Identification and characterization of the virion protein products of herpes simplex virus type 1 gene UL47

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We have identified two encoded proteins with an antiserum raised against a synthetic oligopeptide corresponding to amino acids 671 to 684 of the predicted protein product of gene UL47 of herpes simplex virus type 1 (HSV-1). They have apparent Ms of 82000 and 81000 and are both major virion components located in the tegument. The 82/81K proteins were first detected in infected cells in minor amounts 6 h after infection at 37 °C but were later (from 10 h until 24 h after infection) present in large amounts. UL47 regulation was investigated using phosphonoacetic acid (PAA), an inhibitor of DNA synthesis: the amounts of the 82/81K protein synthesized were compared with those of 65K, an early gene product, and 21K/22K, a true late gene product. The data showed that UL47 is regulated as a true late gene.

Introduction

Over 30 proteins have been detected in virions of herpes simplex virus type 1 (HSV-1) (Spear & Roizman, 1972; Heine et al., 1974; Marsden et al., 1976; reviewed by Dargan, 1986). However, the genes encoding only about half the virion proteins have so far been identified. Research towards identification of the genes encoding others has recently been stimulated by the availability of the complete sequence of the HSV-1 genome (McGeoch et al., 1988). This sequence allows an approach whereby antisera made against synthetic oligopeptides which correspond to short amino acid sequences of predicted gene products are used to detect previously unidentified HSV proteins, or to assign encoding genes to already recognized proteins. Using this approach our laboratory has previously reported the identification of two virion proteins: gG-1, an envelope glycoprotein encoded by gene US4 (Frame et al., 1986a) and a 10K tegument phosphoprotein encoded by gene US9 (Frame et al., 1986b). We now report the identification of the products of gene UL47 as major virion proteins of apparent Ms 82000 and 81000 and show that the 82/81K proteins are regulated as true late proteins.

Methods

Cells. BHK-21 C13 cells (Macpherson & Stoker, 1962) were used throughout and were maintained in Eagle's medium supplemented with 5% tryptose phosphate and 10% newborn calf serum (Gibco) (ETC10).

Viruses. HSV-1 strain 17 syn+ (Brown et al., 1973) was used in these studies. The phosphonoacetic acid (PAA)-resistant mutant PAAr-1 was derived from HSV-1 17 syn+ (Hay & Subak-Sharpe, 1976); the mutation has been mapped within the DNA polymerase gene (Crumpacker et al., 1980).

Infection procedure. Cell monolayers at 75% confluence were infected for 1 h at a multiplicity of 20 p.f.u. in a volume of 0.5 ml of growth medium per 50 mm dish, 1.5 ml per 90 mm Petri dish or 20 ml per roller bottle. To inhibit viral DNA synthesis, cells were maintained from 1 h before and then throughout infection in medium containing 300 μg PAA (Sigma) per ml.

Synthesis of oligopeptides. Oligopeptides YGAAALRAHVSGRRA and YLTPANLIRGDNA were synthesized by continuous flow Fmoc chemistry (for reviews, see Atherton et al., 1979, Sheppard, 1983) using an LKB Biolynx peptide synthesizer. Chemicals for these syntheses were purchased from Biochrom with the exception of dimethylformamide (Rathburn Chemicals), trifluoroacetic acid (Aldrich Chemical Company) and resin to which the first amino acid had been coupled via an acid-labile bond (Peptide and Protein Research). The peptides were synthesized both directly onto the resin and onto a branching lysine core to generate a peptide with eight identical branches (Posnett et al., 1987; Tam, 1988) using two columns of the synthesizer in series. The lysine core was synthesized (onto an alanine residue coupled to the resin) using Fmoc-Lys (Fmoc) pentfluorophenyl ester (purchased from Peptide and Protein Research).

