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Assembly of Herpes Simplex Virus (HSV) Intermediate Capsids in Insect Cells Infected with Recombinant Baculoviruses Expressing HSV Capsid Proteins

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The capsid of herpes simplex virus type 1 (HSV-1) is composed of seven proteins, VP5, VP19C, VP21, VP22a, VP23, VP24, and VP26, which are the products of six HSV-1 genes. Recombinant baculoviruses were used to express the six capsid genes (UL18, UL19, UL26, UL26.5, UL35, and UL38) in insect cells. All constructs expressed the appropriate-size HSV proteins, and insect cells infected with a mixture of the six recombinant baculoviruses contained large numbers of HSV-like capsids. Capsids were purified by sucrose gradient centrifugation, and electron microscopy showed that the capsids made in Sf9 cells had the same size and appearance as authentic HSV B capsids. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis demonstrated that the protein composition of these capsids was nearly identical to that of B capsids isolated from HSV-infected Vero cells. Electron microscopy of thin sections clearly demonstrated that the capsids made in insect cells contained the inner electron-translucent core associated with HSV B capsids. In infections in which single capsid genes were left out, it was found that the UL18 (VP23), UL19 (VP5), UL38 (VP19C), and either the UL26 (VP21 and VP24) or the UL26.5 (VP22a) genes were required for assembly of 100-nm capsids. VP22a was shown to form the inner core of the B capsid, since in infections in which the UL26.5 gene was omitted the 100-nm capsids that formed lacked the inner core. The UL35 (VP26) gene was not required for assembly of 100-nm capsids, although assembly of B capsids was more efficient when it was present. These and other observations indicate that (i) the products of the UL18, UL19, UL35, and UL38 genes self-assemble into structures that form the outer surface (icosahedral shell) of the capsid, (ii) the products of the UL26 and/or UL26.5 genes are required (as scaffolds) for assembly of 100-nm capsids, and (iii) the interaction of the outer surface of the capsid with the scaffolding proteins requires the product of the UL18 gene (VP23).

Herpes simplex virus type 1 (HSV-1) is a member of the *Herpesviridae* family of viruses (36). Like the other members of this family, the HSV-1 virion is composed of four distinct components: an electron-dense core containing the viral DNA, an icosahedral capsid, an amorphous layer termed the tegument, and a trilaminar lipid envelope (8, 33, 36, 40, 50). The capsid consists of 162 capsomers (150 hexavalent and 12 pentavalent capsomers) which lie on a T=16 icosahedral lattice (50). When herpesvirus-infected cells are disrupted, three types of capsid structures, designated A, B, and C, or empty, intermediate, and full, can be isolated by sucrose gradient centrifugation (10, 26–28, 33, 38). Both A and B capsids lack DNA and are found in the nuclei of infected cells, while C capsids contain DNA and mature into infectious virions (26). Pulse-chase experiments with equine herpesvirus have shown that B capsids are the precursors to both A and C capsids (32, 39). A capsids are considered to result from abortive attempts at packaging DNA into B capsids.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis has shown that HSV-1 capsids are composed of seven proteins, VP5, VP19C, VP21, VP22a, VP23, VP24, and VP26, and the genes that encode these proteins are, respectively, UL19, UL38, UL26, UL26.5, UL18, UL26, and UL35 (2–4, 6, 17, 20–22, 29–31, 35, 41). The icosahedral shell of the capsid is composed of at least three of these proteins. The major capsid protein, VP5, accounts for approximately 70% of the mass of the surface shell, while VP19 and VP23 make up the remainder of the shell (23–25, 42, 47). VP5 is the structural subunit of both the hexons and the pentons, while the intercapsomeric fibrils or triplexes that link the capsomers together are made up of VP19 and VP23 (25, 47). The shell structure is common to all three capsid types. B capsids differ from A and C capsids in that they contain two additional polypeptides, VP22a and VP21, that are located inside the shell (10, 23, 24). Packaging of DNA into B capsids results in the concomitant loss of VP22a and VP21. The ability of VP22a to self-assemble into 60-nm structures (toroids) in vitro suggests that VP22a functions as a scaffold in the inner capsid shell around which VP5, VP19C, and VP23 condense to form the capsid shell (24). The location in the capsid of VP24 and VP26 is not known, although studies by Newcomb and Brown (23, 24) indicate that VP26 is located in the icosahedral shell, since it binds to capsomers in a 1:1 complex with VP5.

Although considerable progress has been made in defining where the different capsid proteins reside within the capsid, a full understanding of the assembly mechanisms that specify how the seven proteins interact to form intact particles is lacking. In order to study the interaction of these proteins in the formation of capsids, we have expressed the six HSV-1 capsid genes in insect cells with recombinant baculoviruses and demonstrate assembly of HSV-1-like capsids in cells infected with a mixture of the six viruses. Coinfections with combinations of these recombinant baculoviruses enabled us to examine the interactions of the capsid proteins and to define

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the role of some of these proteins in assembly of HSV-1 B capsids.

MATERIALS AND METHODS

Cells, viruses, and antibodies. Previously described procedures were employed for growth and maintenance of African green monkey kidney cells (Vero, ATCC CCL 81 [14]), C1 cells (44), HSV-1 wild-type strain KOS (14), and the HSV-1 UL28 deletion mutant GCB (44). Baculovirus *Autographa californica* nuclear polyhedrosis virus was grown in *Spodoptera frugiperda* (Sf9) cells (ATCC CRL 1711) as previously described (43). Monoclonal antibody MCA406 (Serotec Inc.) was used to analyze the UL26 and UL26.5 proteins (5). Rabbit polyclonal antiserum CP3 to the UL18 protein was provided by Stan Person, University of Pittsburgh Medical School, Pittsburgh, Pa. (6). TrpE-UL35 rabbit polyclonal antiserum to the UL35 protein was provided by Richard Courtney, Pennsylvania State University College of Medicine, Hershey (21). The UL19 and UL38 proteins were analyzed with a rabbit polyclonal antiserum (Q49) raised by immunizing rabbits with sucrose gradient-purified HSV-1 B capsids. The HSV-1 capsids were isolated as described below (see Transmission EM).

Construction of recombinant baculoviruses. The six HSV-1 capsid genes were subcloned from an *Eco*RI library of the strain KOS cloned in pBR325 (11). The UL18 and UL19 genes were subcloned from pSG16, UL26 and UL26.5 were subcloned from pSG18, UL35 was subcloned from pSG3, and UL38 was subcloned from pSG124. The restriction sites flanking each open reading frame used in the construction of each recombinant plasmid are shown in Fig. 1. The UL18, UL19, UL26, and UL26.5 genes were cloned into the baculovirus transfer vector pAc373 (16, 43). The UL18 (following the addition of *Bam*HI linkers) and UL19 genes were cloned into the *Bam*HI site of pAc373, while the UL26 and UL26.5 (following addition of *Bgl*II linkers to the *Pvu*I site) genes were cloned into the *Bam*HI-*Kpn*I sites of pAc373. PCRs were used to engineer a *Bgl*II restriction site just upstream from the translation start codon of the UL26 gene. The UL35 (blunt-end ligation) and the UL38 (following addition of *Bam*HI linkers to the *Dra*I site) genes were ligated into the *Bam*HI site of the baculovirus transfer vector pVL941 (43). The resulting isolates were then screened for proper orientation of the HSV-1 open reading frames with respect to the baculovirus polyhedrin promoter. Recombinant baculoviruses expressing HSV-1 capsid proteins (BAC-UL18, BAC-UL19, BAC-UL26, BAC-UL26.5, BAC-UL35, and BAC-UL38) were generated as previously described (16), with the baculoGold transfection kit supplied by Pharmingen.

Transmission EM. (i) Thin sections. Monolayers of Vero cells were infected with KOS or GCB at a multiplicity of infection (MOI) of 10, and at 12 h postinfection the cells were harvested. Monolayers of Sf9 cells were infected with BAC-UL18, BAC-UL19, BAC-UL26, BAC-UL26.5, BAC-UL35, and BAC-UL38 individually or with different combinations of these viruses at a MOI of 5 (each virus), and at 64 h postinfection the cells were harvested. Thin sections were prepared for electron microscopy (EM) as described by Tengelsen et al. (44).

(ii) Negative staining. Suspension cultures (100 to 500 ml) of Sf9 cells were infected with the baculovirus recombinants at a MOI of 5 (each virus), and at 64 h postinfection the cells were harvested. Monolayers of Vero cells were infected with KOS at a MOI of 10, and at 12 h postinfection the cells were harvested. The cell pellets from Vero or Sf9 cells were resuspended in 2× capsid lysis buffer (1 M NaCl, 40 mM Tris-HCl [pH 7.5], 2 mM EDTA, 2% Triton X-100), freeze-thawed three times, sonicated, and centrifuged for 5 min at 5,000 rpm in an SA600 rotor, and the cleared lysates were layered onto a 20 to 65% sucrose gradient (made in phosphate-buffered saline [PBS]) and centrifuged at 24,000 rpm in a SW28 or SW41 rotor for 60 min at 4°C. Capsids were observed as visible light-scattering bands and were harvested and concentrated by diluting the sample in PBS or 1× capsid lysis buffer and centrifuged for 1 h at 20,000 rpm in a SW28 or SW41 rotor. The pellet was resuspended in 0.1 to 0.2 ml of PBS overnight at 4°C, and 50 μl of the suspension was mixed with 100 μl of a 2% phosphotungstic acid (pH 6.0)–0.05% bovine serum albumin solution. A drop of this mixture was absorbed onto a carbon grid (Ted Pella, Inc.) and allowed to absorb for 0.5 min. Excess fluid was removed by touching the edge of the grid to filter paper. The samples were visualized on a JOEL 1200ES transmission electron microscope.

