Sequence Variation in the Herpes Simplex Virus U_S1 Ocular Virulence Determinant

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Purpose. The herpes simplex virus type 1 (HSV-1) U_S1 gene encodes host-range and ocular virulence determinants. Mutations in U_S1 affecting virulence are known in strain OD4, but the genomic variation across several strains is not known. The goal was to determine the degree of sequence variation in the gene from several ocular HSV isolates.

Methods. The U_S1 gene from six ocular HSV-1 isolates, as well as strains KOS and F, were sequenced, and bioinformatics analyses were applied to the data.

Results. Strains 17, F, CJ394, and CJ311 had identical amino acid sequences. With the other strains, most of the variability was concentrated in the amino-terminal third of the protein. MEME analysis identified a 63-residue core sequence (motif 1) present in all α-herpesvirus U_S1 homologs that were located in a region identified as structured. Ten amino acids were absolutely conserved in all the α-herpesvirus U_S1 homologs and were all located in the central core. Consensus-binding motifs for cyclin-dependent kinases and pocket proteins were also identified.

Conclusions. These results suggest that significant sequence variation exists in the U_S1 gene, that the α22 protein contains a conserved central core region with structurally variable regions at the amino- and carboxyl termini, that 10 amino acids are conserved in α-herpes U_S1 homologs, and that additional host proteins may interact with the HSV-1 U_S1 and $U_S1.5$ proteins. This information will be valuable in designing further studies on structure-function relationships and on the role these play in host-range determination and keratitis. (*Invest Ophthalmol Vis Sci.* 2011;52:4630–4638) DOI:10.1167/iovs.10-7032

Herpes simplex virus type 1 (HSV-1) is a significant human pathogen causing diseases such as mucocutaneous ulcers, keratitis, and encephalitis. In the United States, HSV-1 is the leading cause of blindness from infection and the leading cause of sporadic encephalitis.[1,2] Studies in animal models have shown that the severity of an HSV-1 infection depends on three factors. The first is the innate resistance of the host. Strains of mice vary widely in their susceptibility, and some host genes involved in this innate immune resistance have been identified.[3–8] The second factor is the host's acquired immune response. Animals with various defects in acquired immunity have difficulty in controlling viruses, resulting in lethal infections.[9–14] The host immune response is crucial because corneal damage results from an immunopathological response.[15–17]

The third factor is the genetic makeup of the virus. Strains of HSV-1 display virulence patterns ranging from no disease to lethal encephalitis (see Refs. 18, 19 for review). The severity of keratitis also varies widely between strains. Although the sequence of one complete HSV-1 genome has been available for some time[20–23] and two more genomes were recently sequenced,[24] little is known about the total sequence divergence and the role most HSV-1 genes play in the severity of an infection. Deletion of certain genes from the virus can have significant effects on virulence, but in nature it is more likely that virulence differences are due to effects of multiple genes and the combination of alleles carried by a given strain of virus. This is supported by a study showing that transferring different combinations of genes from a moderately virulent strain (CJ394) into a highly attenuated strain of virus (OD4)[25,26] resulted in different virulence patterns in mice. At least seven genes were shown to be involved in the virulence differences. One gene that, when transferred from CJ394 into OD4, increased ocular virulence but not neurovirulence was U_S1, and two sequence changes, S34A and Y116C, that must occur together, were suggested to play a role in the difference in virulence.[25,26]

The HSV-1 U_S1 protein (α22) is an immediate early (α) gene that regulates several processes in infected cells. In concert with the U_S3 and U_L13 kinases, it alters the phosphorylation of RNA polymerase II, and this is thought to target Pol II to the viral genome.[27–31] The α22 protein is also responsible for the efficient expression of some late genes, including U_L41, U_S11, U_L47, U_L49, U_L13, and U_L4.[32–36] In addition, it plays a role in determining the composition of virions, possibly through effects on late gene expression,[37] and negatively regulates α-gene expression.[38,39] The α22 protein has also been reported to block B-cell activation of $CD4^+$ T cells.[40] The activities of the α22 protein are mediated by interactions with both viral and host proteins.[27,32,41–46] The α22 protein is also heavily posttranslationally modified by serine and tyrosine phosphorylation, guanylylation, and adenylation, and multiple isoforms (at least seven or eight) are found in infected cells.[25,36,47–52] The functions of each of the isoforms in infection and virulence are not understood.

