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review

Molecular gymnastics at the herpesvirus surface

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This review analyses recent structural results that provide clues about a possible molecular mechanism for the transmission of a fusogenic signal among the envelope glycoproteins of the herpes simplex virus on receptor binding by glycoprotein gD. This signal triggers the membrane-fusion machinery of the virus—contained in glycoproteins gB, gH and gL—to induce the merging of viral and cellular membranes, and to allow virus entry into target cells. This activating process parallels that of γ-retroviruses, in which receptor binding by the amino-terminal domain of the envelope protein activates the fusogenic potential of the virion in a similar way, despite the different organization of the envelope complexes of these two types of viruses. Therefore, the new structural results on the interaction of gD with its receptors might also provide insights into the mechanism of fusogenic signal transmission in γ-retroviruses. Furthermore, the fusion activation parallels with retroviruses, together with the recently reported structural homology of gB with the rhabdovirus envelope glycoprotein indicate that the complex entry apparatus of herpesviruses appears to be functionally related to that of simpler enveloped viruses.

Keywords: membrane fusion; viral entry; virus–cell attachment

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Introduction

A key event in a virus infection is the delivery of the viral genome across the cellular lipid bilayer. Enveloped viruses accomplish this entry step by catalysing a membrane-fusion reaction in which the viral envelope merges with a cellular membrane, resulting in the release of the viral nucleocapsid or nucleoprotein core into the cytoplasm. Specific viral envelope proteins are responsible for the required interactions during entry, which comprise the initial attachment to cells and the subsequent catalysis of membrane fusion. The latter process occurs through the insertion of a fusion peptide—that is, a segment of the polypeptide chain of the fusion effector protein—into the membrane of the target cell during an important change in the protein conformation triggered by interactions with the host cell. In all viruses for which structural information is available, the fusion peptide is buried at an oligomeric interface of the fusion protein on the virion and becomes exposed during the fusogenic conformational change.

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Viral strategies for triggering membrane fusion

Enveloped viruses can be classified according to the strategy they use to induce fusion of the viral envelope with a cellular membrane. Some use direct interactions with cellular receptors to trigger their membrane-fusion machinery, whereas for others the triggering event is the binding of protons in the acidic *milieu* of the endosome. Viruses from the second category require endosomal uptake, which takes place after receptor recognition at the cell surface, so that the interaction with the receptor only indirectly participates in triggering the membrane-fusion process. For viruses in the first category, intracellular uptake is not always necessary, and in many cases the membrane-fusion process takes place directly at the plasma membrane. Many retroviruses—including human immunodeficiency virus 1 (HIV-1)—belong to this category. Their interactions with one or more cellular molecules in the target membrane alter the conformation of a viral receptor-binding protein, which in turn signals the activation of the fusogenic machinery of the virus. Herpesviruses also belong to this class, even though, for certain cells, herpes simplex virus 1 (HSV-1) entry requires endocytosis and low pH.

Cell-attachment and cell-entry receptors

The attachment of virus particles to the cell surface—for example, by binding to glycosaminoglycans—and their specific interaction with a cellular-entry receptor are often two separate processes. The presence of a soluble receptor (that is, only the ectodomain or part of the ectodomain without a membrane anchor) has been reported to allow the entry of several viruses into cells that lack the entry receptor at their surface. In this case, the viral particles must bind to the target cell through alternative attachment factors. Examples are the mouse hepatitis coronavirus (Taguchi & Matsuyama, 2002), and the avian sarcoma and leucosis virus (a subgroup A retrovirus; Damico & Bates, 2000), which are able to infect cells devoid of a surface-entry receptor provided a soluble version is present.

Soluble viral receptor-binding domains rescue infectivity

An interesting situation—partly mirroring that of soluble receptors rescuing the infectivity of cells devoid of the entry receptor—occurs when soluble viral receptor-binding domains (RBDs) allow the entry of viruses lacking the receptor-binding protein. For instance, the soluble gD ectodomain has been shown to allow the entry of engineered

HSV-1 virus particles that lack gD (that is, gD-null mutants; Cocchi *et al*, 2004). For the γ-retroviruses, soluble RBDs that are specific for a receptor present in the target cells can allow the infection of a virus for which the endogenous RBD is defective or is specific for an absent receptor (Lavillette *et al*, 2000). Some important common features of these two systems are noted in the subsequent sections of this review.