Following synthesis, the peptides were cleaved from the resin and side-chain-protecting groups removed with 95% trifluoroacetic acid and 4% (w/v) phenol in H2O using standard protocols. The Ms of the single peptides and the polylysine core were determined by mass spectrometry (M-Scan Ltd) which gave values identical to those expected. The amino acid composition of the branched peptide was determined by amino acid analysis (Cambridge Research Biochemicals) and was in agreement with expectations. The peptides were chosen in part for ease of synthesis and also because they correspond to sequences near the termini of proteins, a location which is favourable to the generation of protein-reactive sera (Palfreyman et al., 1984).
Antiserum. All antisera were raised in rabbits. Antiserum 94497 was raised against YGAAALRAHVSGRRA, which corresponds to amino acids 671 to 684 of the 693 amino acid predicted UL47 gene product (McGeoch et al., 1988) with an additional amino-terminal tyrosine (for protein coupling purposes not relevant to this manuscript), presented as an eight-branched peptide. The branched peptide was injected intramuscularly, 100 μg per immunization per rabbit without an additional hapten, in Freund’s complete adjuvant on day 0 and then in Freund’s incomplete adjuvant on days 10, 30 and 40. Animals were bled on day 50. Antiserum 18826, prepared against a synthetic oligopeptide corresponding to amino acids 476 to 487 of the 65K DNA-binding protein UL46 (McGeoch et al., 1988), has been previously described (Parris et al., 1988), as has antiserum 14327, which was prepared against a synthetic oligopeptide corresponding to amino acids 151 to 161 of the predicted US11 gene product (MacLean et al., 1987).

Radioisotopic labelling of cells. Infected and mock-infected cells were labelled with [35S]methionine (50 μCi/ml) (Amersham) immediately after adsorption in Eagle’s medium containing 20% of the normal concentration of methionine and supplemented with 20% foetal calf serum (Emet/C2). Approximately 24 h later the cells were washed twice with phosphate-buffered saline and suspended in denaturing buffer (0.05 M-Tris–HCl pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol; enough bromophenol blue was added to visualize the dye front) at a concentration of 2.5 × 106 cell equivalents/ml. This suspension was heated at 70 °C for 5 min, stored at −70 °C and boiled immediately before electrophoresis.

Purification of virions. BHK cells in 80 oz plastic roller bottles were infected at 37 °C with 40 ml ETC17 containing 1 p.f.u. HSV-1 strain 17/1500 cells. After 1 h the infection medium was removed and replaced with 40 ml of Emet/C2. After a further 8 h, [35S]methionine was added (25 μCi/ml) and incubation was continued for 2 days. The cells were then shaken into the culture medium and pelleted by low speed centrifugation (10000 g for 10 min at 4 °C). After supernatant clarification by a second low speed centrifugation, the supernatant was removed and mixed with 0.5 volumes of 3 × denaturing buffer (see above); the pellet was suspended by low speed centrifugation (10000 g for 10 min at 4 °C). After supernatant clarification by a second low speed centrifugation, the supernatant virions were pelleted (9000 g for 2 h at 4 °C). The virus pellet was resuspended and further purified by one of two methods. In the first, the resuspended pellet was centrifuged through a Ficoll 400 gradient, the virus band was then collected and pelleted by centrifugation at 20000 g for 1 h (J. F. Szilágy & C. Cunningham, personal communication). In the second, the resuspended pellet was purified by centrifugation through 54% Percoll [0.025 m-sucrose, 0.01% bovine serum albumin (BSA) and 0.5 mM-Tris–HCl pH 7.5] as described by Marsden et al. (1987).

NP40 extraction of HSV-1 virions. Extraction was as described by Frame et al. (1986b) with some modifications. Briefly, NP40 (Pierce Chemical or BDH Chemicals) was diluted to various concentrations in 20 mM-Tris–HCl, 5 mM-NaCl pH 7.5. Unlabelled or [35S]methionine-labelled HSV-1 virions were incubated in the different concentrations of NP40 for 30 min at room temperature then centrifuged at 40000 r.p.m. for 30 min at 4 °C in a TLA100.2 rotor using a Beckman TL-100 table top centrifuge. The supernatant was removed and mixed with 0.5 volumes of 3 × denaturing buffer (see above); the pellet was suspended in denaturing buffer. These suspensions were heated to 100 °C for 5 min immediately before electrophoresis. For those experiments involving iodination of virion proteins the centrifugation step was omitted.

Iodination of virion proteins. Virion proteins were iodinated essentially as described by Markwell & Fox (1978). Briefly, 2 mg Iodo-gen (Pierce) (1,3,4,6-tetrachloro-3,6-diphenylglycouril) was dissolved in 2 ml chloroform immediately before use, 25 μl was transferred to an Eppendorf tube, dried and stored under nitrogen. 125Iodine (200 μCi) (Amersham; IMS 30) was added, followed by 30 μl of Percoll-purified virions which had been diluted in phosphate buffer (pH 7.4) to a final buffer concentration of 10 mM. Incubation was performed for 10 min at between 0 and 4 °C and the reaction stopped by transferring the solution to another tube without Iodo-gen.