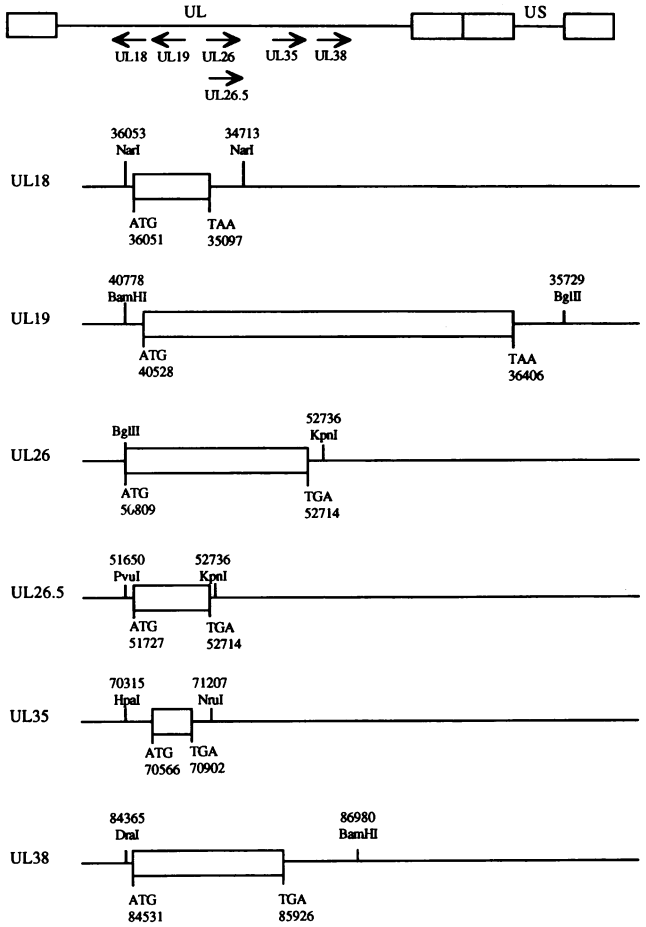


FIG. 1. Schematic representation of the six HSV-1 capsid genes. The HSV-1 genome is shown at the top, with UL and US referring to the long and short unique region sequences, respectively. The approximate location and direction of transcription of the six capsid genes are indicated. The six capsid genes with the region of the complete open reading frame of each gene are displayed at the bottom. The first nucleotide of the translation initiation codon and the first nucleotide of the stop codon are shown along with the restriction sites that were used to clone each gene into recombinant baculovirus. The numbering follows that of McGeoch et al. (20).

Immunoprecipitation. Sf9 cells were infected at a MOI of 5 with recombinant baculovirus. At 24 h postinfection, cells were

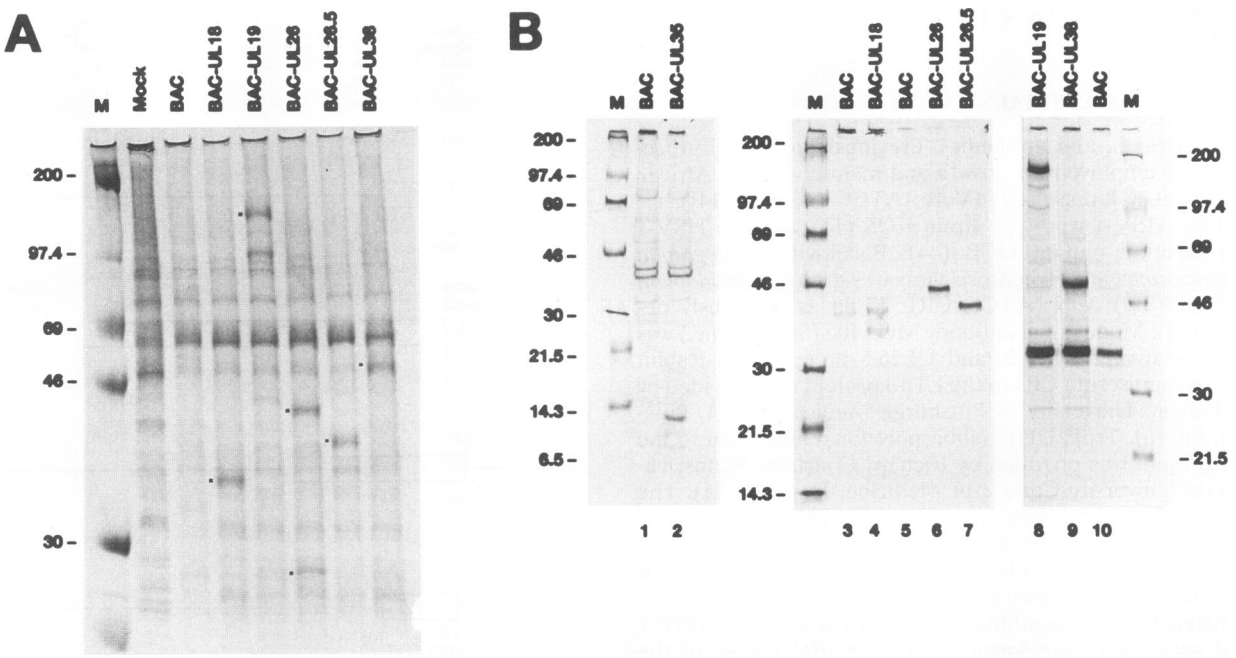


FIG. 2. Polypeptides synthesized by recombinant baculoviruses. (A) Proteins synthesized in mock-infected Sf9 cells (Mock), cells infected with a control baculovirus expressing the cytomegalovirus DNA polymerase (BAC), or cells infected with the indicated virus were separated by electrophoresis through a SDS–10% polyacrylamide gel, and proteins were detected by staining with Coomassie blue. The HSV-1 capsid proteins expressed by each recombinant baculovirus are indicated by dots. (B) Sf9 cells infected with a control virus (BAC) or with the indicated virus were labelled with [^{35}S]methionine from 24 to 48 h postinfection. Proteins were precipitated from lysates of infected cells with rabbit polyclonal antisera TrpE-UL35 (lanes 1 and 2), CP3 (lanes 3 and 4), or Q49 (lanes 8 to 10), or a mouse monoclonal antibody MCA406 (lanes 5 to 7). The source and specificity of these antibodies are described in Materials and Methods. The immunoprecipitated material was separated on either 10 to 20% (lanes 1 and 2), 15% (lanes 3 to 7), or 10% (lanes 8 to 10) polyacrylamide–SDS gels. Lanes M, marker proteins (with molecular mass shown at the sides [in kilodaltons]).

labelled with 50 μCi of [^{35}S]methionine per ml in Grace's methionine-free insect cell medium (GIBCO) containing 5% fetal bovine serum and 10% the normal concentration of methionine. At 48 h postinfection, the cells were harvested and proteins were immunoprecipitated and analyzed by SDS-PAGE as described previously (13).

Western immunoblots. Sf9 cells were infected at a MOI of 5, and at 64 h postinfection the cells were harvested and prepared for Western immunoblots as previously described (44). The Western-Light chemiluminescent detection system (Tropix, Inc., Bedford, Mass.) was used to identify proteins reactive with the UL26- and UL26.5-specific monoclonal antibody, MCA406.

RESULTS

Expression of the six HSV-1 capsid proteins in Sf9 cells. The open reading frames for the HSV-1 UL18, UL19, UL26, UL26.5, UL35, and UL38 genes were cloned into baculovirus expression vectors by using the restriction sites shown in Fig. 1. These vectors were then used to isolate recombinant baculoviruses BAC-UL18, BAC-UL19, BAC-UL26, BAC-UL26.5, BAC-UL35, and BAC-UL38 as described in Materials and Methods. Expression of HSV-1 capsid proteins from these recombinant baculoviruses was initially confirmed in infected-cell lysates. Sf9 cells (T25 flask) were mock infected or infected with 5 PFU per cell of each virus, and at 64 h postinfection total cell proteins were prepared by direct lysis in 0.5 ml of sample buffer. Twenty microliters of each sample was loaded on a 12.5% polyacrylamide gel, and after electrophoresis, the gel was stained with Coomassie blue. Figure 2A shows that with the exception of the UL35 protein (data not shown), the HSV-1 capsid proteins were all expressed at high levels. The products of the UL18, UL19, UL26.5, and UL38 genes exhibited gel mobilities similar to those predicted from DNA sequence analysis (20). As described in the next section, the autoprotease activity of the UL26 protein results in the generation of the two novel polypeptides (45 and 29 kDa) seen in BAC-UL26-infected cells. The gene-specific products of the BAC-UL18, BAC-UL19, BAC-UL26, and BAC-UL38 virus infections were virtually identical in size to the VP23, VP5, VP21, and VP19C proteins found associated with B capsids purified from HSV-1-infected Vero cells (data not shown). To demonstrate that the proteins identified by Coomassie blue staining were HSV-1 capsid proteins, the baculovirus-expressed proteins were examined by immunoprecipitation with antisera specific for each of the capsid proteins. Baculovirus-infected cells were labelled from 24 to 48 h postinfection with [^{35}S]methionine, and HSV-1 capsid proteins were immunoprecipitated with monoclonal or polyclonal serum. The TrpE-UL35 antiserum specifically immunoprecipitated a 12-kDa protein from cells infected with BAC-UL35 (Fig. 2B, lanes 1 and 2), while the CP3 antisera specifically immunoprecipitated a 33-kDa protein from BAC-UL18-infected cells (Fig. 2B, lanes 3 and 4). The MCA406 monoclonal antibody immunoprecipitated a 45-kDa protein from BAC-UL26-infected cells and a 35-kDa protein from BAC-UL26.5-infected cells (Fig. 2B, lanes 5 to 7). The MCA406 antibody recognizes an epitope