Envelope proteins of herpesviruses and retroviruses

The members of the Herpesviridae family are among the most complex enveloped viruses that infect humans. The α-Herpesvirinae subfamily contains HSV-1 and HSV-2, the varicella-zoster virus (VZV), the porcine pseudorabies virus (PrV) and several other veterinary-relevant herpesviruses. The human cytomegalovirus (CMV) and the Epstein–Barr virus (EBV) are members of the β-Herpesvirinae and γ-Herpesvirinae subfamilies, respectively. All of the above—except for PrV—are important human pathogens that are responsible for persistent infections in the majority of the population. Each of these viruses carries about a dozen different envelope glycoproteins on its surface. Three of the glycoproteins essential for entry (gB, gH and gL) are conserved throughout the herpesvirus family. By contrast, gD is restricted to HSV-1, HSV-2, PrV and other α-herpesviruses. It is absent from VZV and from the members of the β- and γ-Herpesvirinae subfamilies.

HSV-1 is the best-studied member of the family. Four of its surface glycoproteins—namely, gB, gD, gH and gL—are necessary and sufficient for cell entry (Turner *et al*, 1998), at least in tissue culture, and induce fusion of the viral envelope with the membrane of the target cell. This complexity is in contrast to most enveloped viruses, which often carry only one or a few envelope proteins on their surface (Kielian & Rey, 2006). Retroviruses, for instance, encode a single envelope glycoprotein precursor, which is a type I transmembrane protein with a bulky amino-terminal ectodomain and a small carboxy-terminal cytoplasmic tail. This precursor is then proteolytically processed into a surface (SU) and a transmembrane (TM) subunit (such as gp120 and gp41, respectively, in HIV-1). In the case of retroviruses, SU—which is only peripherally attached to the virion—carries the receptor (and co-receptor) binding function. The transmembrane subunit is the effector protein responsible for triggering membrane fusion after a conformational change induced by the binding of SU to a receptor. In the case of herpesviruses, the identity of the fusion effector glycoprotein is not known. The candidates for driving membrane fusion—that is, the effector protein containing the fusion peptide—are gB and/or the gH/gL heterodimer.

Possible herpesvirus membrane-fusion effectors

The HSV-1 glycoproteins gC (which is not essential for entry) and gB both bind to heparan sulphate for attachment to cells. The ability of soluble gD to rescue the infectivity of gD-null viruses shows that it is not necessary for attachment, but that its presence in the receptor-bound conformation is essential for entry. As discussed below, the latter conformation is able to interact with the fusogenic machinery of the virus—that is, the remaining three glycoproteins—for triggering fusion. No specific interactions between gD, gB and gH/gL have been identified at the surfaces of either the viruses or the infected cells. However, a recent report described the characterization of a specific transient glycoprotein complex that forms on gD binding to the receptor, in which the gD–receptor complex recruits first gB and then gH/gL to generate a fusogenic complex (Gianni *et al*, 2006b).

The notable syncytial phenotypes conferred by certain mutations in HSV-1 gB imply that this protein is probably a fusion effector (Foster *et al*, 2001). Moreover, the recently reported crystal structure of HSV-1 gB (Heldwein *et al*, 2006) revealed an unexpected structural homology to the envelope glycoprotein of the vesicular stomatitis virus (VSV; Roche *et al*, 2006). The fusogenic properties of this glycoprotein are well established, and its structure displays features of both class I and class II membrane-fusion proteins. These data allow the identification of a putative fusion peptide in gB and suggest that gB may also undergo a triggered conformational change to induce fusion. However, the homologs of the gH/gL heterodimer of CMV (Kinzler & Compton, 2005) and VZV (Cole & Grose, 2003) have been reported to induce syncytia formation when transfected into certain cells, in the absence of any other viral protein. Indeed, although no three-dimensional crystal structure of gH/gL from HSV-1 (or any of its homologues from other herpesviruses) is available, gH has been reported to have certain features that are characteristic of class I fusion proteins (Gianni *et al*, 2005a,b), such as the presence of heptad repeats and a putative fusion peptide region. Moreover, recent data have shown that peptides spanning the heptad-repeat region of gH inhibit the entry of CMV (Lopper & Compton, 2004) and HSV-1 (Galdiero *et al*, 2006; Gianni *et al*, 2006a) into cells, similar to the corresponding entry-inhibitors identified for viruses that have a class I membrane-fusion protein. Therefore, it seems that gB and gH/gL both have properties of membrane-fusion effectors, and further experiments will be necessary to understand their role. Leaving aside the issue of the identity of the fusion effector, the following sections of this review concentrate on the activation of the fusion machinery of HSV-1, discussing the implications derived from the different crystal structures of gD alone and in complex with a receptor.