Gel electrophoresis and Western blotting. SDS–PAGE of proteins was conducted either with 5 to 12.5% gradient gels cross-linked with 5% (wt/wt) N,N′-methylene-bisacrylamide (BIS) or 9% gels cross-linked with 2.5% (wt/wt) N,N′-diallyl tartardiamide (DATD) using the buffer system of Laemmli (1970). Western blots were performed essentially as described (Towbin et al., 1979) with some modifications (Frame et al., 1986b).

Antiserum 14327, 18826 and 94497 were diluted 50-, 100- and 100-fold, respectively, for the experiments described here. Blotted proteins were incubated with antiserum and bound antibody was visualized with 125I-labelled Protein A. Where indicated, the antiserum was incubated with peptide for 1 h before exposure to the blotted protein. The apparent Mr of reactive polypeptides were determined by alignment of the 35S and 125I autoradiographic images (Haar et al., 1985).

Silver staining. Following electrophoresis, gels were fixed for 30 min in 30% ethanol/10% acetic acid. Gels were then removed from the fix solution and incubated for a further 30 min in a solution containing 3% ethanol, 0.5 M-malic acid, 0.5% gluteraldehyde and 0.2% sodium thiosulphate. The gels were then rinsed thoroughly three times in water for 10 min and soaked in 1% silver nitrate/0.02% formaldehyde for 15 to 30 min. Gels were then developed in a solution containing 2.5% sodium bicarbonate/0.01% formaldehyde pH 11.8 for between 5 and 15 min until all bands were visible. Development was stopped by addition of 0.05 M-EDTA for 5 min followed by a further wash in deionized water. All solutions for silver staining were prepared using high-purity filtered HPLC grade water.

Results

Fig. 1 shows the position of the open reading frame of gene UL47 on the prototype arrangement of the HSV-1 genome (McGeoch et al., 1988), together with the position and sequence of the peptide against which antiserum 94497 was raised. The reactivity on Western blots of this antiserum with proteins from HSV-1-infected cells is shown in Fig. 2. The immune serum specifically detects a strong polypeptide band of apparent Mr 82K in 5 to 12.5% gels which is not detected by the pre-immune serum (Fig. 2) and which is not present in mock-infected cells (Fig. 3, lane 1).

We next investigated the regulation of expression of this 82K protein. Cells were harvested at various times after infection and polypeptides were separated by SDS–PAGE, transferred to a nitrocellulose membrane and probed with antiserum 94497. The protein was just detectable at 6 and 8 h after infection, the amount was strongly up-regulated by 10 h and found in largest amounts at 12 h, whereas later the amount of protein present in the cells declined slightly (Fig. 3). This dramatic up-regulation was reproducibly observed and suggests that gene UL47 is regulated as a late gene.

Johnson et al. (1986) suggested an operational definition of true late genes as those whose expression is most severely reduced, compared to all other groups of genes,
under conditions of severely inhibited virus DNA replication. To determine whether gene UL47 was regulated as a true late gene according to this definition, the expression of the 82K protein was examined in the presence of 300 μg/ml PAA. At this concentration, virus DNA replication is reduced to undetectable levels (<5% of the no drug control) in cells infected with HSV-1 strain 17 (Johnson et al., 1986). The PAA-resistant mutant, PAA'^-1, (Hay & Subak-Sharpe, 1976), which induces wild-type levels of virus DNA synthesis in the presence of 300 μg/ml PAA, was included in the experiment as a control. For comparison, the behaviour of two other proteins was also examined: 21K, a true late protein (Johnson et al., 1986) and 65KDBP, an early protein (Schenk & Ludwig, 1989) whose synthesis is reduced three- to four-fold in the presence of PAA (MacLean et al., 1987). The reactivities on Western blots of the rabbit antisera 18826 and 14327 when used to detect expression of 65KDBP and 21K respectively have previously been described (Parris et al., 1988; MacLean et al., 1987). Antiserum 18826 reacts with only a single protein, 65KDBP (Parris et al., 1988), whereas antiserum 14327 reacts with predominantly 21K and proteins of apparent Mr 22K, 17.5K, 14K and 11K, which have been shown to be related to 21K (MacLean et al., 1987).

