Characterization of Herpes Simplex Virus 1 UL51 Self-Interaction

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CHARACTERIZATION OF HERPES SIMPLEX VIRUS 1 UL51 SELF-INTERACTION

by

Samantha Ryken

A thesis submitted in partial fulfillment of the requirements for graduation with Honors in the Microbiology

Richard Roller
Thesis Mentor

Fall 2017

All requirements for graduation with Honors in the Microbiology have been completed.

Linda McCarter
Microbiology Honors Advisor

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Characterization of Herpes Simplex Virus 1 Ul51 Protein Self-Interaction

By Samantha Ella Ryken

Honors Thesis Supervisor:
Dr. Richard Roller

Fall 2017
Abstract

Following reactivation from latency herpesviruses utilize the conserved ability of cell-to-cell spread to evade the host immune response, mediating disease and viral shedding. The UL51 protein of herpes simplex virus 1 is a conserved tegument protein that functions in cytoplasmic assembly and secondary envelopment. Partial deletions of UL51 are associated with strong cell-to-cell spread defects. UL51 has no known enzymatic ability suggesting its function in cell-to-cell spread is likely dependent on specific interactions with other proteins. It has been shown to form complexes with UL7, the gE/gI complex and itself. However, it is unknown whether the self-interaction is a direct interaction or an interaction mediated by another component. A co-immunoprecipitation assay using plasmid expression of differentially tagged UL51 proteins demonstrates that the UL51 protein does not require other viral proteins for self-interaction. Using UL51 truncations coinciding with regions of conservation we began mapping regions important for self-interaction through co-immunoprecipitation and co-localization assays. We expect that determining the mechanism of interaction that UL51 has with itself will lead to the development of ways to determine the significance of the self-interactions for cell-to-cell spread, viral assembly or both.
Introduction

Herpes simplex virus 1 is a large double stranded DNA virus that can latently infect sensory neurons following primary epithelial infection. The virus can then reactivate causing reoccurring symptomatic disease and viral shedding. Herpes simplex virus is ubiquitous; most of the adult population has antibodies to either HSV-1 or HSV-2 [2]. Symptomatic disease caused by HSV-1 most commonly manifests as ulcerative lesions on the epithelia of the lip or genital area. More severe infection can occur in neonates following vertical transmission from mother to child and in immunocompromised individuals [3]. While more rare, herpesviral encephalitis can result in death and herpesviral keratitis is the leading cause of infectious blindness in developed countries [4] [5]. HSV-1 is one of eight human herpesviruses that cause varying levels of disease in both immunocompetent and immunocompromised individuals, all of which share the ability to establish and reactivate from latency. Studying the conserved processes and gene products associated with reactivation in HSV-1 can lead to further understanding of all herpesviruses.

For symptomatic disease to reoccur, HSV-1 must travel from sensory neurons to epithelial cells and between epithelial cells following reactivation from latency [6]. This requires a way for the virus to evade the adaptive immune response mounted during primary infection in order to avoid neutralization. One way that herpesviruses do this is through a conserved process called cell to cell spread. After assembly and envelopment mature virions are trafficked to junctional surfaces of cells where they are released. In this space, antibodies and effector cells of the immune system are unable to inactivate viruses due to steric protection of the cell junction [7-9] Through this immune evasion process HSV-1 can travel from neural ganglia to epithelial cells to cause disease and viral shedding. Cell to cell spread is a conserved ability of all herpesviruses that is necessary for symptomatic reactivation of disease, but details of this process
remain unknown [6, 10-16]. Understanding this process and the conserved viral genes associated with it can lead to the development of ways to inhibit this ability. Novel therapeutics could be developed to stop spread thus inhibiting recurrent disease. Engineering a virus unable to spread from a site of latency may be a viable candidate for the development of a live attenuated vaccine.

Cell to cell spread is considered the final step in the viral assembly and egress pathway. All herpesviruses egress by similar mechanisms of reconstructing host membranes [17]. Capsids are assembled in the nucleus but are too large to be exported through the nuclear pore. In order to exit the nucleus they bud through the nuclear membrane into the perinuclear space and de-envelope at the outer nuclear membrane [17]. The capsids then undergo cytoplasmic assembly where tegument proteins assemble on the capsid and on the membrane of the trans golgi network. The capsids then bud into the trans golgi network in a process known as secondary envelopment where they acquire the final envelope. The mature virion can then be trafficked to the cell membrane for extracellular release or to cellular junctions for cell to cell spread (Figure 1).