HSV-1 gD and its interactions with receptors

An important challenge in contemporary virology is to understand the mechanism of activation of the membrane fusion process upon virus/receptor interactions, and herpesviruses appear to be a good model. HSV1 gD is 369 amino acids (aa) long, with the predicted transmembrane segment lying between aa 317 and 339 and a C-terminal cytoplasmic tail of 30 residues. The ectodomain has a short N-terminal proline-rich region (PRR) between aa 45 and 54, and a long PRR between aa 244 and 311 at the membrane-proximal region. Productive infection by HSV-1 requires the presence of one of three alternative gD receptors (reviewed by Spear, 2004). The first is the herpesvirus entry-mediator molecule (HVEM), which is a member of the tumour necrosis factor-receptor family. The second is nectin 1, which is a cell-adhesion molecule and a member of the immunoglobulin superfamily—interestingly, soluble nectin 1 has recently been shown to enable the entry of HSV-1 into cells that are resistant to infection (Kwon *et al*, 2006), in keeping with the properties discussed above for other viruses. The third gD receptor molecule is a specifically modified heparan sulphate that is present at the surface of some cells.

Crystal structures of the gD ectodomain

One of the puzzles concerning the interactions of gD with receptors is that recombinant gD molecules spanning almost the entire ectodomain (such as the gD306t construct truncated at residue 306) have an affinity for their protein receptor (HVEM or nectin 1) that is similar to that of the solubilized full-length gD. By contrast,