In addition to the 420-amino acid α22 protein, a second protein, $U_S1.5$, is expressed from the U_S1 gene.[53] The $U_S1.5$

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TABLE 1. Herpesvirus Species Names, Gene Designations, and Accession Numbers

Virus Species	Virus Name Abbreviation	Strain	U_S1 Gene Homolog Designation	Accession No. (reference)
Alphaherpesvirinae				
Simplexvirus				
HSV-1	HSV-1	OD4	U_S1	JF511471
HSV-1	HSV-1	994	U_S1	JF511474
HSV-1	HSV-1	CJ394	U_S1	JF511469
HSV-1	HSV-1	TFT401	U_S1	JF511475
HSV-1	HSV-1	CJ311	U_S1	JF511470
HSV-1	HSV-1	CJ970	U_S1	JF511472
HSV-1	HSV-1	KOS	U_S1	JF511473
HSV-1	HSV-1	Strain F	U_S1	GU734771.1*
HSV-1	HSV-1	Strain 17	U_S1	2703435†
HSV-1	HSV-1	HF10	U_S1	DQ889502*
HSV-1	HSV-1	R-15	ORF_07R	AY344654*
HSV-2	HSV-2	HG52	U_S1	1487350†
Cercopithecine herpesvirus 2	SA8	B264	U_S1	3190318†
Mardivirus				
Gallid herpesvirus 2	GaHV-2	Md5	MDV088	4811543†
Varicellovirus				
Human herpesvirus 3	VZV	03–500	ORF63	ABF21862
Independent branch				
Duck enteritis virus	DEV	DEV Clone-03	U_S1	EF524095

* Gene ID not available.
† Gene ID references.

protein is translated in the same reading frame as the α22 protein but is truncated at the *n*-terminus. Translation start sites for $U_S1.5$ have been reported at residues 171,[52] 147,[53] and 90.[54] The reason for the difference is not clear but may be due to the use of different strains of virus (F and KOS) in these studies. The $U_S1.5$ protein is found only at late times, and the levels are upregulated by the U_S3 kinase and downregulated by the U_L13 kinase.[55] The distinct or shared functions of α22 and $U_S1.5$ are not completely understood, but the $U_S1.5$ protein appears to downregulate cyclin expression in infected cells.[46] The $U_S1.5$ protein has a diffuse cytoplasmic location whereas the α22 protein appears as punctate nuclear spots,[32,44,56–58] but how this correlates with the functions of the two proteins is not clear.

Studies on the role of the HSV-1 U_S1 gene in virulence are hampered by a lack of knowledge of the degree of sequence variability between strains. To address these deficiencies, we sequenced the U_S1 gene from eight HSV-1 strains and applied a bioinformatics analysis of the sequence data. The results show that there is significant sequence heterogeneity between the U_S1 genes concentrated primarily in the amino-terminal third of the protein. In addition, we identified a 63-amino acid motif in the center of the protein, determined that 10 amino acid residues are absolutely conserved in all α-herpesvirus U_S1 homologs, and identified putative binding motifs for host proteins. These data provide information critical for designing mutagenesis strategies that can be used to study the structure and function of the α22 protein and the role it plays in virulence and host range.

MATERIALS AND METHODS

Cell Culture and Viruses

Vero cells were cultured in Dulbecco's modified Eagle's medium with 5% serum and antibiotics, as described previously.[59] The viral sequences analyzed in this study are listed in Table 1. The ocular virulence characteristics of HSV-1 strains OD4, CJ311, CJ394, 994, 970, TFT401, and KOS were described previously.[59,60] Briefly, HSV-1 strain OD4 is avirulent with multiple attenuating mutations.[25,59,60] Strain CJ994 causes mild stromal keratitis. Viral strains CJ394 and TFT401 cause moderate stromal keratitis. HSV-1 KOS produces moderately severe keratitis, with <20% mortality in 5- to 6-week-old mice. Mice infected with strain CJ311 exhibit severe blepharitis, and 70% of the mice die of encephalitis before stromal keratitis develops. Strain CJ970 causes severe stromal disease and 50% mortality. Strain F is a commonly used laboratory strain and is neurovirulent after peripheral inoculation.[61]

Viral DNA Isolation

Viral DNA was isolated using a modification of a previously described large-scale preparation.[62] Briefly, 10 confluent 10-cm plates of Vero cells were infected at a multiplicity of infection of 1.0. The plates were scraped 24 hours after they reached 100% cytopathic effect, and the cells were pelleted at 2000*g* for 10 minutes at 4°C. The cell pellet was resuspended in 5 mL of medium, subjected to three freeze-thaw cycles (−80°C/37°C), and centrifuged at 2000*g* to remove debris. The supernatants were then combined, layered onto a 36% sucrose cushion in reticulocyte standard buffer (RSB; 10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$), and then centrifuged for 80 minutes at 13,500 rpm in a rotor (Beckman SW28; Beckman-Coulter Inc., Brea, CA). The viral pellet was resuspended in 5 mL TE buffer (10 mM Tris, pH 7.4, 1 mM EDTA) with 0.15 M sodium acetate and 50 μg/mL RNase A and was incubated 30 minutes at 37°C. Proteinase

TABLE 2. Catalog of Primers Used for Direct Sequencing of the U_S1 Gene Amplicons