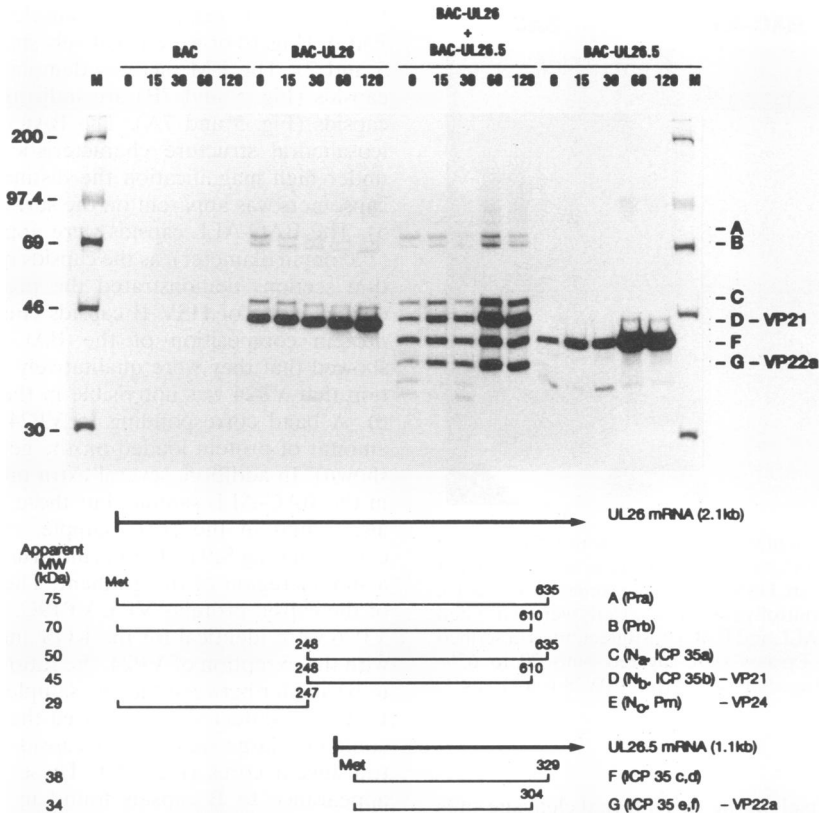


FIG. 3. Pulse-chase analysis of the protease activity associated with the UL26 gene product. (Top) Sf9 cells were infected with a control virus (BAC) or with the indicated viruses. At 48 h postinfection, cells were pulsed with [^{35}S]methionine for 30 min and chased with unlabelled methionine-containing media for the indicated times (in minutes). Labelled proteins were precipitated from lysates of infected cells with monoclonal antibody MCA406, and the immunoprecipitated material was separated on SDS–10% polyacrylamide gels. Lanes M, molecular mass (in kilodaltons) marker proteins. (Bottom) Diagram of the HSV-1 UL26 and UL26.5 genes and their gene products. The different cleaved forms of the UL26 and UL26.5 proteins are shown (size of each product is indicated). The different proteins are listed as A to G along the right margin, and the corresponding protein is indicated in the polyacrylamide gel above. The nomenclatures that Deckman et al. (5) and Liu and Roizman (17–19) have used to describe each protein are indicated in parentheses. The proteins that correspond to HSV capsid proteins VP21, VP22a, and VP24 are indicated.

in the C-terminal portion of the UL26 protein (5), and this accounts for why the 29-kDa N-terminal peptide was not immunoprecipitated from the BAC-UL26-infected cells (see next section and Fig. 3). The Q49 antiserum, which was made by immunizing rabbits with purified HSV-1 B capsids, specifically immunoprecipitated a 150-kDa protein from BAC-UL19-infected cells and a 50-kDa protein from BAC-UL38-infected cells (Fig. 2B, lanes 8 to 10). These results demonstrate that the recombinant baculoviruses are expressing HSV-1 capsid proteins and that these proteins appear to be identical in size to the proteins found associated with HSV-1 B capsids.

Processing of the UL26 and UL26.5 gene products by the protease activity associated with the UL26 protein. The UL26 and UL26.5 genes have identical 3′ ends but different 5′ ends (Fig. 3). The promoter for the UL26.5 gene is contained within the coding region of the UL26 gene. The smaller UL26.5 gene contains a 329-amino-acid open reading frame which is in frame with the 635-amino-acid open reading frame encoded by the larger UL26 gene. The UL26 gene encodes a protease that cleaves itself and the UL26.5 protein at a position 25 amino acids from the C terminus of these two proteins (Fig. 3, bottom). In addition, the UL26 protein cleaves itself at a second site (between amino acids 247 and 248 [Fig. 3, bottom]) to yield two peptides of 45 kDa (VP21) and 29 kDa (VP24). The BAC-UL26 virus expresses an active protease, since the only forms of the UL26 protein that were detected in cell extracts (Fig. 2A) were the cleaved 45- and 29-kDa peptides. In addition, the MCA406 monoclonal antibody failed to immunoprecipitate any full-length UL26 protein after an overnight label, indicating that the protein was rapidly cleaved (Fig. 2B, lane 6). As described above, the MCA406 antibody recognizes an epitope in the C-terminal portion of the UL26 protein that is shared with the UL26.5 protein and accounts for the inability of the 29-kDa UL26 peptide to be immunoprecipitated by MCA406 (Fig. 3).

In order to examine the kinetics and cleavage site specificity of the baculovirus-expressed UL26 protease against itself and the UL26.5 protein, a series of pulse-chase experiments was done (Fig. 3, top). Sf9 cells were infected with BAC-UL26, BAC-UL26.5, BAC-UL26 plus BAC-UL26.5, or a control baculovirus, and 48 h postinfection the cells were pulse-labelled with [^{35}S]methionine for 30 min and chased with cold methionine. Samples were taken at 0, 15, 30, 60, and 120 min after the addition of cold methionine-containing media. The UL26 and UL26.5 proteins were immunoprecipitated with the MCA406 antibody and analyzed by SDS-PAGE. The results for the kinetic analysis are shown in Fig. 3. The UL26 protein

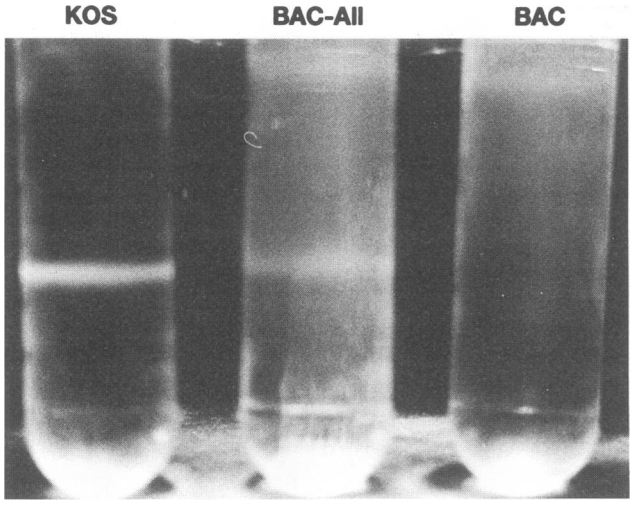


FIG. 4. Rate-velocity sedimentation of capsids from HSV-infected Vero cells (KOS), Sf9 cells infected with a mixture of recombinant baculoviruses expressing the six HSV-1 capsid proteins (BAC-ALL), or Sf9 cells infected with a control virus (BAC). Cells were harvested at 12 h (KOS) or 64 h (BAC-ALL and BAC) postinfection as described in Materials and Methods. Lysates were layered onto 20 to 65% sucrose gradients and centrifuged at 24,000 rpm (SW28 rotor) at 4°C for 1 h.

(75 kDa) rapidly cleaved itself at the two internal cleavage sites to yield three proteolytic products of 70, 50, and 45 kDa. These four peptides were detected at all of the postchase time points. The two cleavage sites within the UL26 protein appear to be cleaved equally, since the fully cleaved 45-kDa form was the predominate species at all times postchase. Cells infected with BAC-UL26.5 accumulated only the unprocessed form (38 kDa) of this protein, but in cells infected with both BAC-UL26 and BAC-UL26.5, the UL26.5 protein was processed to a 34-kDa form which increased at later times postchase. In addition, the doubly infected cells contained all the different proteolytic forms of the UL26 protein that were seen in cells infected with BAC-UL26 alone. These results demonstrate that the baculovirus-expressed UL26 protease cleaves both the UL26 and the UL26.5 proteins at the previously identified processing sites within these proteins (5, 7, 17–19, 48) and that the efficiency of cleavage at the two sites is similar.

Assembly of HSV-1 capsids in Sf9 cells expressing the six HSV-1 capsid genes. To determine whether the six HSV-1 capsid proteins expressed by the recombinant baculoviruses could self-assemble to form HSV-1 capsids, a spinner culture of Sf9 cells (100 ml) was infected at an MOI of 5 with each virus or with a single control virus, and 64 h postinfection the cells were harvested. In addition, Vero cells were infected with the KOS strain of HSV-1, and 12 h postinfection the cells were harvested. Cell extracts were layered onto 20 to 65% sucrose gradients, and after sedimentation, the gradients were visualized (Fig. 4). Lysates of KOS-infected cells yielded a major light-scattering band which corresponded to B capsids. Lysates from Sf9 cells infected with the mixture of baculoviruses expressing the six HSV-1 capsid genes (BAC-ALL) contained a light-scattering band which sedimented in the same region of the gradient as HSV-1 B capsids. The control baculovirus infection (BAC) did not yield a visible band on the sucrose gradient. The bands from the KOS and BAC-ALL gradients were harvested along with the identical region of the gradient from the BAC sample. The samples were subjected to SDS-PAGE (Fig. 6) or were negatively stained for EM analysis (Fig. 5 and 7). The EM pictures demonstrate that the BAC-ALL capsids (Fig. 5 and 7B) are indistinguishable from the KOS capsids (Fig. 5 and 7A). The BAC-ALL capsids showed the icosahedral structure characteristic of HSV-1 capsids, and under high magnification the distinctive configuration of the capsomers was apparent on the surface of these particles (Fig. 5). The BAC-ALL capsids were approximately the same size (100 nm in diameter), as the capsids made in Vero cells and the thin sections demonstrated the presence of the inner core characteristic of HSV B capsids (Fig. 5). Comparison of the protein composition of the BAC-ALL and KOS capsids showed that they were qualitatively identical, with the exception that VP24 was not visible in the BAC-ALL sample (Fig. 6). A band corresponding to VP24 was apparent when the amount of protein loaded on the gel was increased (data not shown). In addition, several extra protein bands were present in the BAC-ALL sample, but these extra protein bands were also found in the BAC sample, indicating that they were contaminating Sf9 cell or baculovirus proteins that sediment in a similar region of the gradient. The electrophoretic mobility of the capsid proteins VP5, VP19C, VP21, VP22a, VP23, and VP26 were identical for the KOS and BAC-ALL capsids, and with the exception of VP24, the ratio of these proteins appears to be similar between the two samples. Finally, thin sections of BAC-ALL-infected cells showed that the nuclei of these cells contained large numbers of capsids that contained electron-translucent cores (Fig. 8C). These particles are identical in appearance to B capsids found in the nuclei of Vero cells infected with either KOS (Fig. 8A) or a mutant virus, GCB (Fig. 8B), that makes only B capsids because of a deletion in one of the genes (UL28) involved in DNA packaging (44). Thus, expression in insect cells of the six HSV-1 capsid genes results in assembly of authentic HSV-1 B capsids.