The results of this experiment are shown in Fig. 4 in which infected cells were harvested at 0, 6 h, 12 h and 24 h after adsorption. Polypeptide 82K is first detected at 12 h after adsorption both in wild-type virus-infected cells in the absence of PAA and in PAA-resistant virus-infected cells in the presence of PAA; in cells infected with wild-type virus in the presence of PAA no synthesis of 82K could be detected at any time after infection (a). This finding is exactly what would be expected for true late proteins and essentially parallels the result with 21K (b), previously found to be a true late protein (Johnson et al., 1986). In contrast, 65KDBP is clearly detectable by 6 h post-infection and is only slightly inhibited by PAA in the wild-type virus-infected cells (c). The sensitivity of detection of 82K by antiserum 94497 was examined by Western blotting twofold serial dilutions of HSV-1-infected cells. The serum could easily detect 0.5% (i.e. 1:256 dilution) of the level of protein induced in cells infected with wild-type virus in the absence of PAA (d). These experiments show that in the absence of viral DNA synthesis the expression of gene UL47 is reduced to less than 0.5% of normal. We conclude from these experiments that UL47, the gene encoding 82K, is regulated as a true late gene.

To test whether the protein product of gene UL47 is a
structural component of virus particles, virions were purified from the supernatant of [35S]methioninelabelled infected cells (Rixon et al., 1988). Fig. 5(a) is an electron micrograph of such a preparation and shows it to be substantially free of contaminating cellular debris. Virion proteins were subjected to gel electrophoresis, transferred to a nitrocellulose membrane and probed with serum 94497. Both 5 to 12.5% gradient gels cross-linked with BIS (Fig. 5, lanes 1 to 6) and 6% gels cross-linked with DATD (lanes 7 to 9) were used because in earlier experiments (Marsden et al., 1976) a polypeptide doublet of apparent Mr's of 82K and 81K had been observed in purified virions. The 6% gels cross-linked with DATD were found to resolve this doublet more clearly. Fig. 5(b) (lanes 1 and 7) show the methionine-labelled virion polypeptides which were transferred to the membrane. The profile is similar to that reported earlier (Heine et al., 1974; Marsden et al., 1976; Dargan, 1986; Rixon et al., 1988) in that the major proteins seen include the major capsid protein (Vmw155), gB, Vmw82, the 65K trans-inducing factor (Vmw65 or 65K_TIF), gD, Vmw51 (VP19C) and Vmw37. It lacks the large tegument protein Vmw273 because the latter is too large to be electroeluted from the gel and transferred to the nitrocellulose membrane (Rixon et al., 1988). Serum 94497 reacts specifically with Vmw82 (lane 2) and Vmw82/81 (lane 8) and this reaction is blocked by the peptide against which the antiserum was raised (lanes 3 to 5 and lane 9) but not by an unrelated peptide (lane 6).

Lane 7 shows virion proteins blotted from a 6% gel cross-linked with DATD in which Vmw82/81 has been resolved as a doublet. Two separate exposures of a strip probed with serum 94497 show that both proteins are recognized by the serum (lanes 8 and 9) and that the reactions are blocked by the peptide against which the antiserum was raised (lane 10). This experiment demonstrates that both proteins are encoded by gene UL47 and identifies the UL47 gene products as the previously recognized Vmw82/81 of HSV-1 virions.

To investigate where in the virion the 82/81K protein is located, purified virions were treated with various concentrations of NP40 and solubilized proteins were separated from larger virion structures by centrifugation. Proteins in both the pellet (P) and the supernatant (S) were resolved by SDS–PAGE using a 5% to 12.5% gel and proteins were visualized by silver staining (Fig. 6).
HSV-1 UL47 gene products

Fig. 5. The 82/81K polypeptide products of gene UL47 are constituents of the HSV-1 virion. (a) Electron micrography of the virion preparation used for the experiment shown in (b). Virions were prepared as described in the text. Virion sample (2 µl) was applied to a parlodion-coated copper grid, allowed to adsorb for 5 min, blotted dry and stained with 3% phosphotungstic acid pH 7.0. (b) HSV-1 virion proteins, labelled with [35S]methionine, were separated by SDS-PAGE using either 5 to 12.5% gels cross-linked with BIS (lanes 1 to 6) or 6% gels cross-linked with DATD (lanes 7 to 9) and then transferred to nitrocellulose membrane strips. Lanes 1 and 7 show autoradiographic images of 35S-labelled virion proteins. Lanes 2 to 6, 8, 9 and 10 were probed with serum 94497 in the absence of polypeptide (lanes 2, 8 and 9) or in the presence of 1 µg/ml (lane 3), 10 µg/ml (lane 4) and 100 µg/ml (lanes 5 and 10) of branched peptide YGAAALRAHVSGRR against which the antiserum was raised or 100 µg/ml of the unrelated branched peptide YLTPANLIRGDNA (lane 6). Bound antibody was visualized with 125I-Protein A (lanes 2 to 6, 8, 9 and 10). Lanes 8 and 9 show two different exposures from the same nitrocellulose strip. The major virion proteins transferred to nitrocellulose are indicated on the left.