Forward or Reverse Primer	Genome Nucleotide Location	Primer Sequence
Forward	132, 363	5′ TTTTGCACGGGTAAGCAC 3′
Forward	132, 782	5′ CAGCCTTGGAGTCTCGAGGTCG 3′
Forward	133, 141	5′ AAGCCCAATGCAATGCTAC 3′
Forward	133, 464	5′ GCAAGCTTCCTTGTTTGGAG 3′
Reverse	133, 006	5′ TGGGGGAATGTCGTCATAAGA 3′
Reverse	133, 245	5′ ACCCGAAACAGCTGATTGAT 3′
Reverse	133, 716	5′ GTCCAGTCAAACTCCCCAAA 3′
Reverse	133, 992	5′ CCGACTTCCTCACATCTGCT 3′

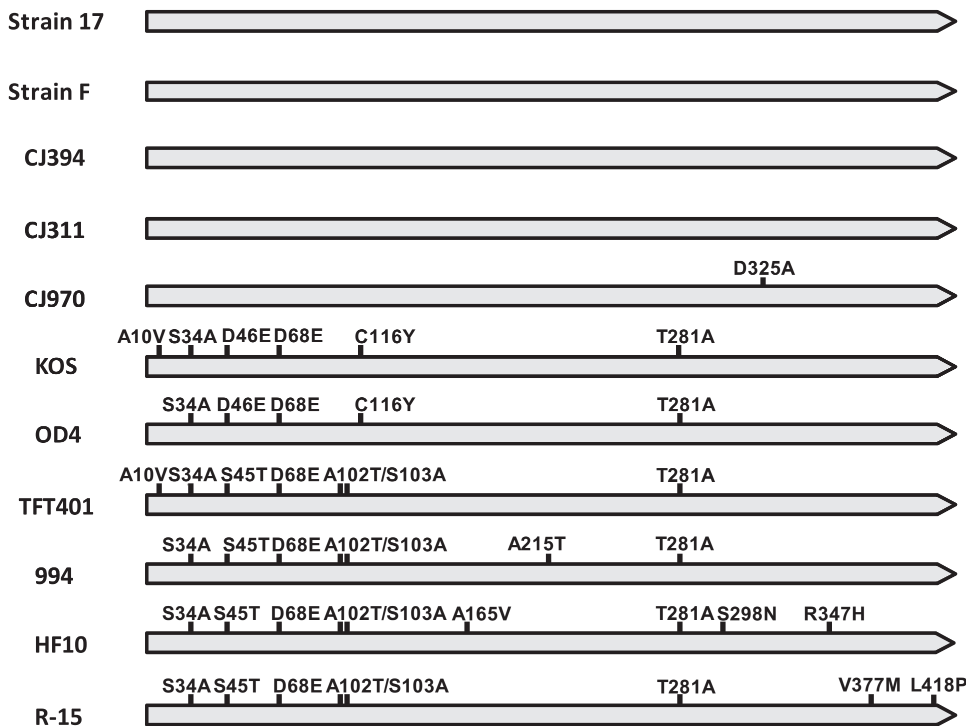


FIGURE 1. Sequence variability in the HSV-1 U_S1 (α22) protein. *Filled arrows*: U_S1 protein for each strain of virus, with amino acid differences indicated by *vertical bars* with the sequence differences noted. Only the amino acids different from the strain 17 sequence are shown.

K and SDS (50 μg/mL and 0.1% respectively) were then added, and the solution was incubated for 30 minutes at 37°C. The viral DNA was then purified by phenol/chloroform extraction and ethanol precipitation, resuspended in deionized water, and stored at −20°C.

Sequencing the U_S1 Gene from Multiple Strains

To obtain the U_S1 sequence from strains F, KOS, OD4, CJ394, TFT401, CJ311, 970, and 994, the U_S1 gene was amplified from purified viral genomic DNA and then directly sequenced. To amplify the U_S1 gene, we performed a PCR reaction consisting of 2.5 μg viral DNA , 1× reaction buffer, 1× enhancer buffer, 1 mM $MgSO_4$, 4 mM dNTP, 0.1 μg each primer, and 1 μL Pfx high-fidelity polymerase (Invitrogen Inc., Carlsbad, CA) in a total volume of 50 μL The cycling conditions were 1 cycle of 94°C for 5 minutes, 30 cycles of 94°C for 15 seconds, 50°C for 30 seconds, 68°C for 2 minutes, and a final cycle of 58°C for 7 minutes. The U_S1 gene extends between genome nucleotides 132,363 and 133,906. The forward primer annealed at nucleotide 132,363 (5′-TTTTGCACGGGTAAGCAC-3′), and the reverse primer annealed at nucleotide 133,992 (5′-CCGACTTCCTCACATCTGCT-3′). The PCR products were directly sequenced using multiple primers (Table 2). The sequencing mixture consisted of 200 μg amplification product, 1× reaction buffer, 0.1 μg primer, and 2 μL dye terminator (Big Dye, v3.1; Applied Biosystems, Foster City, CA) in a total volume of 20 μL. The cycling conditions were 1 cycle of 95°C for 3 minutes, 45 cycles of 95°C for 20 seconds, 45°C for 30 seconds, and 60°C for 4 minutes followed by a final cycle of 72°C for 7 minutes. Sequences were determined at the University of Wisconsin-Madison Biotechnology Center sequencing facility. To reconcile discrepancies in the sequence, chromatograms were visually inspected or sequencing reactions were repeated. The constructs were sequenced and found to match the sequences of the original strains.