The requirements of the seven capsid proteins for assembly of HSV-1 capsids in Sf9 cells. To investigate the need for individual HSV-1 gene products in capsid assembly, we infected Sf9 cells with separate mixtures containing either all six baculovirus recombinants or mixtures from which one or more of the viruses was omitted. At 64 h postinfection, cells were harvested and cell extracts were either layered onto 20 to 65% sucrose gradients or fixed and thin sectioned for EM analysis. After sedimentation, the region of the gradient that corresponded to the HSV-1 B capsid band was harvested (regardless of whether a band was visible or not). The banded samples were diluted, concentrated by centrifugation, and either negatively stained for EM analysis or run on SDS-polyacrylamide gels to examine protein composition. The results of the gel and EM analyses are shown in Fig. 7 to 9, and a summary of the data are presented in Table 1.

UL19 and UL38. The most drastic effect on capsid assembly was observed when the BAC-UL19 or BAC-UL38 viruses (ALL-19 and ALL-38 in Table 1) were omitted. No capsid-like structures were observed in thin sections of cells infected with either virus mixture (Fig. 8F and 8I). Similarly, capsids were not observed when lysates from the same cells were fractionated by sucrose density gradient ultracentrifugation and the B capsid region was examined by EM (data not shown) or by SDS-PAGE (Fig. 9A and B, lanes 2 and 5). We conclude, therefore, that the products of genes UL19 and UL38 are essential for capsid assembly.

UL26, UL26.5, and UL35. When the BAC-UL26, BAC-UL26.5, and BAC-UL35 viruses (ALL-26, ALL-26.5 and ALL-35 in Table 1) were omitted from the infection, a band

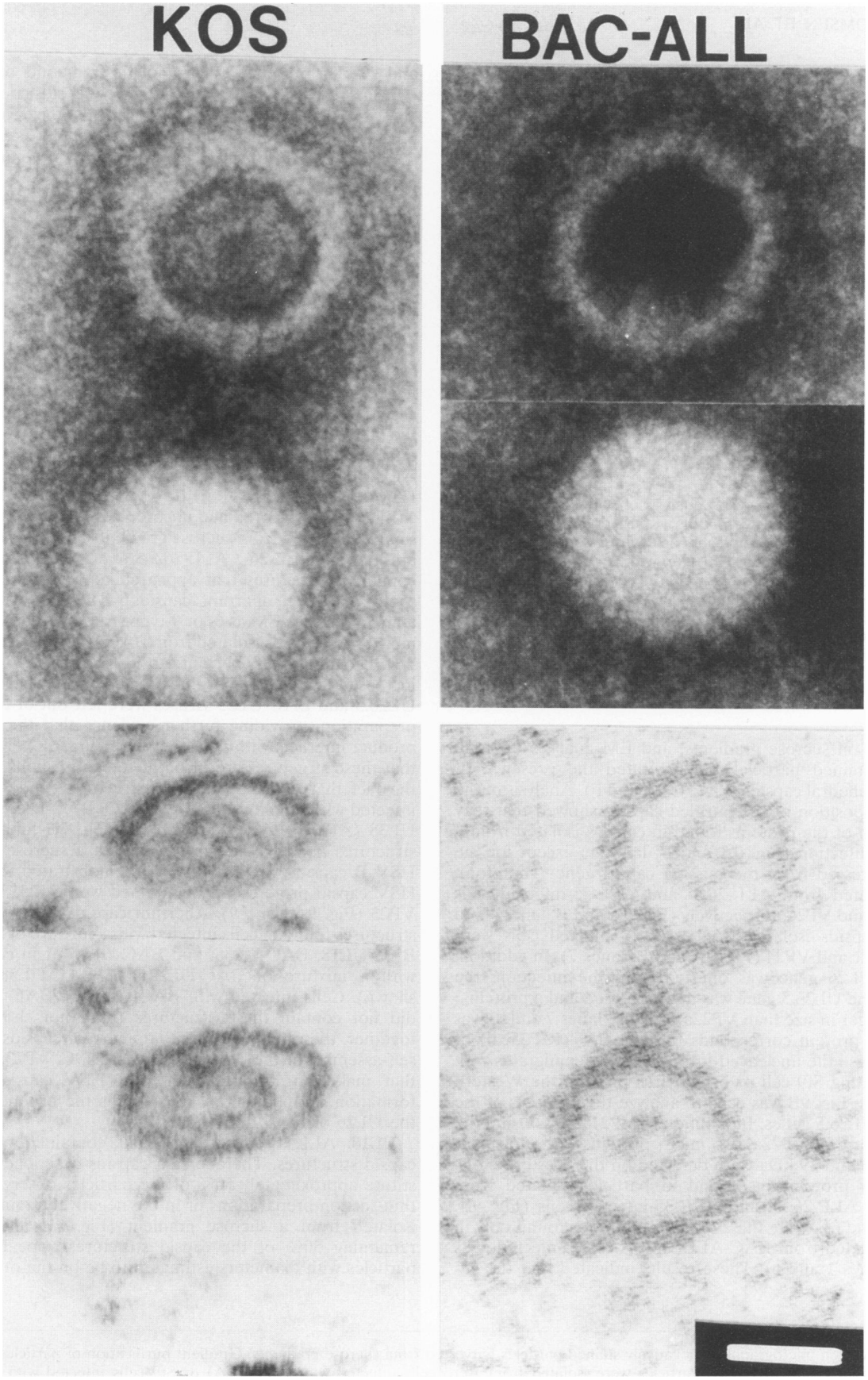


FIG. 5. EM analysis of capsids made in HSV-infected Vero cells (KOS) or Sf9 cells infected with a mixture of recombinant baculoviruses expressing the six HSV-1 capsid proteins (BAC-ALL). (Top) Negatively stained capsids isolated from sucrose gradients (see Fig. 4). (Bottom) Thin sections of infected Vero or Sf9 cells. Bar, 50 nm.

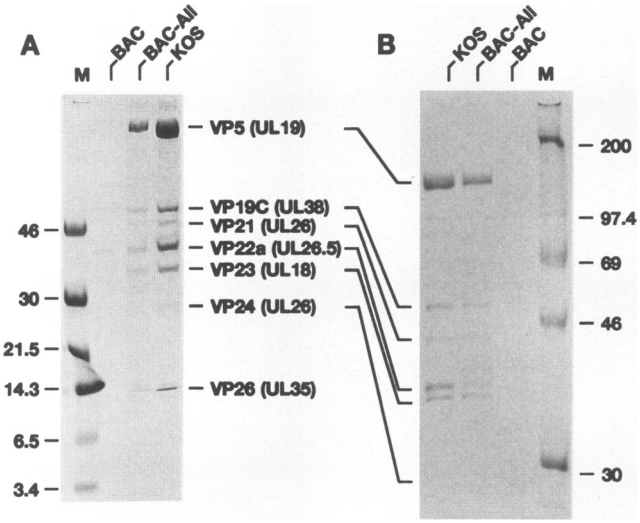


FIG. 6. SDS-polyacrylamide gel analysis of sucrose gradient purified capsids. HSV-infected Vero cells (KOS) or Sf9 cells infected either with a mixture of recombinant baculoviruses expressing the six HSV-1 capsid proteins (BAC-ALL) or with a control baculovirus (BAC) were tested. Capsid proteins were run on 10 to 20% (A) or 10% (B) polyacrylamide–SDS gels, and proteins were visualized by staining with Coomassie blue. The mobilities of the seven capsid proteins are marked, and the HSV-1 gene that codes for each protein is indicated in parentheses. Lane M, molecular mass (in kilodaltons) marker proteins.

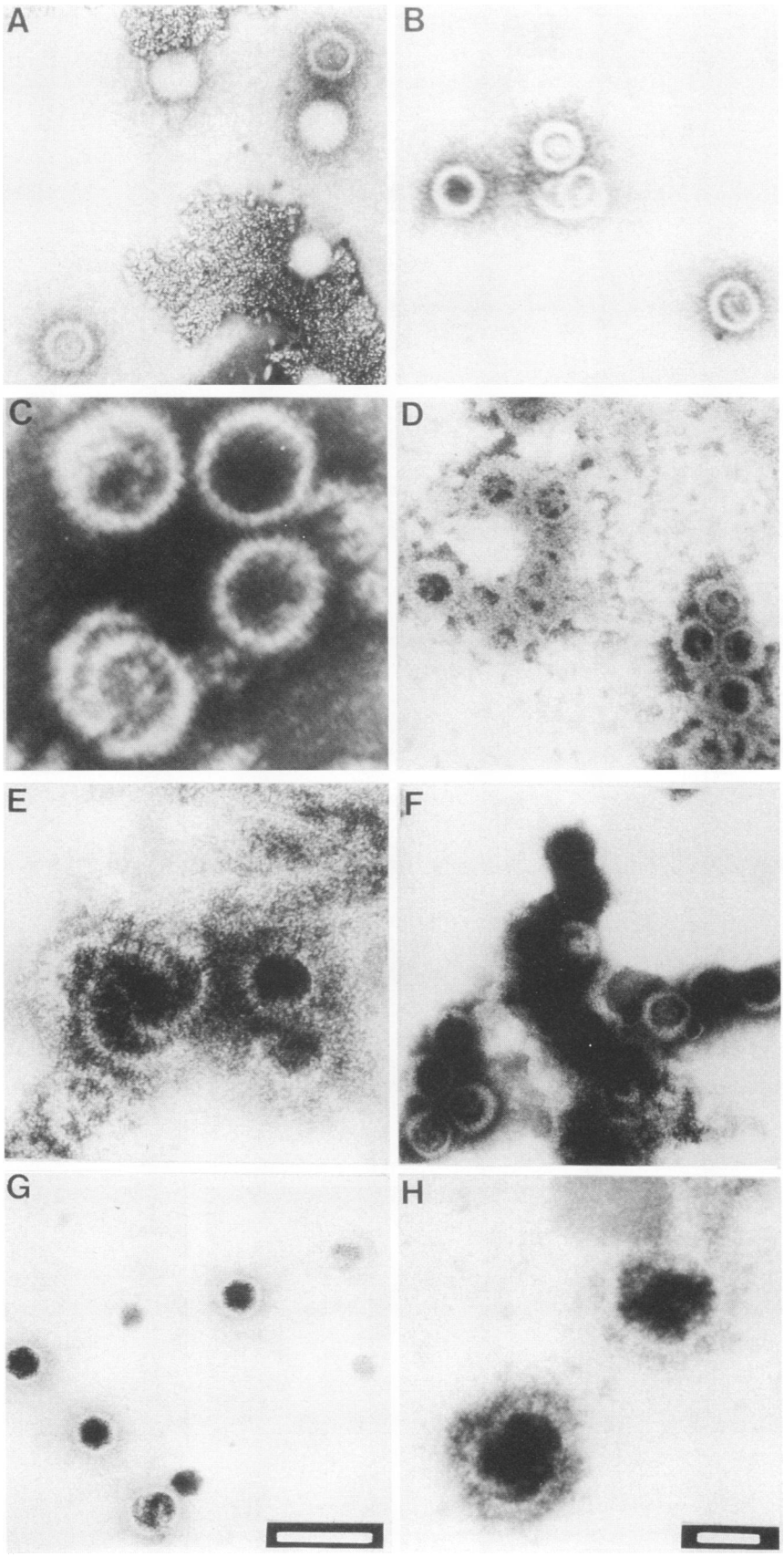
was present on sucrose gradients, and EM analysis of these negatively stained particles demonstrated the presence of 100-nm icosahedral capsids (Fig. 7C, D, and F). Analysis of the protein composition of the purified capsids showed that they contained all of the proteins found in capsids isolated from a BAC-ALL infection (Fig. 9A and B, lanes 1) except for the protein expressed from the missing capsid gene. Therefore, capsids isolated from ALL-26.5- and ALL-35-infected cells lack VP22a and VP26, respectively (Fig. 9A and B, lanes 4 and 6), while capsids isolated from ALL-26-infected cells were missing VP21 and VP24 (Fig. 9A and B, lanes 7). In addition, when the UL26 gene was omitted from the infection, the product of the UL26.5 gene was not processed and a protein 3 to 4 kDa larger in size than VP22a (Fig. 9B, lanes 7 and 8) was found. This protein corresponds to protein F (ICP35c,d) of Fig. 3. Because the uncleaved form of VP22a comigrates with a contaminating Sf9 cell or baculovirus protein, the Western blot shown in Fig. 9B was used to analyze the products of the UL26 and UL26.5 genes. In addition to VP21, VP22a, and the uncleaved form of VP22a, two minor proteins with molecular masses of 33 and 29 kDa were detected on the Western blots. The 33-kDa protein was found in particles isolated from BAC-ALL-, ALL-26.5-, and ALL-35-infected cells (Fig. 9B, lanes 1, 4, and 6), while the 29-kDa protein was found only in particles isolated from BAC-ALL- and ALL-35-infected cells (Fig. 9B, lanes 1 and 6). These results indicated that the 33- and 29-kDa proteins are proteolytic fragments of VP21 and VP22a, respectively, and that since capsids isolated from ALL-26-infected cells contain only the uncleaved form of VP22a (Fig. 9B, lane 7), the protease activity associated with the UL26 gene product must be responsible for generating the 33- and 29-kDa proteins.