Several gene products are involved in HSV-1 cell to cell spread and can be classified into two categories—those required for viral entry into the cell and those required for trafficking viral components and virions to the cell junctions for spread. In the second category, which is the focus

Figure 1. Schematic diagram of HSV-1 egress pathway. Assembled capsids are represented as gray hexagons.
of this project, several gene products have been found to be important. The HSV-1 glycoprotein gE forms a complex with another glycoprotein gI that localizes at cell junctions and is required for efficient cell to cell spread [18]. A complex of UL11, UL16 and UL21 must form on the cytoplasmic tail of gE in order for it to be correctly trafficked to these junctions and function in cell to cell spread [19]. Another conserved gene product, UL7, is a tegument protein involved in virion formation and egress [20]. UL7 forms a complex with another conserved protein, UL51 [21]. UL51 is a conserved tegument protein that has cell type specific functions in viral growth and cell to cell spread [22].

This project focuses on UL51, a 30 kDa palmitoylated tegument protein that associates with Golgi membranes [23]. Strong cell to cell spread defects are associated with partial deletions of UL51 [22, 24]. The protein has no known enzymatic activity and no homology to any enzymes, therefore, UL51 function is likely dependent on interactions with other viral and cellular factors. It is a membrane associated protein making it ideally located for interactions in both secondary envelopment and trafficking of vesicles following packaging and the envelopment compartment (Figure 2). UL51 interacts with the gE/gI complex which is likely important in trafficking of gE to cell junctions during cell to cell spread [22]. However, a deletion of UL51 causes a more severe cell to cell spread defect than gE suggesting that UL51 has other functions in cell to cell spread.

Figure 2. Schematic diagram of hypothetical UL51 function in secondary envelopment. Capsids are denoted by gray hexagons.
that may involve other interactions [22]. UL51 also interacts with UL7 which is necessary for the recruitment of UL7 into the virion during cytoplasmic assembly [21].

UL51 has been observed to interact with itself in virally infected cells, but whether this interaction is directly between two UL51 proteins or facilitated by another viral or cellular factor is unknown [R. Roller unpublished]. The human cytomegalovirus protein UL71, a homolog of UL51, is an important mediator in secondary envelopment and trafficking [25]. UL71 forms a homodimer in a direct interaction that is necessary for secondary envelopment. This interaction is facilitated by a leucine zipper like domain [26]. This provides further evidence that UL51 interacts with itself during secondary envelopment, but since UL51 does not contain a leucine zipper like domain it would do so by a different mechanism.

This evidence suggests that UL51 interacts with itself in the absence of other viral proteins. To test this, we established a co-immunoprecipitation assay using differentially tagged UL51 proteins expressed by plasmids. We then began mapping regions important for this interaction using UL51 truncations that coincided with regions of UL51 amino acid conservation. Once the UL51 self-interaction mechanism is determined we can then determine if this self-interaction is important for cell to cell spread, viral assembly or both.
Materials and Methods

Cell Lines

293T cells were maintained in Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum. Vero cells were maintained as previously described [27].

Plasmid Construction

The plasmid encoding for UL51-HA was constructed by PCR amplification, restriction enzyme digestion and ligation into pcDNA3 UL51-FLAG. The UL51-HA coding sequence was amplified from a BAC containing the HSV-1 strain F genome with an HA tagged UL51. The PCR product was cut with restriction enzymes HincII, BsrGI and Xbal. The restriction enzyme digest product was then ligated into pcDNA3 encoding UL51-FLAG at the BsrGI site in UL51 and XbaI site in pcDNA3.