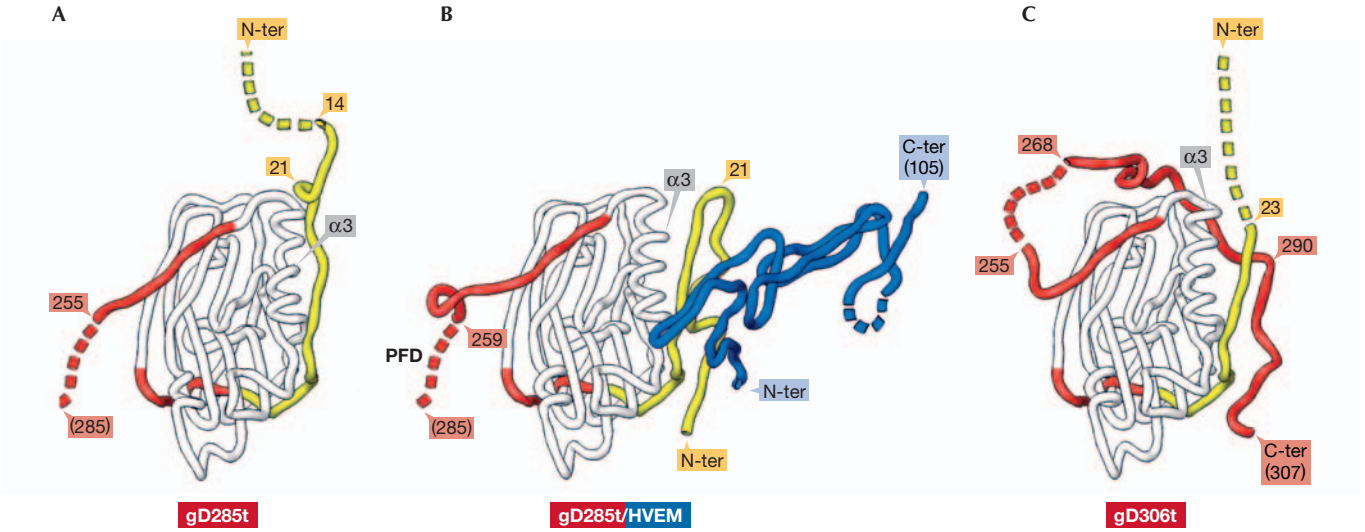

Fig 1 | Ribbon diagrams of different forms of the gD ectodomain and its complex with the herpesvirus entry-mediator molecule. The immunoglobulin-variable domain (IgV)/α3 'core' is depicted in white, the two proline-rich regions (PRRs) are shown in red and the amino-terminal arm that binds to the herpesvirus entry-mediator molecule (HVEM) is depicted in yellow. Disordered regions of the structure are shown as broken tubes. Some key gD amino acids are labelled. (**A**) Structure of the unliganded gD285t. (**B**) Structure of gD285t in complex with HVEM (shown in blue). The region roughly corresponding to the putative pro-fusion domain is labelled. (**C**) Structure of the full ectodomain gD306–Cys 307. The putative pro-fusion domain is constrained by the interaction between segments 290–306 and 23–45. The latter segment is engaged by the very N-terminal end (aa 1–21) on binding by HVEM (**B**) so that the carboxy-terminal segment becomes exposed. C-ter, carboxy terminus; N-ter, amino terminus; PFD, putative pro-fusion domain.

shorter constructs (such as gD285t) bind to the receptor with an affinity that is 100-fold higher (Rux *et al*, 1998; Whitbeck *et al*, 1997; Willis *et al*, 1998). A few years ago, Carfi and colleagues reported the crystal structure of gD285t, both alone and in complex with a soluble version of HVEM (Fig 1A,B; Carfi *et al*, 2001). The structure showed that gD is folded as an immunoglobulin-variable domain (IgV), with N-terminal and C-terminal extensions. The IgV domain comprises aa 55–185, and the only other important secondary structure element is an 18-aa α-helix (α3) that ends at aa 237. This helix packs against the IgV, resulting in a compact domain (Fig 1) flanked by the two PRRs, upstream and downstream. In both structures, the 30 or so C-terminal residues are disordered. HVEM makes physical contact only with residues upstream of the N-terminal PRR (Fig 1), inducing aa 1–33 to adopt a hairpin conformation that packs against helix α3 on one side and HVEM on the other. This region has an extended conformation in the unliganded form, with aa 1–14 being disordered. Receptor binding therefore involves a disorder-to-order transition, in an elegant example of a natively unfolded segment that folds only in the presence of a physiological ligand. However, despite the fact that only the N-terminal segment seems to be involved in binding HVEM, truncated forms of gD containing less than the first 250 aa of the ectodomain cannot interact with it; this shows that the IgV/α3 scaffold is important for presenting the N-terminal segment in such a way that the 'receptor-bound' conformation—a hairpin sandwiched between HVEM and helix α3—can be reached.

Full-length gD forms dimers at the virus surface

The low affinity of gD306t for HVEM compared with shorter constructs led to speculations that the C-terminal end might engage the disordered N terminus in some sort of interaction, making it more difficult for the receptor to bind. These alternative interactions in the gD ectodomain might hold the clue to its role in activating fusion through a conformational change on receptor binding. Furthermore, several functional studies have pointed to an important role for the C-terminal PRR—particularly the gD segment downstream from aa 260, which is known as a putative pro-fusion domain—in the membrane-fusion process (Cocchi *et al*, 2004; Fusco *et al*, 2005; Zago *et al*, 2004). It therefore became apparent that a key issue was to understand the details of the conformation of the full gD ectodomain, although efforts to crystallize the gD306t form failed because of its apparently unstable structure. However, in a recent breakthrough, the Carfi and Cohen/Eisenberg laboratories crystallized two different forms of gD containing the 306 N-terminal residues of the ectodomain (Krummenacher *et al*, 2005). Because previous crosslinking experiments on virus particles indicated that membrane-anchored gD is dimeric (Handler *et al*, 1996), whereas the soluble ectodomain is monomeric, the authors reasoned that the presence of the transmembrane segment might stabilize the dimeric conformation. In this case, the dimers would have to be parallel, with the transmembrane segments in the membrane, such that the C-terminal ends of the ectodomains of the two subunits are likely to be located nearby. The key to obtaining a crystallizable form was adding a cysteine residue after the gD306t C terminus, at position 307, which indeed formed an intersubunit disulphide bond stabilizing the dimer. Furthermore, the gD306t–Cys 307 dimer was recognized by the conformation-sensitive monoclonal antibody AP7, which targets a composite epitope formed by the N-terminal and C-terminal ends of gD (Chiang *et al*, 1994; Minson *et al*, 1986). This indicated that the authors had trapped the gD ectodomain in a physiologically relevant conformation.