Thin-section preparations of ALL-26-infected cells revealed that the nuclei of these cells contained capsids (Fig. 8D) which were identical in appearance to capsids found in the nuclei of BAC-ALL-infected cells (Fig. 8C). In contrast, ALL-26.5-infected cells (Fig. 8E) contained capsids which lacked an internal core structure. The ALL-26.5 capsids are identical in both size and appearance to empty capsids found in thin-section preparations of HSV-infected Vero cells (Fig. 8A, bottom particle). Thin-section preparations of ALL-35-infected cells contained a mixture of both cored and uncored capsids, suggesting that the UL35 gene product interacts with the inner core (Fig. 8H and J). Although we did not attempt to quantitate the number of capsids per cell, ALL-26- and ALL-26.5-infected cells appeared to yield significantly fewer HSV capsids than BAC-ALL- or ALL-35-infected cells.

In addition to cored and uncored capsids, negatively stained samples purified from sucrose gradients and thin-section preparations of ALL-26-, ALL-26.5-, and ALL-35-infected cells contained structures that appeared to be incomplete capsid shells (Table 1, aberrant capsids). These structures, which appeared under EM as 6- or 9-type structures, were also found mixed with capsids isolated from BAC-ALL-infected cells (Fig. 7B). The number of aberrant capsids relative to normal capsids increased significantly in infections in which both the BAC-UL26.5 and BAC-UL35 viruses were left out of the infection preparation, providing further evidence that the UL35 gene product interacts with the inner core (Fig. 8K). The evidence that these structures are incomplete capsid shells comes from the fact that they are the only structures made in Sf9 cells infected with a mixture of BAC-UL19, BAC-UL18, and BAC-UL38 (ALL-26-26.5-35 of Table 1; Fig. 7H and 8L). These structures band in the same region of a sucrose gradient as HSV B capsids, and gel analysis demonstrated that the only HSV capsid proteins they contained were VP5, VP19C, and VP23 (Fig. 9A, lane 9). Aberrant capsids were also the only structures found in cells infected with a mixture of BAC-UL19, BAC-UL18, BAC-UL38, and BAC-UL35 or in cells infected with a mixture of BAC-UL19 and BAC-UL38 (data not shown). Cells infected with BAC-UL19 or BAC-UL38 alone did not contain these structures (data not shown). Taken together, these data indicate that aberrant capsids result from self-assembly of the proteins (VP5, VP19C, VP23, and VP26) that make up the outer shell of HSV capsids and that formation of 100-nm capsids requires the products of either the UL26 or the UL26.5 genes.

UL18. ALL-18-infected Sf9 cells contained two types of capsid structures. The aberrant capsids described above constitute approximately 50% of the particles observed either in thin-section preparations or in the negatively stained particles isolated from a sucrose gradient (Fig. 7E and 8G). The remaining 50% of the capsid structures appear as shelled particles with diameters estimated to be on the order of 50 to

FIG. 7. Electron micrographs of negatively stained particles harvested from sucrose gradients. Gradient purification of particles was done as described in the legend to Fig. 4. Particles were isolated from Vero cells infected with HSV-1 (A) or Sf9 cells infected with a mixture of recombinant baculoviruses expressing the six HSV-1 capsid genes (B) or with all six viruses except BAC-UL26 (C), BAC-UL26.5 (D), BAC-UL18 (E), BAC-UL35 (F), BAC-UL26 and BAC-UL35 (G), and BAC-UL26, BAC-UL26.5, and BAC-UL35 (H). Bar at bottom of panel G represents 200 nm (A, B, D, F, and G), and bar at bottom of panel H represents 50 nm (C, E, and H).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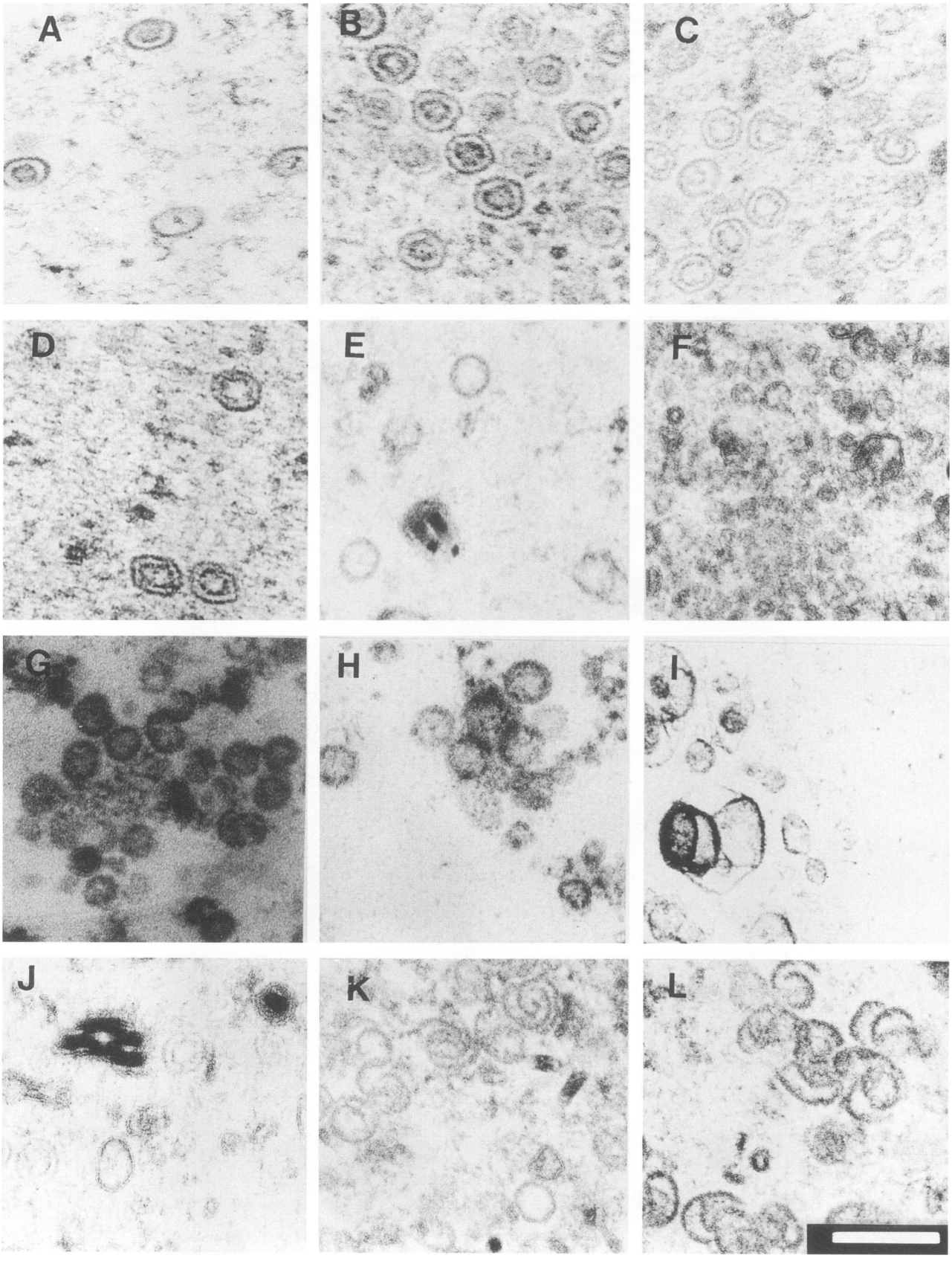
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FIG. 8. Electron micrographs of thin sections of Sf9 cells infected with recombinant baculoviruses or Vero cells infected with HSV-1. Cells were harvested at 12 h (Vero cells) or 64 h (Sf9 cells) postinfection and fixed, and thin sections were prepared for EM. (A) Vero cells infected with HSV-1; (B) Vero cells infected with the UL28 deletion mutant GCB; (C) Sf9 cells infected with a mixture of recombinant baculoviruses expressing the six HSV-1 capsid genes or with all six viruses except BAC-UL26 (D), BAC-UL26.5 (E), BAC-UL19 (F), BAC-UL18 (G), BAC-UL35 (H), BAC-UL38 (I), BAC-UL26 and BAC-UL35 (J), BAC-UL26.5 and BAC-UL35 (K), and BAC-UL26, BAC-UL26.5, and BAC-UL35 (L). Bar, 200 nm.