Gibson assembly was used for the creation of plasmids encoding for 1-244 gD-UL51-FLAG, 1-167 gD-UL51-FLAG and 1-125 gD-UL51-FLAG. A previously constructed pcDNA3

<table>
<thead>
<tr>
<th>Description</th>
<th>Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pcDNA3 UL51-HA</td>
<td></td>
</tr>
<tr>
<td>ULS1 HA forward</td>
<td>ATGCGGTCTTCTTTCTCGGAGGTATATG</td>
</tr>
<tr>
<td>ULS1 HA reverse</td>
<td>TTAAACCTAAATCTGGAGCATCTGATATG</td>
</tr>
<tr>
<td>pcDNA3 gD-UL51-FLAG</td>
<td></td>
</tr>
<tr>
<td>UL51-FLAG forward</td>
<td>GTCCTCGCACCAGCCCTTTTTTACATG</td>
</tr>
<tr>
<td>1-244 UL51-FLAG reverse</td>
<td>ACTTATCGTACGTCTTTTTGTAGTCTTT</td>
</tr>
<tr>
<td>1-166 UL51-FLAG reverse</td>
<td>ACTTATCGTACGTCTTTTTGTAGTGCA</td>
</tr>
<tr>
<td>1-124 UL51-FLAG reverse</td>
<td>ACTTATCGTACGTCTTTTTGTAGCCAT</td>
</tr>
<tr>
<td>pcDNA3 gD-FLAG vector 1 forward</td>
<td>GACTAAAGGAACGATACGAATAGT</td>
</tr>
<tr>
<td></td>
<td>GACTCG</td>
</tr>
<tr>
<td>pcDNA3 gD-FLAG vector 1 reverse</td>
<td>GTAACAAGGCTGGCTGGTGGCGAGG</td>
</tr>
<tr>
<td>pcDNA3 gD-FLAG vector 2 forward</td>
<td>GCTTCTGCTCTCCGATCTGTGTT</td>
</tr>
<tr>
<td></td>
<td>CAGAAGTAATTGGCGCCAGGT</td>
</tr>
<tr>
<td>pcDNA3 gD-FLAG vector 2 reverse</td>
<td>CGATCGAGGACGAGGAGG</td>
</tr>
</tbody>
</table>

Table 1. Oligonucleotide primers used in plasmid construction.
encoding gD-FLAG served as a template for the two vector fragments. A previously constructed UL51-FLAG pcDNA3 was used as the template for the insert fragment. Equimolar amounts of each fragment were joined using Gibson assembly master mix (New England Biolabs) according to the manufacturer’s protocol. Primers used for amplification of all vector fragments and UL51 insert fragments are described Table 1. Plasmids encoding for UL51-FLAG, 1-167 UL51-FLAG, 1-125 UL51-FLAG, 1-90 UL51-FLAG, 91-244 UL51-FLAG, 125-244 UL51-FLAG and 167-244 UL51-FLAG were previously constructed (R. Roller unpublished).

**Immunopurification**

293T cells were seeded into 60 mm tissue culture dishes and transfected using polyethylenimine (PEI). The amount of PEI used was based on a 3:1 DNA (µg) to PEI (µg) ratio. 48-hours post transfection, cells were washed with phosphate buffered saline, scraped into 1 mL of PBS and pelleted at 2000 rpm for 2 minutes. Cells were resuspended and lysed in 500 µl of PI RIPA buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X 100, 5 mM Na Vanadate, 5 mM NaF). The cell lysate was then sonicated for 10 seconds and cell debris was pelleted by centrifugation at 14,000 rpm for 2 minutes. Following removal of 20 µl of the resulting supernatant as a lysate control, 8 µl of α-FLAG magnetic beads suspended in PI RIPA buffer were added to the remaining supernatant. Samples were incubated overnight at 4°C on an end-over-end rotator. Using a magnetic separator, magnetic beads were washed 4 times in 500 µl PI RIPA buffer. Bound protein was eluted on a magnetic separator 3 times in 20 µl elution buffer containing 167 µg/mL 3X FLAG peptide (Sigma).
**SDS-PAGE and Immunoblotting**

Lysate samples and purified protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrically transferred to nitrocellulose by standard methods [28]. Membranes were blocked in 5% nonfat milk with .02% Tween 20 for at least 1 hour. Membranes were probed with either a mouse anti-HA monoclonal antibody (1:500) or a mouse anti-FLAG M2 monoclonal antibody (1:500; Sigma-Aldrich) followed by an alkaline phosphatase-conjugated goat α-mouse secondary antibody (1:1000).