The data show that in the absence of NP40 or with 0.01% NP40 the virions appear to be intact because no proteins are found in the supernatant. With 0.1% and 1% NP40 both gB (an envelope glycoprotein) and Vmw65 (a tegument protein; Roizman & Furlong, 1974; Preston et al., 1988) were solubilized, as was Vmw82/81. In contrast, concentrations of NP40 as high as 1% did not solubilize Vmw155 (the major capsid protein), showing that the capsids remain intact. These data suggest that Vmw82/81 is located in the virion tegument or envelope.

To determine in which of these two structures Vmw82/81 is located, virion proteins were iodinated before or after disruption with 1% NP40. In this experiment, NP40-solubilized proteins were not separated from the remainder of the virion prior to iodination. The first condition (without NP40) should label only protein exposed on the surface of virions whereas the latter condition (with NP40) should label additionally some internal proteins.

Iodinated proteins were analysed by SDS-PAGE (Fig. 7). The positions of gB, Vmw82/81, Vmw65 (trans-inducing factor) and gD were obtained by alignment with [35S]methionine-labelled virion proteins (data not shown). Both gB and gD were labelled in the presence (lane 1) and absence (lane 2) of NP40 as would be expected of surface proteins, whereas Vmw82/81 and Vmw65 (a tegument protein) were labelled only in the presence of NP40. The heavily labelled band just above the 65K trans-inducing factor in lane 1 was also present after iodination of the BSA-containing Percoll solution, and most likely represents BSA. From this experiment, we concluded that Vmw82/81 is located in the virion tegument.
Discussion

Significant differences exist in the published DNA sequences obtained for gene UL47 from HSV-1 strain F (McKnight et al., 1987) and HSV-1 strain 17 (McGeoch et al., 1988). In particular, there is an extra G residue at base 101163 of strain 17 which is not present in the sequence of strain F. As a consequence, the predicted amino acid sequences differ from the aligned prolines at position 650 of strain F and 651 of strain 17; the former continues for a further 14 residues whereas the latter continues for a further 42 residues. The sequence YGAAALRAHVSGRRA, against which antiserum 94497 was raised, corresponds to amino acids 671 to 684 of the UL47 product predicted for strain 17 (McGeoch et al., 1988), with an additional N-terminal tyrosine, but does not exist in the UL47 product predicted for strain F (McKnight et al., 1987). Our data showing that antiserum 94497 specifically identifies the products of gene UL47 of HSV-1 strain 17 as abundant virion proteins with apparent Ms of 82000 and 81000 confirms the correctness of the UL47 DNA sequence obtained for strain 17 but does not exclude the possibility that there is a strain-specific sequence difference between strain 17 and strain F.

We have established that expression of the UL47 gene is regulated in a true late manner. Our findings support earlier studies which reported a 4.7 kb true late mRNA transcribed in a leftward direction from this region of the genome (Hall et al., 1982). The quantitative data reported here (Fig. 4) show that the UL47 protein product levels in the absence of viral DNA synthesis are less than 0.5% of those in a normal infection.

Regulation of HSV-1 gene expression has been most frequently examined at the mRNA level. Evidence exists for nine true late mRNAs (Holland et al., 1980, 1984; Frink et al., Hall et al., 1982; Silver & Roizman, 1985; Johnson et al., 1986). Comparison of the positions and sizes of these transcripts with the DNA sequence obtained by McGeoch et al. (1988) suggests that these true late mRNAs may originate from genes UL22, UL31, UL32, UL38, UL44, UL45, UL47, UL49 and US11, but quantitative data for mRNA regulation has been obtained only for UL49 (Silver & Roizman, 1985) and US11 (Johnson et al., 1986). Quantitative data will have to be obtained for the others to identify these genes unambiguously as true late. At present, the designation of HSV-1 UL38 as a true late gene seems doubtful. Firstly, assembly of capsids is not dependent on virus DNA replication (reviewed by Dargan, 1986) but is dependent on the expression of HSV-1 UL38 (Pertuiset et al., 1989), the product of which is an abundant capsid protein (Rixon et al., 1990). Secondly, qualitative data have been obtained by Yei et al. (1990) suggesting that the HSV-1 gene is not regulated as a true late gene whereas the HSV-2 gene is.