60 nm. We refer to these structures in Table 1 as 50- to 60-nm capsids, since the distinctive configuration of the HSV capsomers was apparent on the surface of these particles (Fig. 7E). SDS-PAGE of particles purified from ALL-18-infected cells showed that they contained only three HSV capsid proteins; VP5, VP19C, and VP26 (Fig. 9A and B, lanes 3). Therefore, in the absence of the UL18 gene product (VP23), neither VP22a nor VP21 was found in the particles isolated from ALL-18-infected cells. This was an unexpected finding which suggested that VP23 either directly or indirectly mediates the interaction of the scaffolding proteins with the proteins that make up the outer shell.

The interaction of the scaffolding proteins with the outer capsid shell requires the product of the UL18 gene. In order to examine the fate of VP22a and VP21 in ALL-18-infected cells, extracts of ALL- and ALL-18-infected cells were analyzed by sedimentation through sucrose gradients. Total-cell lysates were prepared and layered onto 20 to 65% sucrose gradients, and after sedimentation, individual fractions (1 ml) were collected, and 10 μl of each fraction was separated on a SDS–12.5% polyacrylamide gel. The location in the gradient of VP22a and VP21 was then determined by Western blot analysis. Fraction 1 of Fig. 10A and 10B corresponds to the top of the gradients. VP22a and VP21 were predominately found in fractions collected from the bottom of the gradient in extracts prepared from cells infected with all six recombinant viruses (Fig. 10B), while these same proteins were found in fractions collected from the top of the gradient in extracts prepared from ALL-18-infected cells (Fig. 10A). Three additional immunoreactive proteins with molecular masses of 33,

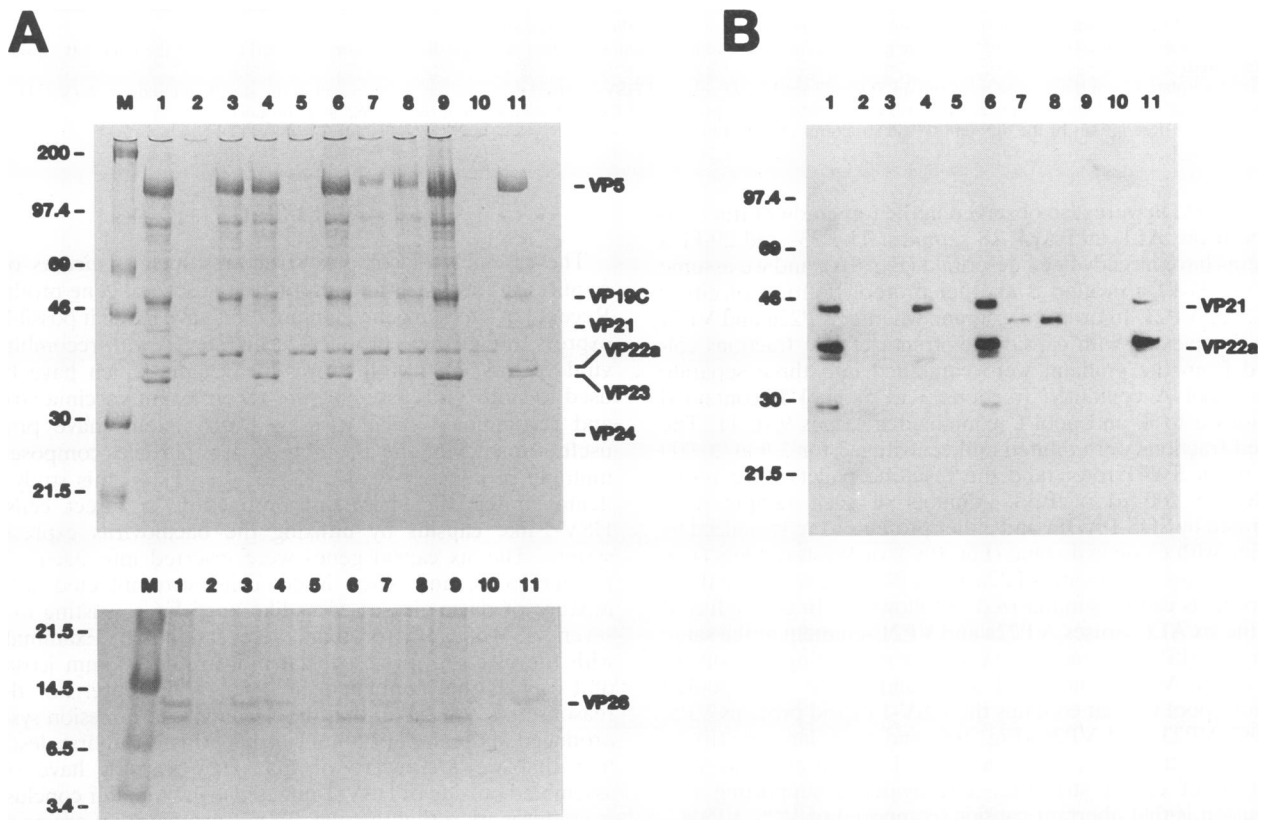
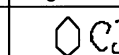
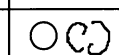
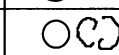
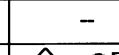
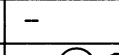
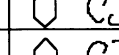
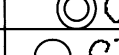
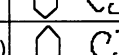
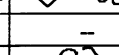
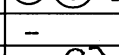
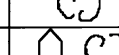
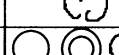
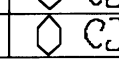
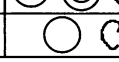
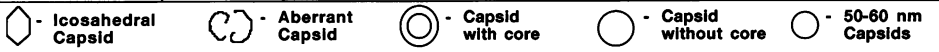


FIG. 9. Protein composition of particles harvested from sucrose gradients. Vero cells were infected with HSV-1 (lanes 11) or Sf9 cells were infected with a mixture of recombinant baculoviruses expressing all six HSV-1 capsid genes (lanes 1) or with all six viruses except BAC-UL19 (lanes 2), BAC-UL18 (lanes 3), BAC-UL26.5 (lanes 4), BAC-UL38 (lanes 5), BAC-UL35 (lanes 6), BAC-UL26 (lanes 7), BAC-UL26 and BAC-UL35 (lanes 8), and BAC-UL26, BAC-UL26.5, and BAC-UL35 (lanes 9), or with a control baculovirus (lanes 10). Cells were harvested at 12 h (Vero cells) or 64 h (Sf9 cells) postinfection, and particles were purified by rate-velocity sedimentation as described in the legend to Fig. 4. When a band was not visible (samples run in lanes 2, 5, and 10), the region of the gradient that corresponded to where HSV-1 B capsids should band was then harvested. (A) Capsid proteins were run on 12% (top gel) or 10 to 20% (bottom gel, which shows only proteins with a molecular mass below 21 kDa) polyacrylamide–SDS gels, and proteins were visualized by staining with Coomassie blue. Lane M, marker proteins (molecular masses in kilodaltons). (B) Western blot of the gel shown in the top part of A, with MCA406 as the primary antibody. Numbers at left show molecular masses (in kilodaltons). The mobilities of the capsid proteins are marked.

TABLE 1. Summary of mixed infections with recombinant baculoviruses expressing the six HSV-1 capsid genes

Recombinant[a] Baculovirus Infection	Band on[b] Sucrose Gradient	Protein Composition[c] by SDS-PAGE	Virus Structure[d] Electron Microscopy	
			Negative Stain	Thin Section
All	+	VP5,VP19C,VP21 VP22a,VP23,(VP24),VP26		
All -18	+	VP5,VP19C,VP26		
All -19	-	–	–	–
All -26	+	VP5,VP19C,ICP35,VP23,VP26		
All -26.5	+	VP5,VP19C,VP21 VP23,(VP24),VP26		
All -35	+	VP5,VP19C,VP21,VP22a,VP23,(VP24)		
All -38	-	–	–	–
All -26 -26.5 -35	+	VP5,VP19C,VP23		
All -26 -35	+	VP5,VP19C,ICP35,VP23		
All -26.5 -35	+	VP5,VP19C,VP21,VP23,(VP24)		



[a] Infection of Sf9 cells was with all six baculoviruses (All) or with all six except the indicated virus.

[b] Cell lysates were loaded on 20 to 65% sucrose gradients, and after sedimentation, the region of the gradient that corresponded to where the HSV-1 B capsid band was harvested.

[c] Samples isolated from the sucrose were run on SDS-polyacrylamide gels, and HSV-1 capsid proteins that were present in the sample are indicated. (VP24), VP24 is present but at reduced levels compared with HSV-1 capsids; ICP35, presence of the uncleaved form of the UL26.5 gene product.

[d] See text for descriptions of the different capsid structures observed by EM.

