Journal of General Virology (1992), 73, 2709–2713. *Printed in Great Britain*

Identification of genes encoding two capsid proteins (VP24 and VP26) of herpes simplex virus type 1

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The capsid of herpes simplex virus type 1 is composed of seven proteins. VP5, VP19C, VP22a and VP23 have been shown previously to be the products of genes UL19, UL38, UL26.5 and UL18, respectively. The genes encoding VP21, VP24 and VP26 have not been identified to date. We have determined amino acid sequences of fragments of isolated capsid proteins generated by partial cleavage with CNBr. The results confirm the gene assignments for VP5, VP19C and VP23. They also show that VP26 is the product of gene UL35 and that VP24 contains the protease domain present in the N-terminal portion of the UL26-encoded protein. VP21 was not investigated, but we suggest that it is the C-terminal portion of the UL26-encoded protein remaining after release of VP24 and that it thus corresponds to a form of VP22a extended at the N terminus.

The capsid of herpes simplex virus type 1 (HSV-1) appears in the electron microscope as an icosahedron containing 150 hexavalent and 12 pentavalent capsomeres (Wildy *et al.*, 1960). Gibson & Roizman (1972) identified six proteins in capsids isolated from infected cell nuclei, and a seventh smaller protein was detected subsequently (Heilman *et al.*, 1979; Cohen *et al.*, 1980). A correlation of published nomenclatures may be found in Rixon *et al.* (1990). In this paper, we employ the nomenclature of Gibson & Roizman (1972) as extended by Newcomb & Brown (1991), designating the seven proteins as VP5, VP19C, VP21, VP22a, VP23, VP24 and VP26. VP22a and possibly VP21 are abundant only in intermediate capsids, which contain a core but lack DNA, but the other proteins are present in intermediate capsids, capsids lacking a core, capsids containing DNA and mature virions (Gibson & Roizman, 1972; Cohen *et al.*, 1980; Rixon *et al.*, 1988; Sherman & Bachenheimer, 1988; Booy *et al.*, 1991). The genes encoding four of the proteins have been identified using a variety of methods.

VP5, the major capsid protein, has an apparent M_r of 155000 and forms the main component of the hexavalent, and probably the pentavalent, capsomeres (Vernon *et al.*, 1981; Schrag *et al.*, 1989; Newcomb & Brown, 1989, 1991). Costa *et al.* (1984) showed that a polyclonal antiserum against VP5 reacted with the *in vitro* translation product of a 6 kb mRNA which was selected by hybridization to a specific HSV-1 DNA fragment. The sequence of this region of the genome was determined by Davison & Scott (1986*b*), and the gene encoding VP5 was designated UL19 by McGeoch *et al.* (1988).

VP19C has an apparent M_r of 53000 and has been shown by argon ion plasma etching to be located near the capsid surface, perhaps as a component of the basal layer in which the capsids are embedded (Newcomb & Brown, 1989). Braun *et al.* (1984*b*) reported that VP19C is able to bind DNA. Rixon *et al.* (1990) determined the protein sequence of the N-terminal region of VP19C isolated from capsids, and showed that it is encoded by gene UL38. Mutants with temperature-sensitive (ts) lesions in UL38 (Pertuiset *et al.*, 1989) or UL19 (Weller *et al.*, 1987) fail to assemble capsids at the non-permissive temperature.

VP23, which has an apparent M_r of 33000, is also located near the capsid surface and may be a component of the trimeric structures linking adjacent capsomeres (Schrag *et al.*, 1989; Newcomb & Brown, 1989). Direct amino acid sequencing of the N-terminal region showed that VP23 is encoded by gene UL18 (Rixon *et al.*, 1990).

VP22a is a highly processed protein comprising many forms of which several are phosphorylated (Gibson & Roizman, 1974; Braun *et al.*, 1983, 1984*a*; Preston *et al.*, 1983). The major species have apparent M_rs in the region of 40000. VP22a is a component of the capsid core (Newcomb & Brown, 1989) and is able to assemble into toroidal structures *in vitro* (Newcomb & Brown, 1991). The transient association of VP22a with capsids during morphogenesis led Newcomb & Brown (1991) to re-emphasize the suggestion of Casjens & King (1975) that

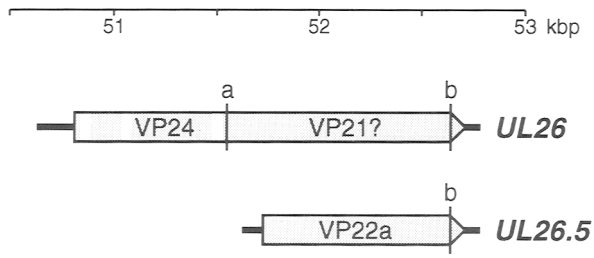

Fig. 1. Arrangement of genes UL26 and UL26.5 in the HSV-1 genome. The scale indicates locations in the HSV-1 DNA sequence (McGeoch *et al.*, 1988). The two rightward oriented mRNAs mapped by Liu & Roizman (1991*a*) are shown as horizontal lines overlaid by shaded arrows denoting protein-coding regions. Sites of proteolytic cleavage (a and b) are indicated by vertical lines. Locations of the three amino acid sequences obtained from VP24 are shown as white rectangles in the gene UL26 protein-coding region.