Sequence Analysis

The sequences used for analysis were derived from this work and from sequences available in GenBank, as shown in Table 1. The nucleotide and amino acid sequences were aligned with ClustalW[63] assuming no gaps. Phylogenetic analyses were performed with the Molecular Evolutionary Genetics Analysis package (MEGA4[64]). The nucleotide-based bootstrap consensus tree was constructed using the neighbor-joining algorithm[65] and the Kimura 2-paramter method[66] and was derived from 1000 replicates.[67] The bootstrap consensus tree constructed with amino acid sequence data was generated using the neighbor-joining algorithm and the JTT matrix-based method[68] derived from 1000 replicates. Analysis with minimum evolution (ME) and maximum-parsimony methods produced similar results. Multiple EM for Motif Elicitation (MEME;[69]) analysis used the zoops (zero or one motif) algorithm to determine the presence and absence of motifs in the target sequences. The number of motifs (n = 10) reported was limited to motifs that had the potential to be phylogenetically informative. The optimum motif width specified in analysis ranged from 35 to 80, and the optimum number of sites for each motif varied from 2 to 50. Motifs revealed in the summary MEME analysis were conservatively excluded to limit the number of false-positive motif placements. The ratio of synonymous to nonsynonymous substitutions for the HSV-1 U_S1 sequences was per-

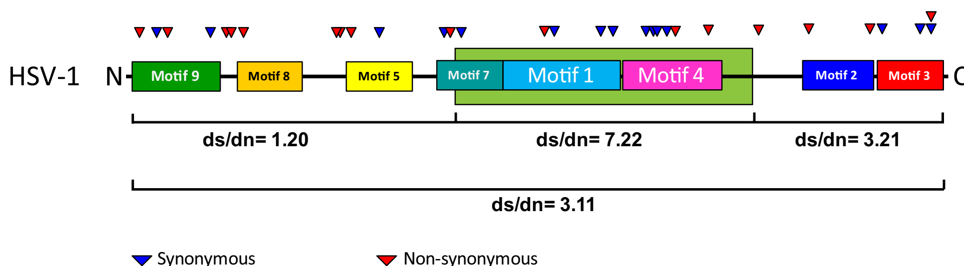


FIGURE 2. Diagram plotting both synonymous and non-synonymous substitutions along the length of the HSV-1 α22 protein. The ratio of synonymous to non-synonymous substitutions was determined for both the whole protein and the major segments.

formed using SNAP (http://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html[70]).

Protein Folding and Structural Feature Prediction

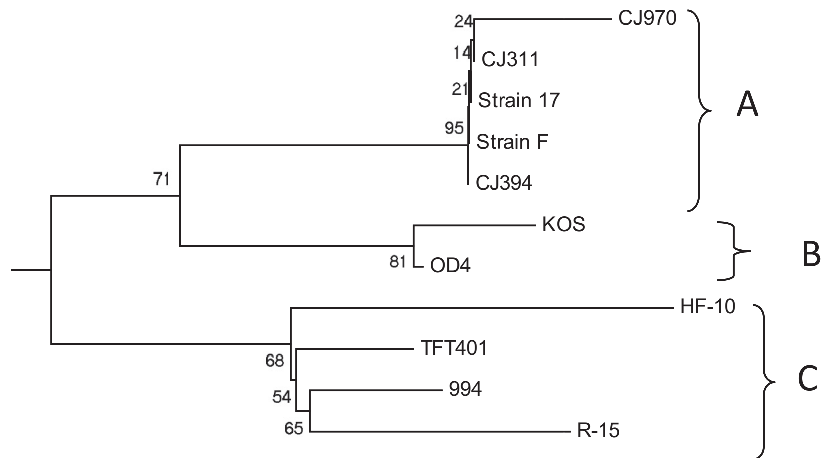
Identification of putative folded globular regions of the α22 protein was performed using Globplot2 (http://globplot.embl.de[71]). Short functional site prediction using the Strain 17 sequence was performed using ELM (http://elm.eu.org[72]).

Results

The U_S1 genes from six independent low-passage ocular isolates of HSV-1 and the laboratory strains KOS and F were sequenced. The U_S1 sequence from our strain F matched the recently deposited strain F, and we refer to that accession number. This information was combined for analysis with available sequence data for strain 17 and other α-herpesvirus U_S1 homologs, as shown in Table 1.