The gD306t–Cys 307 crystals allowed the determination of the three-dimensional structure of this dimeric form. The segment from aa 255–268, connecting the N-terminal domain with the C-terminal end, was disordered; however, the polypeptide chain running from aa 268–306 in the C-terminal PRR was clearly resolved in the new structure, running along the surface of the IgV/α3 scaffold, with the last 20 aa at the C-terminal end making a roughly parallel interaction with aa 23–32 (Fig 1C). The C-terminal end of the ectodomain therefore occupies the same location as the first 16 N-terminal gD aa in the structure of the complex with HVEM, in a conformation that seems to be stabilized by dimer contacts. In this dimeric conformation, the 22 N-terminal aa are disordered. Therefore, the C-terminal end does not engage the N terminus of gD, but instead blocks a space that has to be occupied by the N terminus on binding to HVEM, thereby maintaining this segment buried at the gD dimer interface. The resulting picture of the gD transition from the unbound dimeric form to the receptor-bound monomeric form, which is illustrated schematically in Fig 2, indicates that receptor binding brings in the disordered N terminus and displaces the C-terminal region, allowing it to interact with the fusion machinery. Because gD285t (similar to gD306t) can allow the entry of HSV-1 particles lacking a functional gD, whereas gD260t cannot (Cocchi *et al*, 2004), it is believed that the putative pro-fusion domain mentioned above—or, most likely, an important element of it—is located between aa 260 and 285 (that is, in the region immediately preceding the segment observed in interaction with the N-terminal end). Similarly, binding to nectin 1 involves some of the residues occluded by the C-terminal segment in the dimeric gD structure, implying that the interaction with this alternative receptor will also displace it from its buried location in the closed form of gD.

Important residues in the closed conformation of gD

The crystal structure of the gD306t–Cys 307 dimer identifies two key aa, Pro 291 and Trp 294, which lie in a pocket lined by aa 23–27 and seem to be responsible for locking the molecule in the observed closed conformation. A mutation of Trp 294 to Ala in the context of the full-length gD or gD306t results in a molecule that binds to receptor with high affinity—comparable with gD285t—indicating that the mutant is in the open form and highlighting the role of Trp 294 in stabilizing the closed conformation. Furthermore, a mutant in which an internal disulphide bridge was introduced to covalently lock the ectodomain in its closed conformation—by simultaneously mutating positions 37 and 302 (based on the three-dimensional structure) to cysteine—resulted in a molecule that was unable to bind either receptor, but which was recognized by the conformation-sensitive antibody AP7. Krummenacher and colleagues also crystallized this 'closed' mutant, and found that it is monomeric, thereby showing that the intra-subunit disulphide bond stabilizes the closed conformation in the absence of dimeric interactions. Although the engineered disulphide bond affects the local environment around aa 302, the overall conformation is otherwise identical to that of the gD protomer in the covalent dimer, further supporting the conclusions derived from the crystal of the gD306t–Cys 307 dimer.

Parallels with γ-retroviruses

The information provided by the structure of the gD ectodomain in its closed conformation is in agreement with the wealth of functional data (Cocchi *et al*, 2004; Fusco *et al*, 2005; Zago *et al*, 2004) available on the role of the C-terminal PRR in activating the HSV-1