VP22a is analogous to the scaffolding protein of dsDNA bacteriophages. The region of the genome encoding VP22a is transcribed into two mRNAs which have identical 3′ ends but different 5′ ends, as illustrated in Fig. 1 (Liu & Roizman, 1991*a*). The smaller mRNA is specified by gene UL26.5 and encodes VP22a. The larger mRNA is transcribed from gene UL26 and encodes a protein with an apparent M_r of 75000 whose C-terminal region is identical to VP22a. This protein can proteolytically cleave itself and the gene UL26.5 protein at a site near the C terminus (site b in Fig. 1; Liu & Roizman, 1991*b*; Preston *et al.*, 1992). Proteolysis is essential for capsid maturation, since a ts mutant with a lesion in the portion of gene UL26 upstream from gene UL26.5 exhibits reduced processing of VP22a and fails to package DNA into capsids (Preston *et al.*, 1983).

Of the remaining three capsid proteins, for which the genes have not been mapped, VP26 is a component of the capsid exterior and VP21 and VP24 are core proteins that resemble VP22a in their responses to argon ion bombardment (Newcomb & Brown, 1989, 1991).

In previous attempts to map capsid protein genes by N-terminal sequencing of isolated intact capsid proteins, we obtained data for VP19C and VP23 (Rixon *et al.*, 1990). We attributed our inability to obtain sequences for other capsid proteins to N-terminal blocking. To overcome this problem, we decided to treat isolated capsid proteins with CNBr, which reacts with methionine residues, and sequence the N-terminal regions of partial cleavage products. We present data for VP5, VP19C, VP23, VP24 and VP26.

Intermediate capsids were isolated from the nuclei of BHK-21 cells infected with HSV-1 strain 17 and purified on sucrose density gradients as described by Gibson & Roizman (1972). A second preparation was purified further on Renografin density gradients as described by Perdue *et al.* (1974). Capsids were subjected to SDS–PAGE on a 5 to 15% (w/v) gradient gel, which was then fixed, stained with Coomassie blue and destained overnight. Stained bands were excised and placed in 1·5 ml microcentrifuge tubes containing 1 ml 70% (v/v) aqueous formic acid. Up to 15 gel slices (2 × 10 × 1·5 mm) containing a total of up to 30 μg of protein were placed in each tube. The gel slices were macerated using a microspatula, and approximately 0·2 μg CNBr was added per μg of protein (i.e. a ratio of 5000 moles of CNBr per mole of methionine residues). The tubes were flushed gently with nitrogen and incubated overnight in the dark at room temperature. They were centrifuged at 12000 ***g*** for 5 min and the supernatants were transferred to clean tubes. Twenty μl of 10% (w/v) SDS was added to each tube to reduce protein loss by adsorption to the plastic during subsequent reduction of sample volumes to about 50 μl in a centrifuged evaporator. Five-hundred μl of distilled water was added to each sample and the volume was again reduced to 50 μl. This step was repeated to remove residual formic acid. Fifty μl of SDS-gel loading buffer were added and the samples were boiled for 10 min. The CNBr-generated fragments were then subjected to SDS–PAGE on a 16·5% (w/v) gel which was electrophoresed employing a Tris–Tricine buffer system (Schägger & von Jagow, 1987), and electroblotted to Applied Biosystems' Problott membrane (Matsudaira, 1987) using 50 mM-glycine, 50 mM-Tris–HCl pH 8·3, 10 mM-DTT containing 20% (v/v) methanol for transfer. Coomassie blue-stained bands from VP5, VP19C, VP23, VP24 and VP26 were excised and sequenced using an Applied Biosystems 475 sequencer. The individual sequences of fragments present as mixtures in some bands were discerned routinely by virtue of their presence in unequal proportions.

Table 1 shows the correlation of sequences obtained with protein sequences predicted from the HSV-1 DNA sequence (McGeoch *et al.*, 1988). A single band containing two fragments was analysed for each of VP5, VP19C and VP23. The results confirm that these proteins are encoded by genes UL19, UL38 and UL18, respectively.