31, and 29 kDa were also observed in the top gradient fractions for both the ALL and ALL-18 samples. The 33- and 29-kDa proteins have already been described (Fig. 9B), and we assume that the 31-kDa protein is another proteolytic form of either VP22a or VP21. In order to examine whether VP22a and VP21 were complexing with capsid-like structures, the fractions collected from the gradient were combined into three separate pools. Pool A contained fractions 3 to 5, pool B contained fractions 6 to 8, and pool C contained fractions 9 to 11. The pooled fractions were diluted and centrifuged for 1 h at 20,000 rpm in an SW41 rotor, and the resulting pellets were resuspended in 100 μl of PBS. Aliquots of each sample were separated by SDS-PAGE, and either proteins were visualized by staining with Coomassie blue (Fig. 10C), or Western blots (Fig. 10D) were done to locate VP22a and VP21. The results of these experiments can be summarized as follows. (i) In cells infected with the six ALL viruses, VP22a and VP21 sediment at the same density as HSV B capsids. The evidence supporting this conclusion is that VP22a and VP21 are found in the same pooled fraction (pool C) that contains the HSV-1 capsid proteins VP5, VP19C, VP23, and VP26 (Fig. 10C and 10D, lane 6). (ii) In ALL-18-infected cells, VP22a and VP21 do not associate with any type of capsid structure. The evidence supporting this conclusion is that aberrant capsids (composed of VP5, VP19C, and VP26) assembled in ALL-18-infected cells are found in pool B (Fig. 10C, lane 2) while VP22a and VP21 are in pool A (Fig. 10A). In addition, neither VP22a nor VP21 was found in the concentrated pool A sample (Fig. 10D, lane 1), further indicating that they do not associate with capsid structures in the absence of VP23. It should be noted that a prominent protein with a molecular mass of 38 kDa comigrates with VP22a on these gels; therefore, the Western blots were used to identify VP22a (Fig. 10D).

DISCUSSION

The assembly of complex structures such as viruses often requires the synthesis and interaction of several gene products. Recent advances in gene manipulation have made it possible to express foreign gene products at high levels with recombinant viral vectors. Two viral expression vectors which have been used to study virus assembly are recombinant vaccinia viruses and recombinant baculoviruses. Both systems have proved useful for studying the assembly of virus particles composed of multiple proteins (1, 9, 12, 15, 37, 46, 51). In this study, we demonstrated the successful production in insect cells of HSV-1-like capsids by utilizing the baculovirus expression system. The six capsid genes were inserted into baculovirus recombinants, and when insect cells were infected with a mixture of these viruses, virus-like particles consisting of the seven HSV-1 capsid proteins were assembled. Examination with the electron microscope demonstrated 100-nm icosahedral capsids that contained an inner 50-nm core. For these reasons, it is concluded that the baculovirus expression system produced authentic HSV-1 B capsids. This is the first description that we are aware of that HSV capsids have been assembled outside of HSV-1-infected cells. A major conclusion from this work is that the only HSV-1 proteins that are needed for assembly of B capsids are the products of the UL18, UL19, UL26, UL26.5, UL35, and UL38 genes. The development of the baculovirus-based assembly system allowed us to explore the roles of the different capsid proteins in the assembly process.

Icosahedral shell. A prominent structure found in insect cells expressing the HSV-1 capsid genes was incomplete capsids (aberrant capsids [Table 1]). With the exception of the ALL-19 and ALL-38 infections, these structures were found in

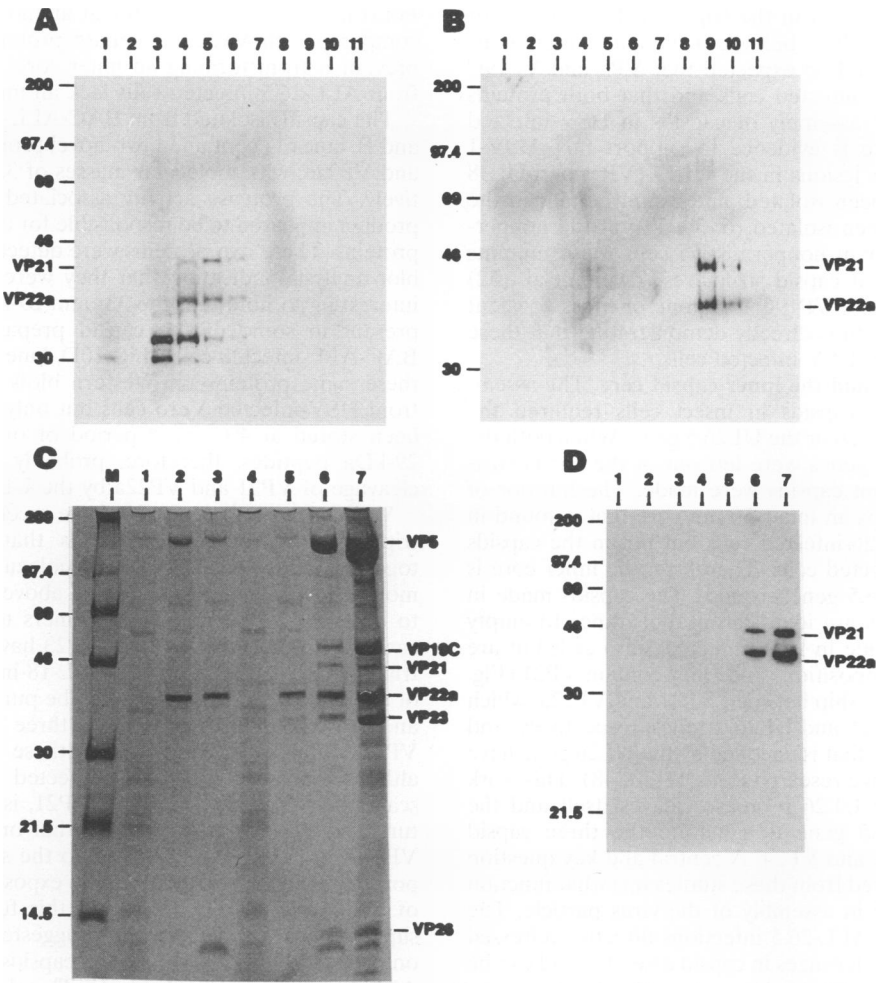


FIG. 10. Western blot analysis of sucrose gradient-fractionated cell lysates from baculovirus-infected Sf9 cells. Sf9 cells were infected with a mixture of recombinant baculoviruses expressing the six HSV-1 capsid genes (B) or with all six viruses except BAC-UL18 (A), and cells were harvested at 64 h postinfection. Lysates were layered onto 20 to 65% sucrose gradients and centrifuged at 24,000 rpm (Beckman SW41 rotor) at 4°C for 1 h. Fractions (1 ml) were collected, 10 μl of each fraction was separated on SDS–12% polyacrylamide gels, and reactive proteins were revealed by Western blotting with MCA406 as the primary antibody. The direction of sedimentation is from left to right. In order to examine the protein makeup of the particles separated on the sucrose gradient, selected fractions were pooled, diluted with 1× capsid lysis buffer, and concentrated by centrifugation at 20,000 rpm (SW41) at 4°C for 1 h, and the pellets were resuspended in 100 μl of PBS. (C) Ten microliters of each sample was run on a SDS–10 to 20% polyacrylamide gel, and proteins were visualized by staining with Coomassie blue. Lanes 1 to 3, gradient A combined fractions 3-5, 6-8, and 9-11, respectively; lanes 4 to 6, gradient B combined fractions 3-5, 6-8, and 9-11, respectively; lane 7, BAC-ALL capsid standard; lane M, marker proteins. (D) Western blot of the gel shown in panel C, with MCA406 as the primary antibody. Numbers at left show molecular masses (in kilodaltons). The mobilities of the capsid proteins are marked.

all of the mixed infections that we tested (Table 1). Aberrant capsids can be purified by sucrose gradient sedimentation; SDS-PAGE analysis of the purified particles demonstrated that they contain from two to four HSV capsid proteins. The results from the mixed infections demonstrated that VP5 and VP19C are required for assembly of aberrant capsids and that VP23 and VP26, although not essential, appear to stabilize these structures, since the number of aberrant capsids increased when these two proteins were present. Similar types of structures have been observed in cells infected at the nonpermissive temperature with several HSV-1 temperature-sensitive (*ts*) mutants (38), suggesting that aberrant capsids form in HSV-infected cells. The ratio of aberrant capsids to complete capsids increased when either the UL26 or the UL26.5 gene was left out of the infection preparation, and when both genes were absent, aberrant capsids were the only structures formed. Thus, in the absence of the scaffolding proteins, the proteins (VP5, VP19C, VP23, and VP26) that make up the outer capsid shell self-assemble into these defective capsid structures. The abundance of these structures in insect cells is probably due to the overexpression of the capsid proteins from the recombinant baculoviruses.

VP5 is the structural subunit of both the hexons and the pentons, and VP19C and VP23 form the triplex or intracapsomeric fibrils that hold the capsomers together (25, 47). Although the location of VP26 has not been determined, results of Newcomb and Brown (24) and Newcomb et al. (25) suggest that this protein binds in a 1:1 ratio with VP5. The fact that VP26 is found in aberrant capsids supports their conclusion. The results presented here support a model in which capsomers (VP5) can be linked together to form shell-like structures through their interaction with VP19C. In other

words, VP23 is not required in the triplex to form the outer shell (see discussion of VP23 below). If the above arguments are correct, then it would be expected that VP5 and VP19C would interact in HSV-infected cells and that both proteins would be essential for assembly of capsids in HSV-infected cells, and indeed there is evidence to support this. HSV-1 mutants that contain *ts* lesions in the UL19 (VP5) and UL38 (VP19C) genes have been isolated, and a null mutant in the UL19 gene has also been isolated (6, 30, 49); at the nonpermissive temperature or in nonpermissive cells these mutants fail to make any type of capsid structures. Zweig et al. (52) have shown that VP5 and VP19C form one or more covalent bonds with each other, thus directly demonstrating that these two proteins interact in HSV-infected cells.