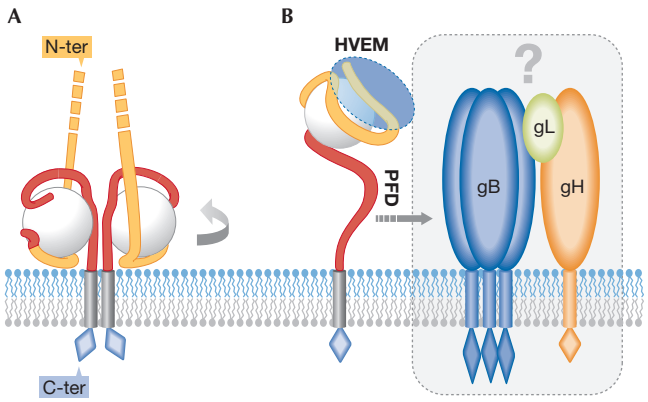

Fig 2 | Schematic diagram illustrating the activation of the membrane-fusion machinery of herpes simplex virus 1 on gD interactions with receptors. The gD immunoglobulin-variable domain (IgV)/α3 core is represented by a white sphere, the amino-terminal arms are depicted in yellow and the proline-rich regions are depicted in red (as in Fig 1). The viral lipid bilayer is drawn with blue lipids facing the external side, and with grey lipids comprising the inner leaflet. The transmembrane helices of the viral proteins are drawn as cylinders (grey for gD) and the cytoplasmic tail is represented by a diamond shape. (**A**) gD is drawn in its putative dimeric conformation at the virus surface (extrapolated from the structure of the Cys 307 dimer, with the disulphide linking the two protomers in the region immediately preceding the transmembrane helices; see the main text), with the twofold axis vertical in the plane of the membrane. In this conformation, the N-terminal end is able to interact with the receptor, and the pro-fusion domain is involved in dimer interactions, as well as with the region spanning amino acids 24–45 (Fig 1). (**B**) The interaction with the receptor (herpes virus entry-mediator molecule (HVEM) in this case) is proposed to disrupt the dimer interface and induce the flipping of the gD core away from the viral membrane, resulting in exposure of the putative pro-fusion domain to activate the HSV-1-fusion machinery. gB and the gH/gL heterodimer are depicted inside broken lines with a question mark, to indicate that the stoichiometry and the gD interaction site are not known. C-ter, carboxy terminus; N-ter, amino terminus; PFD, putative pro-fusion domain.

membrane-fusion machinery. There is an interesting parallel here with γ-retroviruses, including the murine leukaemia viruses Friend and Moloney (Fr-MLV and Mo-MLV, respectively), which have a class I fusion protein in the form of an SU/TM hetero-hexameric complex that is proteolytically derived from a trimeric envelope precursor, as in all retroviruses. Indeed, the crystal structure of the extraviral region of TM from Mo-MLV in its post-fusion conformation was determined a decade ago (Fass *et al*, 1996), and showed a typical class I coiled-coil-stabilized trimeric conformation. The RBD is located at the N terminus of SU, separated by a PRR from the C-terminal domain (Lavillette *et al*, 1998). Receptor binding by the RBD transmits a signal to the rest of the protein, triggering the fusogenic conformational change. The PRR apparently acts as an adaptor between the variable RBD and the conserved C-terminal domain of SU, which in turn interacts with the conserved transmembrane subunit. Swapping the PRR from different viruses results in high syncytium-formation phenotypes, which at the same time give rise to non-infectious virus particles. This implies that the exogenous PRR cannot prevent the fusogenic conformational

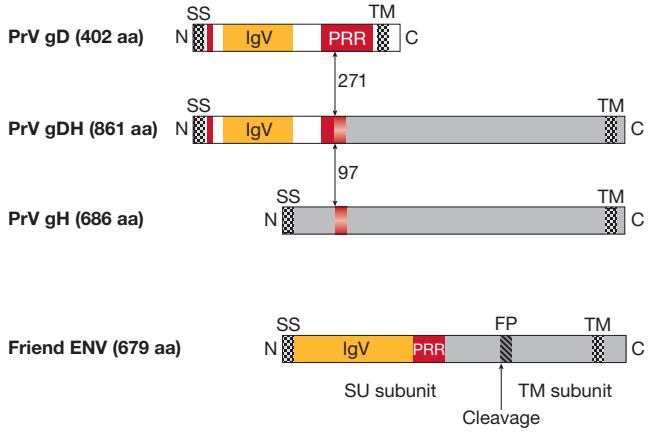

Fig 3 | Schematic diagram of the organization of envelope proteins gD, gH and gD/gH of the porcine pseudorabies virus compared with that of the envelope protein of the murine leukaemia virus Friend. The immunoglobulin-variable domain (IgV)/α3 core is depicted in yellow, the proline-rich regions (PRRs) are depicted in red, and the signal sequence (SS) and transmembrane (TM) segments are cross-hatched. The fusion peptide at the amino-terminal end of the transmembrane subunit of Fr-MLV is diagonally striped. aa, amino acids; C, carboxy terminus; ENV, single envelope glycoprotein precursor; Fr-MLV, Friend murine leukaemia virus; PRV, porcine pseudorabies virus; N, amino terminus.

change from taking place too soon (before budding), such that the particles incorporate envelope proteins that are already inactivated (Lavillette *et al*, 1998).