At the protein level, the only gene previously shown quantitatively to be regulated in a true late manner is US11 (Johnson et al., 1986). Its product is a 21K non-structural protein (Rixon & McGeoch, 1984) which localizes to the nucleoli of infected cells (MacLean et al., 1987) and binds directly or indirectly to DNA, most likely to the 'a' sequence (Dalziel & Marsden, 1984; MacLean et al., 1987).

We have now shown that the 82/81K products of UL47 are abundant structural proteins and, because they are readily released from virions by NP40, they must be components of the tegument or envelope. Iodination in the presence but not in the absence of NP40 indicates that Vmw82/81 is located in the tegument. Examination of the amino acid sequence predicted for UL47 reveals no features characteristic of a membrane protein, which would support this conclusion.

By examination of earlier studies (Roizman & Furlong, 1974; Heine et al., 1974; Honess & Roizman, 1973), and comparison of the apparent Ms, relative intensity and virion location of the proteins described there, we
speculate that Vmw82/81 corresponds to virion proteins 13 and 14. However, this speculation remains to be experimentally tested.

The tegument is a poorly defined structure which lies between the capsid and the envelope and constitutes approximately 65% of the virion by volume (Schrag et al., 1989). Many tegument proteins are poorly characterized but some appear to function at the early stages of infection. Prominent among these is 65K_TIF, a protein of apparent Mr, 65,000, which stimulates transcription from immediate early genes (Post et al., 1981; Batterson & Roizman, 1983; Campbell et al., 1984). It has been reported (McKnight et al., 1987) that the products of genes UL46 and UL47 act to modulate (positively and negatively, respectively) the activity of 65KTIF. It is of interest that two proteins originate from UL47. It is therefore of interest that the 82/81K proteins of gene UL47 should be present in virions in amounts comparable to that of 65K_TIF (Vmw65). 65K_TIF is also of importance as a structural component of the virion (this might account for its abundance in virions) because a temperature-sensitive mutation in the gene encoding this protein confers decreased thermostability on virions (Moss, 1989). Whether Vmw82/81 will also have an equivalent structural role and whether it is essential for virus growth and assembly remain to be determined.

It is of interest that two proteins originate from UL47. In a previous analysis using two-dimensional (2D) gel electrophoresis (Marsden et al., 1983) we identified two polypeptides (spots 24 and 25) of apparent Mr, 81,000 and one polypeptide (spot 402) of apparent Mr, 82,000. We cannot unambiguously equate spots 24, 25 and 402 with either of the 82K or 81K polypeptides but, because no other spots having these apparent Mr's were detected on 2D gels and because all three spots are labelled following electrophoresis (Marsden et al., 1987) that the products of genes UL46 and UL47 act to modulate (positively and negatively, respectively) the activity of 65K_TIF. It is therefore of interest that the 82/81K products of gene UL47 should be present in virions in amounts comparable to that of 65K_TIF (Vmw65). 65K_TIF is also of importance as a structural component of the virion (this might account for its abundance in virions) because a temperature-sensitive mutation in the gene encoding this protein confers decreased thermostability on virions (Moss, 1989). Whether Vmw82/81 will also have an equivalent structural role and whether it is essential for virus growth and assembly remain to be determined.

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Note in proof: HSV-1 mutants in which gene UL47 has been deleted can grow in tissue culture [Barker, D. E. & Roizman, B. (1990). *Virology* 177, 684-691]. Misra and Carpenter (personal communication) have identified the gene encoding VP8, a major 107K tegument protein of bovine herpesvirus 1 [Marshall et al. (1986). *Journal of Virology* 57, 745-753] and have shown by sequencing the gene that VP8 is homologous to UL47 of HSV-1. They have also shown that VP8 is a phosphoprotein which is expressed late in cells infected with BHV-1.

References


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