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Vennard, LM, Atanasiu, D, Saw, WT et al. (3 more authors) (2018) Recent insights into the structural characterization of herpes simplex virus fusion protein, gB. Future Virology, 13 (1). pp. 5-7. ISSN 1746-0794

https://doi.org/10.2217/fvl-2017-0124

Recent insights into the structural characterisation of Herpes Simplex Virus fusion protein, gB

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Herpesviruses infect many vertebrate and at least one invertebrate hosts. They include over 100 viruses, of which eight cause human infections, with Herpes Simplex Virus (HSV) being one of them. 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. HSV inflicts lifelong infections by establishing latency in the host and undergoes periodic reactivations that can spread the virus. 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 152Kb packaged tightly in an icosahedral capsid, which is 15nm thick and 125nm in diameter. The capsid itself is encased in a matrix of 20 proteins (the tegument), that lies beneath a host derived lipid envelope decorated with 10-12 glycoproteins [2]. Therefore, the size and 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 close together, 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 pre-fusion conformation to the more energetically favoured post-fusion conformation, lead to membrane curvature and disruption of cellular membranes, resulting in viral capsid release into the host cell [5].

Several structures of gD exist , including unliganded gD and in complex with its receptors (reviewed in [6]) and for a partially activated form of gH/gL [7]. However, only the post-fusion structure of gB has been solved [8]. This is because all purified forms of gB adopt the post-fusion conformation, and attempts to change this have been unfruitful [9]. This leaves an important gap in the knowledge of the HSV lifecycle.

HSV-1 gB is comprised of 904 residues and is a trimer in the post-fusion conformation. Side views depict it as a three-lobed structure. The truncated post-fusion structure identifies five domains that place the two fusion loops 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 lob, 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 groups: 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 post-fusion form shares features similar to gB [11, 12]. Based on the structures of pre- and post-fusion G, Gallagher et al. created an in silico model for pre-fusion gB [13, 14]. To generate it, they proposed that gB’s pre-fusion domain arrangements are similar to G in its pre-fusion 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 post-fusion 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 pre-fusion 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 post-fusion form, and a compact form, putatively pre-fusion gB. They then calculated a 3D average of the compact form, fitting two post-fusion 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 post-fusion 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 pre-fusion 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 pre-fusion 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 post-fusion 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 pre-fusion structure of gB and its transition to the post fusion form, thereby elucidating the mechanism of fusion.

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

Acknowledgements

This work was supported by the University of Leeds (University Academic Fellow scheme) and the National Institutes of Health grant R01-AI-18289 (G.H.C.).

References

1. WHO. Herpes Simplex Virus. 2017 [cited 2017 17/01]; Available from: http://www.who.int/mediacentre/factsheets/fs400/en/.
2. Grunewald, K., et al., Three-dimensional structure of herpes simplex virus from cryo-electron tomography. Science, 2003. **302**(5649): p. 1396-8.
3. Chernomordik, L.V. and M.M. Kozlov, Mechanics of membrane fusion. Nat Struct Mol Biol, 2008. **15**(7): p. 675-83.
4. Harrison, S.C., Viral membrane fusion. Nat Struct Mol Biol, 2008. **15**(7): p. 690-8.
5. Eisenberg, R.J., et al., Herpes virus fusion and entry: a story with many characters. Viruses, 2012. **4**(5): p. 800-32.
6. Krummenacher, C., et al., Entry of herpesviruses into cells: the enigma variations. Adv Exp Med Biol, 2013. **790**: p. 178-95.
7. Chowdary, T.K., et al., Crystal structure of the conserved herpesvirus fusion regulator complex gH-gL. Nat Struct Mol Biol, 2010. **17**(7): p. 882-8.

8. Stampfer, S.D., et al., Structural basis of local, pH-dependent conformational changes in glycoprotein B from herpes simplex virus type 1. J Virol, 2010. **84**(24): p. 12924-33.
9. Vitu, E., et al., Extensive mutagenesis of the HSV-1 gB ectodomain reveals remarkable stability of its postfusion form. J Mol Biol, 2013. **425**(11): p. 2056-71.
10. Cooper, R.S. and E.E. Heldwein, Herpesvirus gB: A Finely Tuned Fusion Machine. Viruses, 2015. **7**(12): p. 6552-69.
11. Roche, S., et al., Structures of vesicular stomatitis virus glycoprotein: membrane fusion revisited. Cell Mol Life Sci, 2008. **65**(11): p. 1716-28.
12. Roche, S., et al., Structure of the prefusion form of the vesicular stomatitis virus glycoprotein G. Science, 2007. **315**(5813): p. 843-8.
13. Atanasiu, D., et al., Dual split protein-based fusion assay reveals that mutations to herpes simplex virus (HSV) glycoprotein gB alter the kinetics of cell-cell fusion induced by HSV entry glycoproteins. J Virol, 2013. **87**(21): p. 11332-45.
14. Gallagher, J.R., et al., Functional fluorescent protein insertions in herpes simplex virus gB report on gB conformation before and after execution of membrane fusion. PLoS Pathog, 2014. **10**(9): p. e1004373.
15. Zeev-Ben-Mordehai, T., et al., Two distinct trimeric conformations of natively membrane-anchored full-length herpes simplex virus 1 glycoprotein B. Proc Natl Acad Sci U S A, 2016. **113**(15): p. 4176-81.
16. Fontana, J., et al., The Fusion Loops of the Initial Prefusion Conformation of Herpes Simplex Virus 1 Fusion Protein Point Toward the Membrane. MBio, 2017. **8**(4).