# DISCUSSIONS

UL9 is a multifunctional protein required for herpes simplex virus type 1 (HSV-1) replication *in vivo*. It is believed that this protein plays a key role in the initiation of viral DNA replication and for the assembly of the rest of the viral replication proteins at the origin of replication, but many questions about its precise mode of action have not been resolved.

The Origin Binding Protein might be an important target for the design of new drugs, because it is dispensable for the initiation of the replication of the virus. It is not reported until now that there is not an homolog protein in humans, so these taking this facts together, make UL9 a good candidate for research.

In this study we showed that there are some important motifs like SH2, WD40 and LC8 in UL9 that affects its function.

The SH2 motif mutant did not complement UL9 deficient virus in the complementation assays and the truncated protein also exhibited reduced ability to interact with the C-terminus of the UL9 protein. This SH2 motif is within the N-terminal region in residue 304 which is mapped as important UL9-UL9 interaction domain. The truncations with the point mutation may be decreasing the ability of UL9 N-terminus to interact with its UL9 C-terminus. N-terminal fragments containing the SH2 mutations exhibited significant decreases in their ability to interact with the C-terminus compared to wild type fragment so this suggests that this motifs may be important for UL9-UL9 interaction.

SH2 truncations in the N terminal part of the protein (where it is not the DNA binding domain), showed that wild type UL9 has about 60% number of plaques while the SH2 truncation only 20%, so we can see a transdominant effect on it, suggesting that even if the DNA binding domain is in the C terminus of the protein, there might be crosstalk between domains, and the N terminal domain might be influencing the functionality of the C terminal domain. The Plaque Reduction Assay for full length mutations in SH2 did not showed much difference compared to wild type.

The truncations do not include the DNA binding domain, so they did show a strong effect on relief of inhibition. Although, there was some effect in inhibition of replication compared to wild type same size fragment in the N terminal domain. This may be suggesting a transdominant effect in the protein, observing how mutations in one domain may be affecting the functional abilities of the other.

SH2 motifs are found in tyrosine kinases and proteins involved in phosphorylation as well as association. It has also been reported previously that UL9 is phosphorylated during infection (Isler and Schaffer, 2001) and phosphorylation followed by proteasomal degradation might be an important way of regulating UL9 during infection (Lehman, 2003). Taken these together it can be suggested that this motif might be important for the interaction of the N-terminus with the C-terminal part of UL9. Moreover, this motif might be involved in the phosphorylation of this protein, but further studies are required to explore these possibilities.

Regarding WD40 mutants, the mutant for the first motif of WD40 (SS304/305 AA) in UL9 complemented partially the UL9 deficient virus, while the second one (SS 380/384 AA) complemented totally (compared to the UL9 wild type protein) this UL9 deficient virus. This means that the second motif is not important for the origin binding protein, but that the first one might be affecting some function of it. As it was mentioned before, many regulatory proteins have WD40 domains that mediate protein-protein interactions, so these mutation might be affecting an interacting region.

As shown in the co-immunoprecipitation assay, it was observed that this mutant was defective in its ability to interact with the C-terminal part of the protein, therefore this motif is important in the UL9-UL9 interaction. The N-terminal fragments containing the WD40-1 mutations exhibited significant decreases in their ability to interact with the C-terminus compared to wild type fragment so this suggests that also this motif may be important for dimmer formation. In the other hand the WD40-1 motif mutant did not have a considerable effect on inhibition compared to wild type. The full length WD40 and SH2 motif mutants have the DNA binding domain on them so, they were not

causing a big effect as well. The Plaque Reduction Assay for full length mutations in WD40-1 motifs also did not show much difference compared to wild type.

The truncations do not include the DNA binding domain, so they did show a strong effect on relief of inhibition. Although, there was some also, the same as in the SH2 motif, an effect in inhibition of replication compared to wild type same size fragment in the N terminal domain. This may be suggesting a transdominant effect in the protein, observing how mutations in one domain may be affecting the functional abilities of the other. Further experiments are required to find out whether if this WD40-1 motif is important in interaction of UL9 with some other viral or cellular protein.

The LC8 motif mutants did not disrupt any UL9-UL9 interactions, as shown by co-Immunoprecipitation they seem not to be important in interaction, but on the other hand they were defective in origin binding.

The Plaque Reduction Assay also showed a relief of inhibition in the case of these mutations.
It was shown by the number of plaques that LC8 deletion was causing a considerable relief on inhibition compared to the C-terminal wild type fragment of UL9. DNA Binding Assay showed LC8 motif mutants were defective for their ability to bind to the origin. These results are consistent indicating that the residues or this motif are important for DNA Binding ability of UL9. These sites may disrupt completely the ability of UL9 to bind to DNA and HSV-1 replication also might be affected.

It was reported previously by Deb and Deb (1991) that DNA binding domain is localized within residues 564-832. They reported that the N-terminal boundary of the DNA binding domain is within residues565 - 596 and the C-terminal boundary is within 805 – 832 and they need to be together to support DNA binding. The LC8 motif is located between residues746-750 and the deletion mutant showed complete defect in DNA binding which might open the new possibility that this region is very important for the DNA binding ability of this protein.

The MDM2 motif mutants also did not disrupt UL9-UL9 interactions, but they were little defective in DNA Binding and also relieved some inhibition. These motif might be not only playing a role in DNA binding, but also in phosphorylation and ubiquitination of the protein, and thus regulating it in the second stage of infection. Also further studies are required to explore these possibilities

Very serious efforts to recapitulate an origin unwinding reaction in vitro with double stranded origin containing DNA and UL9 have failed. One interpretation of this failure is that "something" is missing. Although several potential interaction partners for UL9 have been identified, there have been no comprehensive attempts to complete the "UL9-proteome".

The mechanism of regulation of UL9 levels and activity will be essential to the understanding of the regulation of HSV infection as a whole and is likely to provide important paradigms for other replication systems like, the ability of viruses to degrade host and viral function is currently of important interest not only in the virus world but also to cancer researchers since many viruses have evolved to interact with key cellular pathways which also become dis-regulated during oncogenic transformation in terms of regulating DNA replication, repair, cell cycle check points and induction of apoptotic pathways

UL9 is a complex and a multifunctional protein. In this work we explored the importance of some motifs on it. The structural data for the full length UL9 protein is difficult to obtain by x-ray crystallography or nuclear magnetic resonance because it's a large 94 kda protein, but the approach like this to define functional motifs will be useful to understand better the multiple roles played by this protein during DNA replication of HSV-1.