Recent insights into the structural characterization of herpes simplex virus fusion protein, gB

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“Using cryo-EM, we imaged vesicles expressing full-length gB bound to monovalent antibody fragments that do not possess an Fc region (Fabs) and to whole antibodies, along with gB containing genetically encoded FP insertions. Since the Fabs, antibodies and FPs were visible by cryo-EM, we used them as landmarks to map the position of gB domains in its prefusion conformation. According to our experimental data, we proposed that, initially, gB has the fusion loops pointing toward the viral membrane”

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The herpesviridae family replicate within a wide range of vertebrate hosts and one invertebrate host. The family includes over 100 viruses, of which eight cause human infections such as herpes simplex virus (HSV). HSV is a model system for the herpesvirus family and has two serotypes, HSV-1 and HSV-2, that globally infect approximately 90% of the population [1]. HSV inflicts lifelong infections by establishing latency in the host and undergoing periodic reactivations that cause the virus to spread. These infections normally manifest as mucocutaneous infections including keratitis, gingivostomatitis and genital warts. Furthermore, infection with HSV-2 increases the risk of acquiring and transmitting HIV [1]. Specific antivirals limit the impact of HSV but none cure infection. Coupled with the lack of a preventative vaccine, this virus will continue to afflict the population, making it a global health burden of high priority.

HSV has a linear DNA genome of approximately 152 kb packaged tightly in an icosahedral capsid, which is 15 nm thick and 125 nm in diameter. The capsid itself is encased in a matrix of 20 proteins (the tegument), that lies beneath a host-derived lipid envelope surrounded by 10–12 glycoproteins [2]. Therefore, the size and diverse complexity of HSV make structural studies extremely challenging.

As with all enveloped viruses, HSV infection begins with entry, a process that requires fusion of cellular and viral membranes. While the molecular details are still not known, all events are thought to follow the fusion-through-hemifusion pathway [3]. The basic principle posits that the fusion of two lipid membranes is thermodynamically favorable and that the high kinetic barrier is overcome when free energy is released as the fusion protein undergoes a series of conformational changes. These changes bring the membranes in close proximity, inducing membrane curvature, hemifusion (where only the outer leaflets are fused) and finally full fusion [4].

HSV membrane fusion is mediated by four glycoproteins: the primary receptor binding protein gD, a covalently linked heterodimer gH/gL and the fusion protein, gB. HSV fusion begins with the interaction of gD with a cellular receptor. This interaction induces a conformational change in gD, prompting gH/gL to activate gB. Successive rearrangements of gB, from its initial metastable prefusion conformation to the more energetically favored postfusion conformation, leads to membrane curvature and disruption of cellular membranes, resulting in viral capsid release into the host cell [5].

Several structures of HSV glycoproteins exist, including unliganded gD, gD in complex with its receptors [6] and a partially activated form of gH/gL [7]. However, only the postfusion structure of gB has been solved [8]. This

is because all purified forms of gB adopt the postfusion conformation, and attempts to change this have been unsuccessful [9]. This leaves an important gap in the knowledge of the HSV lifecycle.

HSV-1 gB is comprised of 904 residues and acts as a trimer in the postfusion conformation. Side views depict the protein as a three-lobed structure. The truncated postfusion structure identifies five domains, with the two fusion loops residing in domain I. Both domains I and V are at the 'base' of the protein, in close proximity to the viral membrane. Domain II, the central lobe, is postulated to mediate interactions with gH/gL and is connected to the trimeric coiled-coil domain III. Domain IV, the 'crown', resides at the top, tethered to domain II by domain III [10]. The N-terminus (residues 31–102, putatively domain VI) is not resolved in the crystal structure due to its flexibility. Amino acids 730–904, which are missing in the purified proteins used for crystallographic studies, include the cytoplasmic tail, the transmembrane domain and the membrane-proximal region, all of which are involved in virus fusion and infectivity.

Viral fusion proteins are categorized into three distinct classes: I, II and III. As a class III fusion protein, gB is composed of α -helices and β -sheets, and contains two fusion loops per protomer. Class III fusion proteins are found in herpesviruses, vesicular stomatitis virus (VSV) and baculovirus. The VSV fusion protein, G, is the best characterized class III fusion protein and its postfusion form shares features similar to gB [11,12]. Based on the structures of pre- and postfusion G, Gallagher *et al.* created an *in silico* model for prefusion gB [13,14]. To generate it, they proposed that gB's prefusion domain arrangements are similar to G in its prefusion conformation, and accordingly gB's fusion loops would point toward the viral membrane. Therefore, by analogy to G, during the transition from its pre- to postfusion conformation, the fusion loops would first relocate to the top of gB to interact with the target membrane. Further conformational changes would position the fusion loops of gB close to the transmembrane domains, leading to the merging of the cell and virus membranes. This model is supported by an in-depth structural study using fluorescent proteins (FP) to map gB's domains, which suggested that regions allowing insertion of the FPs are exposed [14].

A second model of prefusion gB was recently proposed by Zeev-Ben-Mordehai *et al.* [15]. This was generated using cryo-electron microscopy (cryo-EM) to image microvesicles expressing full-length gB. Cryo-EM allows imaging of specimens at atomic or molecular resolution in close-to-native conditions. gB expressed in microvesicles adopted two different conformations: an elongated postfusion form, and a compact form, putatively prefusion gB. They then calculated a 3D average of the compact form, fitting two postfusion domains of gB (domains I and II) into the average. Based on VSV G, and like Gallagher *et al.*, they assumed that the domains of gB are similar in the pre- and postfusion conformations. The resulting model suggests that gB's fusion loops (within domain I) point away from the viral membrane. Therefore, to produce fusion, gB would extend so that the fusion loops could reach the target membrane, and then conformational changes, similar to the ones proposed by Gallagher *et al.*, would merge the cell and virus membranes.

Recently, we augmented the microvesicle strategy [15] to produce gB in its prefusion form [16]. Using cryo-EM, we imaged vesicles expressing full-length gB bound to monovalent antibody fragments that do not possess an Fc region (Fabs) and to whole antibodies, along with gB containing genetically encoded FP insertions. Since the Fabs, antibodies and FPs were visible by cryo-EM, we used them as landmarks to map the position of gB domains in its prefusion conformation. According to our experimental data, we proposed that, initially, gB has the fusion loops pointing toward the viral membrane [16], thereby agreeing with the model proposed by Gallagher *et al.* Additionally, some samples trapped intermediate conformations of gB, providing insights about how the pre- to postfusion transitions could take place. Based on these intermediate conformations, we suggested that the fusion loops of gB, which initially point toward the viral membrane, are relocated to the top of the molecule as a second step in the fusion process, while gB maintains a compact conformation. This intermediate conformation would therefore be similar to the one proposed by Zeev-Ben-Mordehai *et al.*, reconciling the two models for two conformations of gB. More data will be needed to unequivocally unravel the prefusion structure of gB and its transition to the postfusion form, thereby elucidating the mechanism of fusion.

In conclusion, although the prefusion structure of HSV gB still poses a challenge to structural biologists, the advances in structural determination techniques and the ability to produce gB in conformations other than postfusion, are bringing us closer to the answer. This structure will help with rational drug design and vaccine development to tackle HSV infection.

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