Two separate bands containing single fragments were analysed for VP26, and yielded sequences present in the gene UL35 protein (Table 1). This protein has a predicted M_r of 12095 (McGeoch *et al.*, 1988), in excellent agreement with the estimated M_r of 12000 for VP26. This result does not support the speculation of Barker & Roizman (1992) that VP26 is encoded by a previously unidentified small HSV-1 gene which they designated $U_L49.5$. On the contrary, this gene probably encodes a transmembrane protein (Barnett *et al.*, 1992).

Gene UL35 is the 3′ member of three rightward-oriented HSV-1 genes (UL33, UL34 and UL35) that

Table 1. *N-terminal sequences of CNBr-generated partial cleavage products of HSV-1 capsid proteins and correspondence to protein sequences predicted from DNA sequence data*

Protein	Sequence obtained*	Gene	Residues	Sequence predicted†
VP5	QRYLDNGRLA	UL19	202–211	(M)QRYLDNGRLA
	APAtIAAvRG	UL19	607–616	(M)APATIAAVRG
VP19C	MKTNPLPATPsV-Ggs	UL38	1–16	MKTNPLPATPSVWGGS
	KTNPLPAT	UL38	2–9	(M)KTNPLPAT
VP23	MLADGFETDIAI	UL18	1–12	MLADGFETDIAI
	LADGFETDIAIP	UL18	2–13	(M)LADGFETDIAIP
VP24	PRGPFFVGLIA-VQL	UL26	77–91	(D)PRGPFFVGLIACVQL
	EEPLPDRAVPIYVAGFLAL	UL26	11–29	(M)EEPLPDRAVPIYVAGFLAL
	LRDR-sLVAERRRQA-IAG	UL26	223–241	(M)LRDRWSLVAERRRQAGIAG
VP26	RGLVLATnn-Q	UL35	23–33	(M)RGLVLATNNSQ
	DN--PHPQGTQ	UL35	37–47	(M)DNNHPHPQGTQ

* Bracketed sequences were derived from a mixture of two fragments in a single band. Lower case letters denote tentative assignments. Hyphens denote residues for which no assignments were made.

† The preceding residue in the predicted sequence is shown in parentheses.

have recognizable counterparts in other alphaherpesviruses: varicella-zoster virus (VZV) genes 25, 24 and 23 (Davison & Scott, 1986*a*) and equine herpesvirus 1 (EHV-1) genes 27, 26 and 25 (Telford *et al.*, 1992). Counterparts of these genes in the gammaherpesvirus Epstein–Barr virus (EBV) and the betaherpesvirus human cytomegalovirus (HCMV) are more difficult to identify, since this region of the genome appears to have diverged considerably during evolution (Baer *et al.*, 1984; Chee *et al.*, 1990). Considerations of gene size, orientation and encoded amino acid similarities indicate that gene UL33 corresponds to HCMV gene UL51 and to a previously unidentified EBV gene (which we designate BFRF1A) the protein-coding region of which extends from nucleotides 58524 to 58931 in the EBV genome. Gene UL34 corresponds to HCMV gene UL50 and to EBV gene BFRF1. Gene UL35 corresponds to EBV gene BFRF3 and possibly to a previously unidentified HCMV gene (which we designate UL49A) the protein-coding region of which extends from nucleotides 70407 to 70180 in the HCMV genome. The latter gene is predicted to encode a protein with an M_r of 8480. The EBV counterpart of VP26 has not been described, but HCMV capsids contain an abundant protein with an apparent M_r of 11000 which is assumed to be the equivalent of VP26 (Irmiere & Gibson, 1985). The region between the counterparts of genes UL34 and UL35 is occupied in EBV and HCMV by the related genes BFRF2 and UL49, respectively.

Two bands isolated from different capsid preparations were analysed for VP24, and yielded the sequences listed in Table 1, which are present in the gene UL26 protein at the locations illustrated in Fig. 1. One had an approximate M_r of 18000 and gave a sequence which commenced with a proline residue preceded in the predicted protein by an aspartic acid rather than a methionine residue. This fragment was probably produced by hydrolysis of the aspartic acid–proline peptide bond under the acidic CNBr cleavage conditions (Aitken *et al.*, 1989). The other band, obtained from capsids which had been purified sequentially on sucrose and Renografin density gradients, had an approximate M_r of 24000 and contained a mixture of two fragments (Table 1). These data indicate that VP24 contains the region extending at least from residues 10 to 241 in the UL26-encoded protein. Assuming that it commences at residue 1, the M_rs of VP24 (25000) and two of the three sequenced fragments indicate that the C terminus of the capsid protein is located in the region of residue 250. The third fragment, which yielded a sequence from residue 223 in a cleavage product with an M_r of 24000, is apparently inconsistent with this conclusion. A possible explanation is that cleavage of CNBr-modified methionine residues in certain sequence contexts sometimes fails to take place efficiently and instead may occur during subsequent treatment of the excised membrane with trifluoroacetic acid at the initiation of sequencing (Aitken *et al.*, 1989). We know of no reported example, however, corresponding to the methionine residue at 222, which is set in the context of a methionine residue at 221 and a leucine residue at 223.