The structure of the RBD of Fr-MLV (Fass *et al*, 1997) showed that, similar to the gD N-terminal part, it is folded as an IgV domain, with long insertions in some of the loops between strands. The IgV is directly followed in sequence by a PRR, similar to the C-terminal PRR of gD. The residues in the IgV scaffold are conserved, but the loops are variable among viruses and provide the receptor specificity. The N terminus of the RBD contains a strictly conserved Ser-Pro-His-Gln motif. Deletion of the His residue results in a mutant for which receptor binding does not trigger membrane fusion (Barnett & Cunningham, 2001; Barnett *et al*, 2001; Lavillette *et al*, 2000, 2001). Interestingly, the addition of soluble RBD containing the intact His motif rescues infectivity, allowing membrane fusion to proceed. The available experimental evidence indicates that receptor binding promotes an interaction between RBD and another region in the SU–transmembrane complex, which triggers the fusogenic conformational change (Barnett & Cunningham, 2001; Barnett *et a*l, 2001; Lavillette *et al*, 2001). Therefore, receptor binding by the RBD triggers the fusion machinery of Fr-MLV in a way that is conceptually similar to the action of gD in HSV-1. In both cases, there is no need for these activating molecules to be attached to the virus particle. In the structure of the Fr-MLV RBD, the N-terminal end is located close to the C terminus, indicating that in the full-length molecule the His motif can be in physical contact with the PRR, which is immediately downstream. The results described by Krummenacher *et al* (2005) therefore seem to provide a new model for γ-retroviruses. The scenario would be more similar to that of the nectin 1 receptor, in which the receptor displaces a region of the PRR such that the signal is transmitted downstream in the polypeptide chain, as there are no N-terminal extensions that would be used by an HVEM-type interaction. Conversely, the parallel with MLV hints at a possible role for residues other than those in the putative pro-fusion domain, located in the more compact region of gD, similar to that of the conserved N-terminal histidine of γ-retroviruses.

A gD–gH fusion protein in the porcine herpes virus

A further parallel between the two viruses came from studies in which a revertant virus—rescuing the entry default of an engineered gL-null PrV mutant—was reported (Klupp & Mettenleiter, 1999). Interestingly, the revertant virus had acquired a fusion protein in which the PrV-gD ectodomain was fused to the N-terminal region of PrV-gH. As indicated in Fig 3, this mutant connected aa 271 at the beginning of the C-terminal PRR of PrV gD to a PRR present in PrV-gH. Indeed, aa 97, in which the polypeptide fusion takes place, is the first in a row of proline residues (97-ProProPro-99) in PrV-gH, in a region that has many additional proline residues. This gD–gH fusion protein was able to interact with PrV-gB for entry, substituting for gD, gH and gL. As shown in Fig 3, this configuration is similar to that of the γ-retrovirus envelope protein, with an RBD (PrV-gD271t) connected through the PRR to the 'fusion' protein (PrV-gH). Furthermore, in the case of bovine herpesvirus (BHV), which is similar to HSV-1 and HSV-2, a mutation compensating for a BHV gD-null virus defect in cell-to-cell spread was also found to map to BHV-gH (Schroder *et al*, 1997), indicating a possible interplay between the two proteins during entry, as would be expected from a γ-retrovirus model (Fig 1).

The parallel with γ-retroviruses is a further clue linking gH to class I viral membrane-fusion proteins. However, it does not necessarily mean that the effector protein is gH, as the activation can equally well take place *in trans*, with the RBD activating gB from its location at the N terminus of gH. Interestingly, the fact that in herpesviruses the RBD is located in a different protein, gD, suggests that in the γ-retroviruses, the RBD could also act *in trans*, by activating a neighbouring envelope trimer instead of the trimer to which it belongs.

Conclusions

The structure of the HSV-1 gD ectodomain in its closed conformation provides important insights into the process of activation of the viral fusogenic machinery on receptor binding. Although the details will certainly differ, it seems that, conceptually, γ-retroviruses and herpesviruses have overall mechanistic similarities involving the transmission of a signal generated by receptor binding, through a PRR, to the fusion machinery. All of the available data strongly suggest that, despite the involvement of four glycoproteins in an altogether different system, the herpesvirus fusion proteins are related to the envelope proteins of simpler viruses. The clear structural homology of gB to the VSV glycoprotein is one indication, and the fusion activation parallel with viruses bearing class I fusion proteins is another, possibly linking gH to the latter. Further studies are necessary to identify the precise role of each of the members of the conserved triad of glycoproteins involved in membrane fusion by herpesviruses. In addition, future work should aim to identify whether the use of alternative entry routes to infect different cells implies the activity of one or another of the components, even though the simultaneous presence of four glycoproteins is necessary in the case of HSV-1.

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