Sequence Divergence between HSV-1 U_S1 Genes

A schematic diagram summarizing the amino acid sequence differences between all of the 11 HSV-1 U_S1 genes available for analysis is shown in Figure 1. Four strains—17, F, CJ394, and CJ311—had identical U_S1 sequences. Strain CJ970 differed from the strain 17 group by only one amino acid



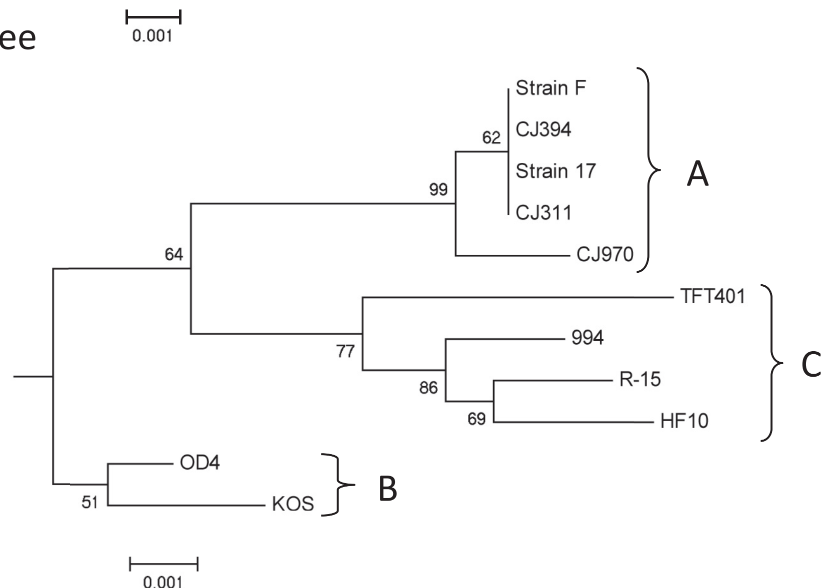


Figure 3. Phylogenetic relationships among U_S1 sequences of HSV strains. (**A**) Neighbor-joining tree based on amino acid sequence data. (**B**) Neighbor-joining tree generated with nucleotide sequence data. Each tree was generated using 1000 bootstrap replicates. The sequence of the HSV-2 strain HG52 U_S1 gene was used as the outgroup, but only the HSV-1 strains are shown. The three clades are denoted A, B, and C. The branch lengths are proportionate to the amount of evolutionary change and the scale to either nucleotide or amino acid replacement per position, respectively. The sequences of strains CJ970, CJ311, CJ394, F, KOS, OD4, TFT401, and 994 were generated for this study. All the HSV-1 strains, except F and KOS, are low-passage clinical isolates obtained from the Seattle, Washington, area. All other sequences were obtained from the NCBI GenBank database (Table 1).

(D325A). The remainder of the strains had between 5 and 9 amino acid differences compared with strain 17. These results and others shown suggest that U_S1 has a central conserved core and that most of the variability occurs in the amino-terminal third of the protein. To confirm this, we compared the ratio of synonymous to non-synonymous mutations. As shown in Figure 2, the central core clearly had the highest ratio ($ds/dn = 7.22$), the carboxyl-terminal third was conserved but less so ($ds/dn = 3.21$), and the amino-terminal third had a ratio near 1 ($ds/dn = 1.2$).

Phylogenetic Analysis of U_S1 Genes

Figure 3A shows the neighbor-joining tree for the HSV-1 U_S1 amino acid sequences using HSV-2 strain HG52 as the out group. Similar results were obtained using maximum-parsimony and maximum-likelihood methods, indicating the tree was robust. The maximum sequence divergence between the HSV-1 strains was 1.2%, and the HSV-1 strains clearly separated into three distinct groups or clades. The three-clade structure was seen previously, but the clades based on the U_S1 gene differ.[73,74] Group A included strains 17, CJ970, CJ394, CJ311, and F. Only two strains (KOS and OD4) were in group B, and four strains (HF10, TFT401, 994, and R-15) were in group C. As shown in Figure 3B, the phylogenetic analysis based on the nucleotide sequences yielded the same three clade structures of amino acid sequences, although the topology of the tree was slightly different.

MEME Analysis for Conserved Motifs

MEME analysis, which identifies statistically significant motifs in a data set, was used to identify 10 phylogenetically informative amino acid sequence motifs (Figs. 4, 5). A core motif (motif 1) was identified in all α-herpesvirus U_S1 genes and their homologs and is, thus, distinctive for all α-herpesviruses. This motif had the lowest expectation value (2×10^{-56}) and was, therefore, highly statistically significant. The three reported start sites for the $U_S1.5$ protein lie in motifs 5, 7, and 8.[52–54] Motifs 5 and 9 each contain one of the nuclear localization signals, and motif 8 contains a putative nucleotidylation site.[49] The consensus tyrosine phosphorylation site[51] (Y193) mapped to motif 1. In the Old World simian virus group (e.g., SA8), only motifs 1, 2, 3, 5, and 8 were conserved; thus, MEME analysis can clearly be used to distinguish simian α-herpesviruses from human viruses. The avian and varicellovirus groups are characterized by the presence of motif 6. The presence of motif 10 was variable; it was present in GaHV-2 and VZV but not in the duck enteritis virus.