Recently, Liu & Roizman (1992) reported, using plasmid transfection experiments, that the N-terminal 287 residue portion of the UL26 protein lacks proteolytic activity. However, in characterizing the gene UL26 counterpart in simian cytomegalovirus (SCMV) by a similar approach, Welch *et al.* (1991*b*) showed that the N-terminal 249 residues of the encoded protein contain an active protease domain. The reason for this difference in the apparent extent of the protease domain is unclear, but expression of the transfected plasmids was not monitored directly in either study. Welch *et al.* (1991*b*)

(*a*)	HSV-1	Y	L	Q	A^S
	EHV-1	Y	L	Q	A^S
	VZV	Y	L	Q	A^S
	ILTV	Y	L	Q	A^N
	EBV	Y	L	K	A^S
	HCMV	Y	V	K	A^S
	SCMV	Y	V	K	A^S
(*b*)	HSV-1	L	V	N	A^S
	EHV-1	T	V	D	A^S
	VZV	A	V	E	A^S
	ILTV	T	V	D	A^S
	EBV	L	V	Q	A^S
	HCMV	V	V	N	A^S
	SCMV	V	V	N	A^S

Fig. 2. Alignment of potential sites of proteolytic cleavage in the HSV-1 gene UL26 protein and its counterparts in other herpesviruses. Parts (*a*) and (*b*) correspond to sites a and b in Fig. 1. Proposed cleavage sites are indicated by arrowheads. Sequence data are from: HSV-1, McGeoch *et al.* (1988); EHV-1, Telford *et al.* (1992); VZV, Davison & Scott (1986*a*); infectious laryngotracheitis virus (ILTV), Griffin (1990); EBV, Baer *et al.* (1984); HCMV, Chee *et al.* (1990); SCMV, Welch *et al.* (1991*a*). The alignment is based on that presented by Welch *et al.* (1991*b*), and includes an improved alignment for VZV site b.

located a cleavage site near the C terminus of VP22a between an alanine and a serine residue which Gibson *et al.* (1990) had shown previously are conserved in other herpesviruses (site b in Fig. 1). They also noted a similar conserved sequence between the protease and VP22a domains in the UL26-encoded protein, and suggested that the protease is able to release itself from the UL26 protein by cleaving at this site (site a in Fig. 1). An alignment of the two sites at which the protease is proposed to act is shown in Fig. 2. The consensus is V/L-X-A-S, where X is a polar residue (Q, N, K, D or E in the examples shown) and proteolysis occurs between the A and S residues. Cleavage at these sites in the 635 residue HSV-1 gene UL26-encoded protein would result in a 247 residue protease domain with a predicted M_r of 26618 from the N-terminal region, a central 363 residue protein and a 25 residue C-terminal peptide. Cleavage of the 329 residue UL26.5-encoded protein would result in a 304 residue VP22a and the C-terminal peptide. This scheme is illustrated in Fig. 1 and is fully consistent with the identification of the protease domain as VP24. The 363 residue protein thus represents a form of VP22a extended at the N terminus, and may correspond to VP21, which has been proposed to be a slowly migrating form of VP22a (Braun *et al.*, 1984*a*). Direct proof of this is lacking, however, and is likely to arise from immunological studies. It is not known whether the 25 residue protein is present in capsids.

The results of our analysis indicate that a potentially active protease is present in capsids and virions. Synthesis of the gene UL26 protein as a fusion of the protease and VP22a domains suggests that it associates with VP22a (produced from gene UL26.5), forming a core for assembly of intermediate capsids. Although there is no evidence that core formation precedes capsid assembly, the observation that proteolysis can occur in the absence of capsid formation (Liu & Roizman, 1991*b*; Welch *et al.*, 1991*b*; Preston *et al.*, 1992) suggests that the protease may act during formation of the core. Inclusion of the protease as an internal capsid component further indicates that it may function after capsid formation has commenced, perhaps facilitating release of VP22a from intermediate capsids prior to DNA packaging. Moreover the apparent retention of VP24 within the mature virion suggests that it either has a direct role in capsid structure or may act during the initial stages of infection and capsid disaggregation. Clearly, elucidation of the precise roles of the protease is now a matter of pressing interest.

The authors thank John Subak-Sharpe for his continuing interest, Valerie Preston for useful discussions and critical reading of the manuscript and Clare Addison for technical assistance.

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(*Received 2 April 1992; Accepted 11 June 1992*)