**Indirect Immunofluorescence**

Vero cells for imaging were grown in 24-well plates on coverslips until 50% confluent. 500 ng of DNA was transfected using Lipofectamine reagent according to the manufacturer’s protocol. Preparation for immunofluorescence was performed as previously described [29]. Cells were probed with mouse anti-HA monoclonal antibody (1:500) and mouse anti-FLAG M2 monoclonal antibody (1:500; Sigma-Aldrich). Secondary antibodies used were donkey anti-mouse AlexaFluor 488 (1:1000) and donkey anti-goat AlexaFluor 568 (1:1000). Cells were mounted on coverslips using ProLong Diamond Antifade Mountant (Invitrogen) according to the manufacturer’s protocol. Images for gD-UL51-FLAG co-localization with UL51-HA were acquired using confocal microscopy and ImageJ Software as previously described [30]. Images for UL51-FLAG truncation localization were acquired using a Leica epifluorescence microscope and ImageJ Software.
Results

*UL51 does not require other viral factors for self-interaction*

To determine whether UL51 interacts with itself in the absence of other HSV-1 viral proteins we performed a co-immunoprecipitation assay of transfected cell lysate containing differentially tagged UL51 proteins. Plasmids containing UL51-HA and UL51-FLAG were co-transfected into 293T cells. UL51-FLAG was purified from cell lysate using anti-FLAG magnetic beads and presence of both proteins were detected by immunoblot (Figure 4). UL51-HA and UL51-FLAG were both successfully transfected and expressed well in the lysate and UL51-FLAG

![Co-immunoprecipitation of UL51-HA and UL51-FLAG in transfected cells.](image)

Figure 4. *Co-immunoprecipitation of UL51-HA and UL51-FLAG in transfected cells.* Digital images of immunoblots are shown. Cells transfected with indicated constructs were lysed, separated by SDS-Page and immunoblotted as described in methods. Left hand panels show lysate proteins before immunopurification and right hand panels show proteins in purified fractions.
was enriched following FLAG purification. UL51-HA was detected in the anti-HA immunoblot following pull down of UL51-FLAG in co-transfected cells (Lower right panel, Figure 4). This suggests that UL51-HA was interacting with UL51-FLAG in transfected cells without the presence of other viral proteins.

When UL51-HA was transfected alone, it was also present following purification for FLAG (Figure 4). This background suggests that there is non-specific binding occurring between UL51-HA and the anti-FLAG magnetic beads. However, band intensity in the co-transfected UL51-HA/UL51-FLAG lane was significantly greater than the background. This was true for 10 immunoblots showing self-interaction, each showing various levels of background. UL51 undergoes several post-translational modifications including palmitoylation and phosphorylation. In a transfected cell lysate several species of UL51 are present due to the proteins being at different stages of modification. The UL51 species are slightly different sizes and run as multiple bands on SDS-PAGE gels around 30 kDa (Figure 4).

*Amino Acids 91-244 are sufficient for UL51 self-interaction*

The UL51 protein can be broken down into four regions of conservation (Figure 5A). In order to determine the importance of these regions in UL51 self-interaction, truncations whose boundaries coincide with the boundaries of the regions of conservation of UL51 were created (Figure 5B).

To begin mapping regions of the protein important for self-interaction we repeated the co-immunoprecipitation assay used previously with UL51-HA and the N terminal UL51-FLAG truncations. Full length UL51-FLAG (1-244) expressed well in the lysate. 91-244 UL51-FLAG and125-244 UL51-FLAG were expressed less than the full-length protein in the lysate and 167-
244 UL51-FLAG was not detected by immunoblot in the lysate (Figure 6). All FLAG tagged UL51 proteins were enriched by purification with anti-FLAG magnetic beads. 91-244 and 125-244 were enriched enough following purification to be able to compare their pull-down ability of UL51-HA. Once again, UL51-HA was detected in the anti-HA immunoblot after purification with full length UL51-FLAG suggesting self-interaction. Similarly, UL51-HA was detected in the 91-244 lane as a more intense band than the background. UL51-HA had similar band intensity in the 125-244, 167-244 and UL51-HA only lanes (Figure 6). This suggests that any UL51-HA detected in these lanes is due to non-specific binding of UL51-HA to the anti-FLAG magnetic beads. These results suggest that amino acids 91-244 are sufficient for self-interaction and amino acids 125-244 are not.
To gather additional evidence supporting self-interaction we performed a co-localization assay using N terminal UL51-FLAG truncations and full length UL51-HA (Figure 7). Full length UL51-HA localizes to the golgi apparatus in transfected cells. The N terminal truncations are all missing the site of palmitoylation at amino acid position 9 and are therefore unable to associate with membranes in transfected cells. They localize diffusely throughout the cytoplasm and nucleus when transfected alone (R. Roller unpublished). Upon co-transfection, UL51-HA was able to recruit 91-244 UL51-FLAG to the golgi apparatus (A-C Figure 7). The 125-244 truncation was unable to recruit the full length UL51-HA and remained diffused throughout the cytoplasm (D-F). Interestingly, the 167-244 truncation showed recruitment by UL51-HA in some of the cells imaged (G-I). These results support the claim that amino acids 91-244 are sufficient for self-interaction and 125-244 is not. However, the results also suggest that amino acids 167-244 are sufficient for this self-interaction as well.
UL51-FLAG C terminal truncations express poorly in transfected cells