When the GlobPlot2 program was used to examine potential folding of the HSV-1 strain 17 U_S1 protein (Fig. 5), we found that only the central portion of the protein could be folded into a putative globular domain. This region overlapped motif 1 in all the α-herpesvirus $\alpha22$ proteins. These results are consistent with the central portion of the $\alpha22$ protein as a core conserved region.

FIGURE 4. MEME analysis for conserved motifs within the α-herpesvirus $\alpha22$ protein. Motif detection was limited to a phylogenetically relevant $n = 10$. The sequence of each of the motifs is presented; the larger the letter, the greater the conservation.

Discussion

The HSV-1 U_S1 gene encodes an immediate early (α) protein that carries out a number of functions in infected cells and is clearly a host range virulence determinant.[25,26,34,75–77] We previously identified two sequence changes in the $\alpha22$ protein of the avirulent strains OD4, S34A, and Y116C[25,26,59] that, when reverted to wild type, conferred the ability to cause severe keratitis in a mouse model. At that time, only the strain 17 sequence was available for comparison. In this study, we sequenced the U_S1 gene from six low-passage ocular HSV-1 isolates as well as KOS and F and used these new sequence data to carry out a bioinformatic analysis and to determine the sequence variability in the α-herpesvirus U_S1 homologs. When we analyzed the amino acid sequence variability in the HSV-1 $\alpha22$ protein, we found several differences between the strains. Sequence variability was highest in the amino-terminal third of the protein. Multiple functions have been mapped to the carboxyl-terminal portion, including modification of Pol II.[46,57] Thus, the clustering of several functions in the carboxyl-terminal half of the protein may account for the sequence conservation in this region.

