatitis virus and herpes simplex virus, show combinations of these structural motifs and lack a major priming event. The reovirus small proteins that induce cell-cell, but not virus-cell fusion have been proposed to represent a fourth class of viral fusogens [8,9].

One could consider viruses as evolutionarily optimized nanodevices, primed to enter and take over a host to ensure their continued existence [10]. The different viral fusion systems encountered in nature each represent elegant solutions to a biophysically challenging problem: the catalysis of the kinetically highly unfavorable merging of two bilayers. This review will discuss our current knowledge of the mechanistic operating principles of the influenza fusion machinery, arguably the most intensively studied viral fusion system. In particular, we will observe the problem through a biophysical lens; we will review structural knowledge on the influenza fusion system and discuss recent approaches relying on molecular modeling and single-particle microscopy that describe the fusion process. These biophysical studies suggest an intricate orchestration of the activity of a large number of fusion proteins as a key requirement for membrane fusion, suggesting that full understanding of viral fusion will need to come from both a detailed knowledge of the structural and chemical properties of the fusion proteins and a more holistic treatment of the interactions between larger numbers of fusion proteins connecting the viral and target membrane.

Influenza virus is a canonical example of an enveloped virus that has caused world-wide pandemics [11]. Because it inhabits multiple hosts and readily mutates, the threat of a new pandemic is real. The fusion of the viral and host cell membranes is mediated by hemagglutinin, a class I trimeric fusion protein. Viral entry is initiated by the virus binding to host-cell receptors via an interaction with a subdomain of the hemagglutinin and followed by cellular uptake into an endosomal compartment [12]. The low-pH environment of the matured endosome initiates a conformational change in the hemagglutinin structure causing it to extend and insert a hydrophobic N-terminal peptide into the target membrane. A subsequent refolding of the protein results in the two membranes to be pulled together and fuse.

Membrane fusion generally is not a spontaneous process on a biological timescale: the merger of two lipid membranes is thermodynamically favorable but has several kinetic barriers [13]. The essential characteristic of a biological membrane is the combination of a polar, hydrophilic exterior formed by the lipid headgroups and an apolar, hydrophobic interior containing the lipid tails [14]. Major players in membrane arrangement are the hydrophobic effect, giving rise to a poorly understood but strong interaction, polar and polarizing (Van der Waals) forces, and the interaction with water (hydration force) [15,16]. The interplay between these forces and the geometries of the system, such as lipid shape and membrane curvature, are key to the energetics of the membrane fusion process.

After being brought into close proximity, both the viral and target membranes have to be stripped of the hydration layer, the water layer that is tightly interacting with the polar headgroups of the lipids. Fusion of the two membranes then proceeds through a hemifusion stalk, an intermediate in which the proximal leaflets have merged. The final steps of fusion are the opening of a pore, so that aqueous contents on both sides of the membrane are connected, and subsequent expansion of the pore [13,17,18]. The progression from two separate membranes into a single contiguous one may be supported by lowering of the transition barriers, i.e. through the interaction with a catalyst or enzyme, or by the input of additional free energy through work [17,18].

This review aims to highlight the recent insights into the action of the influenza hemagglutinin as a catalyst and workhorse of this intricate membrane fusion process, and the role played by the kinetic steps and spatial distribution of HA as elucidated by single-particle studies. We will first discuss our current knowledge of the structural states and conformational dynamics of HA acquired from structural, computational and biochemical studies. As a central component of this review, we will then provide a description of single-particle methodologies and the insight they have given us, and discuss how collaboration of multiple hemagglutinins overcomes the membrane fusion barrier.

2. Hemagglutinin structure and conformational rearrangement

2.1. Hemagglutinin-mediated membrane fusion

The influenza A hemagglutinin (HA) is intensively studied and has since long served as a model system for viral fusion proteins [19]. The HA glycoprotein is synthesized as an inactive precursor, designated HA0 [20]. Cleavage in the *trans*-Golgi network by a host-cell protease results in a metastable, disulfide-bonded complex of HA1 and HA2 [21,22]. The crystallization of both the prefusion [23] and postfusion [24,25] structures of HA2 has brought tremendous insight into the large conformational changes involved in the fusion process. Biochemical and computational work has helped to fill in many details, including the role of HA1, the fusion peptide and possible intermediate states.

The global rearrangements of the trimeric HA1/HA2 complex and their hypothesized relations to the different steps of membrane

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