To continue mapping regions of UL51 important for self-interaction we attempted to carry out another co-immunoprecipitation assay using UL51-HA and the C terminal UL51-FLAG truncations. Full length UL51-FLAG was transfected and expressed efficiently (Figure 8). 1-167
UL51-FLAG was not able to be detected in the lysate by immunoblot and was only slightly enriched following purification. 1-125 UL51-FLAG was not detected in the lysate immunoblot or the immunoblot following purification. These results suggest that the C terminal truncations are not being expressed at a high enough level in transfected cells to successfully do the established co-immunoprecipitation assay previously used with the N terminal truncations.

**Figure 8. UL51-FLAG C terminal truncation expression in transfected cells.** Digital images of immunoblots are shown. Cells were transfected with UL51-FLAG constructs with indicated UL51 length. Lysate and purified protein fractions were separated by SDS-Page and immunoblotted as described in methods.

**gD-UL51 fusion proteins do not interact with UL51-HA**

To obtain better expression of the UL51-FLAG C terminal truncations we fused UL51 to the highly expressed glycoprotein, gD. All constructs were expressed well in the lysate and enriched by immunoprecipitation (Figure 9A). However, UL51-HA was not detected in any lanes following purification with anti-FLAG magnetic beads (Figure 9A). Since it was previously shown that the full length UL51-FLAG is able to co-purify UL51-HA, the negative results in the full length (1-244) gD-UL51-FLAG lane suggests that gD-UL51-FLAG fusion proteins are not suitable candidates for interaction with UL51-HA.
In order to confirm these results, the full length gD-UL51-FLAG was co-transfected with UL51-HA and localization of the proteins was visualized by immunofluorescence. When transfected alone, UL51-HA has been shown to localize to the golgi apparatus and gD-UL51-FLAG localizes to the endoplasmic reticulum. When co-transfected gD-UL51-FLAG was unable to recruit UL51-HA and vise versa (Figure 9B). These results confirm that when gD is fused to UL51-FLAG, UL51 is unable to self-interact in transfected cells. This makes these constructs unsuitable for a UL51 self-interaction co-immunoprecipitation assay or a co-localization assay in transfected cells.

![Figure 9](image)

**Figure 9. gD-UL51-FLAG does not co-localize with UL51HA in transfected cells.** (A) Digital images of western blots are shown. Cells were co-transfected with gD-UL51-FLAG constructs with indicated UL51 length and UL51-HA. Lysate and purified protein fractions were separated by SDS-Page and immunoblotted as described in methods. (B) A representative image selected from ten randomly selected cells showing efficient transfection is shown. Cells were co-transfected with indicated constructs and prepared for immunofluorescence imaging as described in methods.
Discussion

UL51 is a palmitoylated tegument protein conserved across all herpesviruses [31]. Palmitoylation of the protein allows for membrane association and has been shown to facilitate localization to the golgi apparatus in transfected and infected cells [23]. Incorporation of UL51 into the tegument of mature virions suggests that UL51 localizes to the exterior of cellular membranes. This places UL51 in a prime position for participating in both virion assembly and trafficking interactions. UL51 has previously been shown to interact with UL7 which is required for UL7 incorporation into the virion making it an important interaction in viral assembly [21]. Also, UL51 interacts with the gE/gI complex which plays a role in virion trafficking[22]. Previous studies suggest that UL51 may also interact with itself [32]. Due to the known role of UL51 in cell to cell spread it is likely that this self-interaction is also an important interaction in the assembly and egress pathway of herpesviruses.