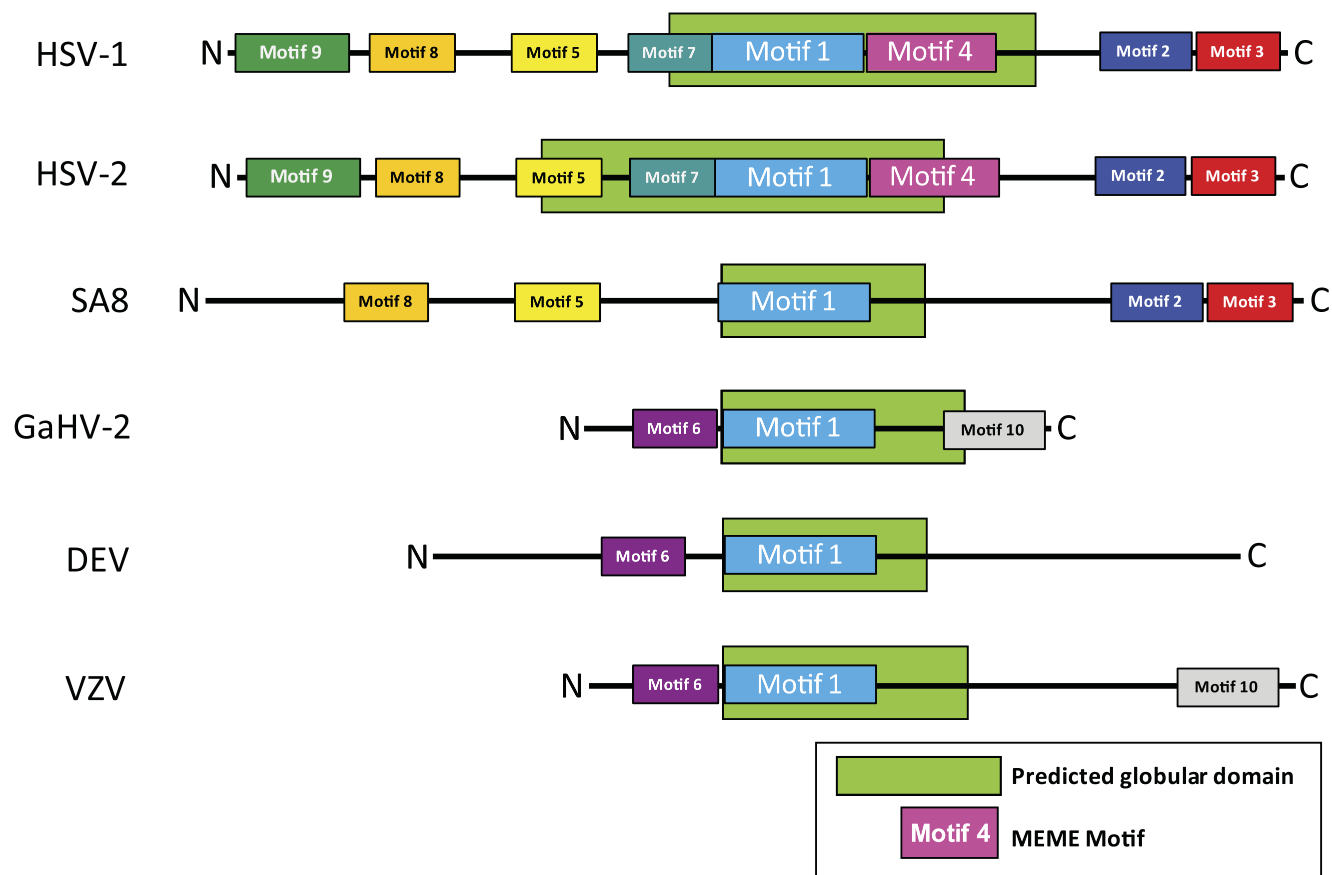


Figure 5. Map of MEME analysis for conserved motifs in the α-herpesvirus α22 protein. The motifs are numbered 1 through 10 and are color coded for visualization. Motifs discovered in the summary MEME analysis were conservatively excluded to limit the number of false-positive motif placements. The location of the predicted globular domain of the protein is denoted by the *green hatched bars*. Note that motif 1 is located in the putative globular domain in all the groups.

A second protein, $U_S1.5$, which plays a role in the host range of the virus, is involved in optimal expression of ICP0 and γ-genes and is distributed differently in infected cells compared with α22.[56,78] It is encoded within the U_S1 gene, and is identical with the carboxyl terminus of the α22 protein.[54,78] Thus, the clustering of multiple functions in the C-terminal half of the protein and the presence of an overlapping protein could also explain the higher sequence conservation in this part of the α22 protein compared with the amino-terminal third. The potential effects of sequence variability on the function, or functions, of the $U_S1.5$ protein depend on the start site. Currently, three start sites—M90, M147, and M171—have been reported for the $U_S1.5$ protein, and it is unclear whether all three sites are used or whether the start site differs between strains.[52–54] Based on the reduced variability in the C-terminal half of the sequence, if $U_S1.5$ begins at residue 147 or 171,[52,53] the variability would have less effect. However, if translation of $U_S1.5$ begins as residue 90,[54] then the sequence variability would be greater and may affect the function of the $U_S1.5$ protein. Additional studies are clearly required.

The S34A and Y116C changes in OD4 had previously been shown to be partially responsible for the attenuation of strain OD4 in ocular infections.[25,26] Six of the strains—KOS, OD4, TFT401, CJ994, R-15, and HF-10—had the S34A sequence change. However, both changes were required for the loss of virulence in strain OD4; thus, the presence of only S34A would not be expected to change ocular virulence. However, KOS had both changes and causes keratitis.[59] There are several possible explanations for this apparent discrepancy. One we currently favor is related to observations that the α22 protein interacts with multiple viral and cellular proteins (see Introduction) and that its role in virulence very likely depends on at least some of these interactions. This may be an example of epistasis or, more specifically, sign epistasis, in which the phenotype of a particular mutation depends on the context in which it is expressed.[79] Sequence changes in other KOS proteins could compensate for the U_S1 changes and maintain virulence. The KOS U_S1 protein also has an additional sequence difference compared to OD4 (A10V), and this could be compensatory. Additional studies with KOS and OD4 are needed to address this issue.

The HSV-1 strains could be separated into three groups or clades. Previous phylogenetic analyses using the HSV-1 gC, gG, gE, and gI genes also indicated that there were three clades.[73,74] However, the clades identified using the gC, gG, gE, and gI genes were not the same as those identified using the U_S1 gene. For example, strains F and 17 were in the same clade based on U_S1 sequences, but they sorted in different clades based on the gE/gI sequences. The differences in clade structure seen when analyzing single genes or a small subset of genes point to a weakness with this approach because it may bias the actual genetic relationships between the strains. The most likely explanation is that this is the result of recombination events between strains, but further work is needed to confirm this.

The MEME analysis, which identifies phylogenetically useful motifs, revealed a number of interesting structural features in the α22 protein. First, unique patterns of motifs were identified