Scaffolding proteins and the inner capsid core. The assembly of 100-nm HSV-1 capsids in insect cells required the product of either the UL26 or the UL26.5 gene. When both the UL26 and the UL26.5 genes were left out of the baculovirus infections, only aberrant capsids were made. The interior of HSV B capsids contains an inner 50-nm core that is found in capsids made in ALL-26-infected cells but not in the capsids made in ALL-26.5-infected cells. Therefore, the inner core is composed of the UL26.5 gene product. The capsids made in ALL-26.5-infected cells are identical in appearance to empty capsids (A capsids) made in HSV-infected Vero cells but are different in protein composition, since they contain VP21 (Fig. 9B, lane 4). The relationship between VP21 and VP22a, which are encoded by the UL26 and UL26.5 genes, respectively, and the associated protease that is encoded by the UL26 gene have been an area of extensive research (5, 7, 17–19, 48). This work demonstrated that the UL26 protease cleaves itself and the product of the UL26.5 gene to generate the three capsid proteins VP21, VP22a, and VP24. A central and key question that remained unresolved from these studies was what function do the cleavages serve in assembly of the virus particle. The results of ALL-26 and ALL-26.5 infections directly addressed the role of some of the cleavages in capsid assembly and can be summarized as follows. First, cleavage of the UL26.5 gene product by the UL26 protease is not required for assembly of B capsid. Capsids isolated from ALL-26-infected cells are identical in appearance to HSV B capsids but contain only the uncleaved form of the UL26.5 gene product. Evidence that processing of the UL26.5 gene product is not required for capsid assembly was previously indicated in studies with a *ts* mutant (*ts*1201) that maps to the UL26 gene (31, 34). Capsid assembly was observed at the nonpermissive temperature, but only the uncleaved form of the UL26.5 gene product was present in the capsid core. This mutant fails to package viral DNA, supporting the conclusion that C-terminal cleavage of the UL26.5 gene product is required for DNA packaging (31, 34). Second, both the cleaved and the uncleaved forms of the UL26.5 gene product will form the inner 50-nm core, since the inner core is present in ALL (cleaved form)- and ALL-26 (uncleaved)-infected cells. VP22a purified from capsids has the ability to self-assemble into 60-nm structures, suggesting that scaffolds form prior to assembly of the capsid shell (24). Third, VP21 will serve as a scaffolding protein in capsid assembly, since ALL-26.5-infected cells contain 100-nm capsids. Since there is no inner core in these capsids, either VP21 is performing the scaffolding function in a different way than is VP22a or VP21 forms a shell that is not readily apparent by EM analysis. The shell formed by VP21 may be larger than the shell formed by VP22a, making it difficult to distinguish it from the outer (icosahedral) shell. Alternatively, the shell formed by VP21 may not be a solid sphere but may have a more "cage-like" structure which would again be difficult to visualize by EM. Fourth, the extra 59 N-terminal amino acids found on VP21 compared with VP22a (compare proteins D to G in Fig. 3) prevent it from forming an inner core, since capsids isolated from ALL-26.5-infected cells lack an inner core.

The capsids isolated from BAC-ALL-infected cells (Fig. 9A and B, lanes 1) contained two novel proteolytic forms of VP21 and VP22a, with molecular masses of 33 and 29 kDa, respectively. The protease activity associated with the UL26 gene product appeared to be responsible for the appearance of both proteins. These two proteins were detectable only by immunoblot analysis, indicating that they were minor proteins. It is interesting to note that the 33- and 29-kDa proteins were not present in some of the capsid preparations isolated from BAC-ALL-infected cells (Fig. 10D, lane 6). We have observed these same proteins on Western blots of B capsids isolated from HSV-infected Vero cells but only after the capsids had been stored at 4°C for a period of time (45). The 33- and 29-kDa peptides, therefore, probably result from aberrant cleavage of VP21 and VP22a by the UL26 protease.

VP23. Newcomb et al. (25) have recently proposed that the triplex or intracapsomeric fibrils that link the capsomers together are composed of two molecules of VP23 and one molecule of VP19C. As discussed above, only VP19C appears to be required for linking capsomers together. If this is the case, then what function does VP23 have as a member of the triplex in capsid assembly? In ALL-18-infected cells, two types of defective capsid structures can be purified: aberrant capsids and 50- to 60-nm capsids. Only three capsid proteins, VP5, VP19C, and VP26, are found in these structures. Therefore, although present in ALL-18-infected cells, neither of the scaffolding proteins, VP22a or VP21, is found in these structures, leading to the conclusion that one of the functions of VP23 is to link the capsid shell to the scaffolding proteins. A portion of VP23 would have to be exposed on the interior side of the capsid shell to carry out this function, and evidence supporting this conclusion was suggested by the fact that the only instance of 50- to 60-nm capsids being found was in ALL-18-infected cells (Table 1). The absence of 50- to 60-nm capsids from all the other mixed infections suggests that VP23 sterically hindered the growing capsid shell from circularizing into smaller capsid structures.

VP26. Although not required for capsid assembly, the product of the UL35 gene (VP26) is important for formation of 100-nm capsids that contain an inner core. As discussed above, the present results have demonstrated that VP26 is located in the outer (icosahedral) capsid shell. Therefore, VP26 may play an important (but not essential) role in the interaction of the scaffolding proteins with the outer capsid shell. This conclusion is supported by the fact that when both the UL35 and the UL26.5 genes were omitted from the mixed infections (Table 1, ALL-26.5-35), more aberrant capsid structures were formed than when just the UL26.5 gene was missing (Table 1, ALL-26.5). Alternatively, VP26 may be required for maintaining the integrity of the capsid shell. Capsids isolated from ALL-35-infected cells appear to take up more stain (Fig. 8F) than do BAC-ALL capsids, suggesting that in the absence of VP26 the capsid shell is compromised. The numbers of capsids produced in ALL- and ALL-35-infected cells are nearly the same, but few if any empty capsids (capsids without an inner core) were found in cells infected with the six ALL recombinant baculoviruses, while ALL-35-infected cells contained a mixture of cored and uncored capsids. The loss of the inner core may therefore result after B capsids are assembled in ALL-35-infected cells because of the absence of VP26 from the outer shell.

Scheme for HSV-1 capsid assembly. Figure 11 summarizes

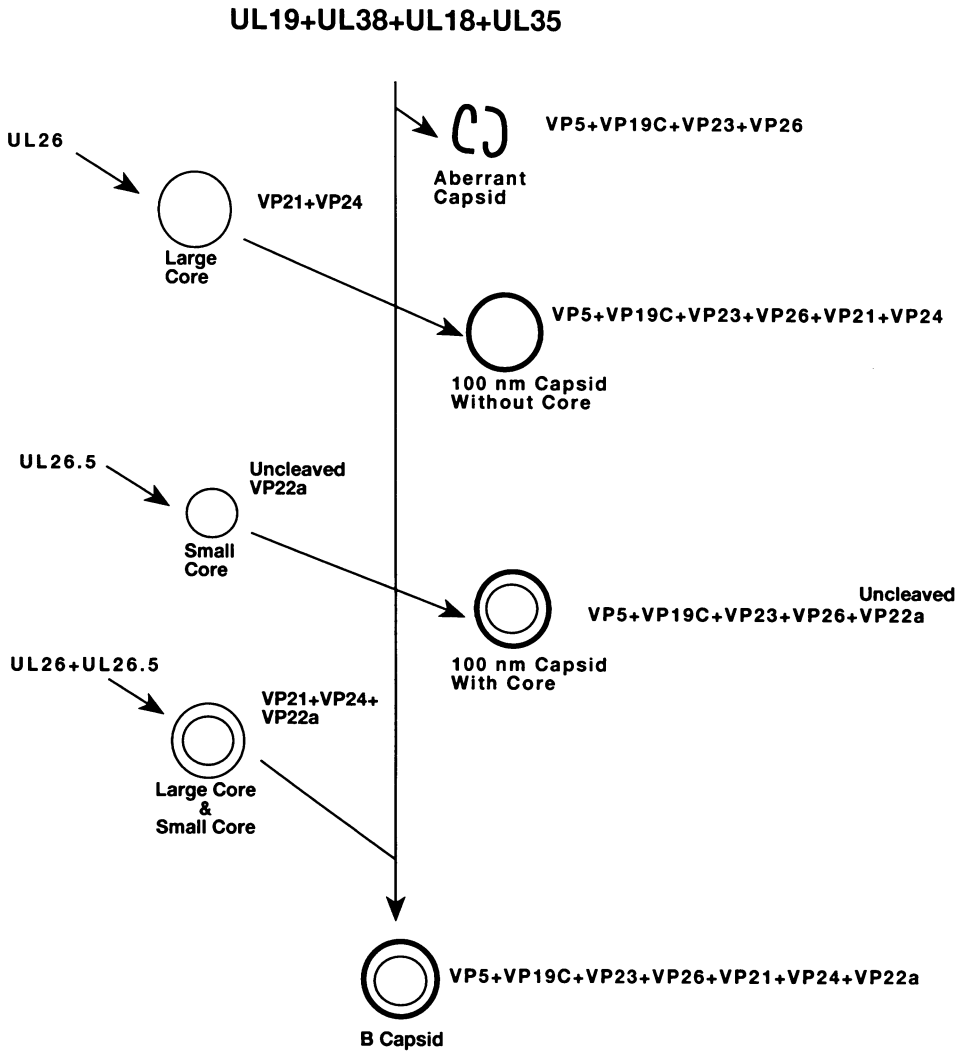


FIG. 11. Schematic diagram of the possible pathways for assembly of 100-nm HSV-1 capsids. This scheme assumes that the scaffolding proteins self-assemble into core structures. In the absence of the scaffolding proteins, the products of the UL19, UL38, UL18, and UL35 genes self-assemble to form aberrant capsids. If the products of the UL26 and/or the UL26.5 gene are also present, then 100-nm capsids are formed. The capsid proteins that make up each particle are indicated. It is assumed that the large core formed by the UL26 gene product is not visible by EM when present in 100-nm capsids, either because of its cage-like structure or because of its location beneath the outer capsid shell. For details, see the text.

the possible pathways for assembly of 100-nm HSV-1 capsids as defined by the results of this study. The scheme shown in Fig. 11 proposes that when expressed alone, the products of the UL26 and UL26.5 genes will self-assemble to form large-core (UL26) and small-core (UL26.5) particles that function as scaffolds around which the products of UL19, UL38, UL18, and UL26 self-assemble to form the outer (icosahedral) shell of the capsid. When expressed together, the UL26 and UL26.5 gene products self-assemble to form a double-core scaffold around which the outer shell is formed. Assembly of double-core scaffolds must be very efficient, since 100-nm capsids without cores are rarely found either in cells infected with HSV-1 mutants that are deficient in DNA packaging (44) or in insect cells infected with the six ALL recombinant baculoviruses. If this is the case, then we should be able to detect the inner core particles when the UL26 and/or the UL26.5 gene is expressed in insect cells. Although we have not yet been able to detect these cores (45), the fact that the products of the UL26 and UL26.5 genes band about halfway into the sucrose gradient shown in Fig. 10 suggests that the scaffolding proteins are assembling into some type of particle. We were not able to concentrate these particles by ultracentrifugation, suggesting either that these particles are unstable or that they require specific conditions (e.g., ionic strength or metal ion concentration) for them to be pelleted. Finally, in this scheme the pathway that leads to assembly of HSV B capsids is apparently the most favored since, as mentioned above, few if any aberrant or empty capsids are made in either HSV-infected cells or insect cells infected with the six HSV capsid genes.

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