Here, we confirm UL51 self-interaction and show that this interaction does not require other viral factors. What remains unknown is whether this interaction is a direct interaction between two UL51 proteins or if there is a cellular component mediating the interaction. In order to differentiate these two scenarios, further studies could be performed using two bacterially derived UL51 fusion proteins. Fusion of UL51 to glutathione S-transferase and maltose binding protein would provide a means for establishing a co-immunoprecipitation assays similar to the one used in this study. Interaction observed in this environment would confirm that UL51 is directly interacting with itself in the absence of viral proteins and cellular proteins.

Determining the importance of this interaction, whether direct or indirect, requires a way to inhibit it. This would allow for observation of the phenotype of a virus expressing UL51 that is unable to interact with itself. In order to create such a UL51 mutant that inhibits only self-
interaction function but no other interactions we aimed to determine the amino acids important for interaction. By using both C terminal and N terminal truncations of UL51 that coincide with regions of amino acid conservation of the protein we could potentially determine which region is important for self-interaction.

The N terminal truncation UL51 constructs expressed well enough to be able to preliminarily determine that amino acids 91-244 are sufficient for the interaction. Our results from both the co-immunoprecipitation assay and co-localization assay suggest that amino acids 125-244 are not sufficient for self-interaction, indicating that amino acids 91-125 are important for interaction. Interestingly, in the co-localization assay some cells transfected with the 167-244 truncation showed co-localization with full length UL51-HA at the golgi. If confirmed that the 1-167 UL51 truncation is able to self-interact this may suggest that there are two possible interactions sites- one in amino acids 91-125 one in 167-244. It is difficult to predict the effect that truncating a protein may have on secondary and tertiary structure. It is possible that the 125-244 truncation folds improperly and blocks the interaction site located in the 167-244 area, thus blocking self-interaction. The 167-244 truncation may still be able to fold properly in order to self-interact. To confirm these results C terminal truncations would be necessary to show that constructs containing the 91-125 region would be sufficient for self-interaction but that amino acids 1-90 are insufficient. Interestingly, UL51 amino acids 30-60 have been shown to be sufficient for interaction with UL7. If the UL51 self-interaction domain is indeed not in amino acids 1-90 this could suggest that UL51 is able to interact with both itself and UL7 at the same time.

Expression of the C terminal truncations was not high enough to be able to perform the established co-immunoprecipitation assay. In an attempt to increase expression of these
constructs we used a method previously used when characterizing the interaction between UL51 and UL7. There were previous issues expressing 1-90 UL51-FLAG so the truncation was fused to the highly expressing glycoprotein, gD. This fusion increased expression and changed localization in transfected cells from the golgi to the endoplasmic reticulum. UL7 is a soluble protein and localizes diffusely in the cytoplasm and nucleus. A co-localization assay was performed which showed recruitment of UL7 to the endoplasmic reticulum suggesting interaction between the UL51 truncation and UL7. We hypothesized that this method could be used for the rest of the UL51 truncations and used in co-localization and co-immunoprecipitation assays with UL51-HA. However, the gD fusion proteins were unable to pull down UL51-HA and did not co-localize in the cell. The exact reason why this occurred is unknown. It may be due to the fact that UL51 and gD are both membrane associated proteins that localize to different compartments. Therefore, the proteins are sterically hindered from interacting with each other. Fusing gD to UL51 may also disrupt folding of UL51, inhibiting self-interaction.

Another method to determine which C terminal truncations are able to self-interact is to do further co-localization assays. The C terminal truncations, like full length UL51, would localize to the golgi in transfected cells. An HA tagged UL51 mutant with a C9V mutation could be used as bait for the assay. This mutation prevents palmitoylation of UL51 causing the protein to localize diffusely in the cytoplasm. Upon co-transfection of full length UL51-FLAG and the mutant UL51-HA, we would expect recruitment of C9V UL51-HA to the golgi apparatus. This can then be compared to recruitment by the C terminal truncations which would provide evidence as to which amino acids are contributing to self-interaction.

The eventual goal of these experiments is to determine whether this interaction is important in cell to cell spread, viral assembly or both. Once a region of interaction is
determined, point mutants of the protein could be created and screened for loss of self-interaction function. This mutant UL51 could then be incorporated into a recombinant virus and viral growth and spread could be measured. This knowledge would further characterize the importance of UL51 interactions with viral proteins in the assembly and egress pathway of herpesviruses.
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Literature Cited