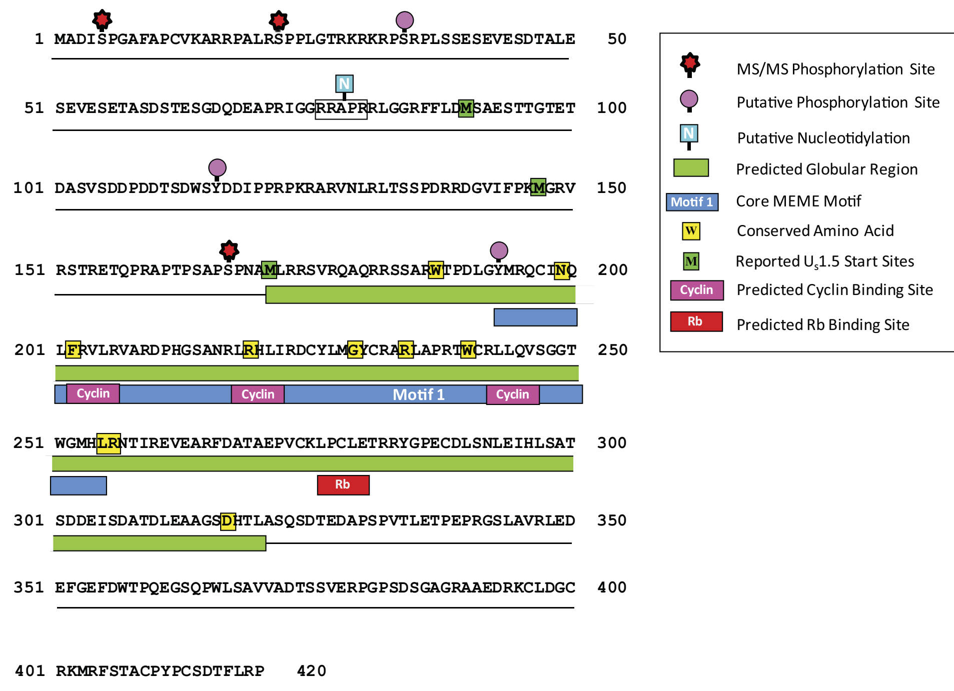


FIGURE 6. Schematic diagram of the HSV-1 α22 protein showing predicted and confirmed phosphorylation sites (unpublished data, 2010), the location of MEME motif 1, structural features and consensus sequence motifs, predicted functional regions, and amino acid residues that are conserved in all the strains analyzed. Note that we have shown all three of the reported start sites for the U$_S$1.5 protein. The legend denotes features that have been experimentally confirmed versus those that are predicted.

for the human viruses, Old World monkey viruses, and the varicella viruses. For example, motifs 4, 7, and 9 were unique to HSV-1 and HSV-HG52, which would be expected given their close evolutionary relationship. Some motifs are predominantly cationic whereas others are primarily anionic. Several of the motifs were hydrophobic. Hydrophobic residues tend to be conserved in regions involved in folding and the maintenance of the tertiary structure, suggesting that these motifs might be important for stabilizing the α22 protein structure. As revealed in Figure 4, each of the motifs has several highly conserved residues, and these motifs can now serve as the focus of mutational studies to determine structure-function relationships in the α22 protein.

The analysis also identified a motif (motif 1) that was conserved in all α-herpesvirus U$_S$1 homologs. Motif 1, which has 63 residues, was contained within a larger region previously identified as conserved across the α-herpesvirus subfamily.[57,80] Thus, our analysis more precisely defines this core conserved sequence. We identified several residues using the ClustalW alignment program (Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-7032/-/DCSupplemental) that are absolutely conserved in the U$_S$1 homologs analyzed, including W187, N198, F202, R219, G229, R234, W240, L255, R256, and D317, which were located in the predicted globular domain (Fig. 6). The conservation of residues within this region would be consistent with it functioning as a conserved core or scaffold for variable amino- and carboxyl-terminal domains.

When we applied the protein folding program GlobPlot2 to the HSV-1 α22 sequence, we found that only the very central region was predicted to fold into a globular domain and that motif 1 was centered in this region. This behavior is characteristic of proteins that can adopt alternative conformations that are regulated by posttranslational modification such as phosphorylation.[81] The fact that the α22 protein has multiple functions, is present in at least seven or eight isoforms in infected cells, and is phosphorylated would be consistent with such behavior. We propose that the U$_S$1 protein consists of a central core scaffold region centered on motif 1 that has amino- and carboxyl-terminal domains that can adopt different conformations, depending on specific posttranslational modifications. These different conformers would then carry out specific functions.

As shown in Figure 6, we compiled the available data on the structural features of the HSV-1 α22 protein. This analysis reveals a number of important features and serves as a guide for site-directed mutagenesis studies. We included both predicted and confirmed modification sites, recognizing that additional studies using physical methods are needed to confirm modification sites. Note that the phosphorylation of serines 5, 22, and 167 have been confirmed using ms/ms (Hermann Bultmann and Curtis Brandt, unpublished data, 2010). Predicted cyclin and Rb protein binding sites were identified and reside in the central globular region. The presence of cyclin binding sites is consistent with previous reports that α22 interacts with CDK9.[27,82]

In summary, for the first time, we show that there is significant sequence variability in the HSV-1 α22 protein and that most of the variability lies in the amino-terminal third of the protein. We have also identified 10 conserved residues in the protein and that a core 63 amino acid sequence is located in the central part of the α22 protein. This information is critical for designing targeted mutational studies of the structure and function of the α22 protein and its role in virulence.

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