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Characterization of ICP6::*lacZ* Insertion Mutants of the UL15 Gene of Herpes Simplex Virus Type 1 Reveals the Translation of Two Proteins

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The herpes simplex virus type 1 (HSV-1) UL15 gene is a spliced gene composed of two exons and is predicted to encode an 81-kDa protein of 735 amino acids (aa). Two UL15 gene products with molecular masses of 75 and 35 kDa have been observed (J. Baines, A. Poon, J. Rovnak, and B. Roizman, J. Virol. 68:8118–8124, 1994); however, it is not clear whether the smaller form represents a proteolytic cleavage product of the larger form or whether it is separately translated. In addition, an HSV-1 temperature-sensitive mutant in the UL15 gene (*ts*66.4) is defective in both cleavage of viral DNA concatemers into unit-length monomers and packaging of viral DNA into capsids (A. Poon and B. Roizman, J. Virol. 67:4497–4503, 1993; J. Baines et al., J. Virol. 68:8118–8124, 1994). In this study, we detected two UL15 gene products of 81 and 30 kDa in HSV-1-infected cells, using a polyclonal antibody raised against a maltose binding protein fusion construct containing UL15 exon 2. In addition, we report the isolation of two HSV-1 insertion mutants, *hr*81-1 and *hr*81-2, which contain an ICP6::*lacZ* insertion in UL15 exon 1 and exon 2 and thus would be predicted to encode C-terminally truncated peptides of 153 and 509 aa long, respectively. *hr*81-1 and *hr*81-2 are defective in DNA cleavage and packaging and accumulate only B capsids. However, both mutants are able to undergo wild-type levels of DNA replication and genomic inversion, suggesting that genomic inversion is a result of DNA replication rather than of DNA cleavage and packaging. We also provide evidence that the 81- and 30-kDa proteins are the products of separate in-frame translation events from the UL15 gene and that the 81-kDa full-length UL15 protein is required for DNA cleavage and packaging.

Most double-stranded DNA (dsDNA) bacteriophages and animal viruses share common features in DNA replication as well as DNA maturation. DNA replication in many dsDNA bacteriophage systems results in the formation of a large head-to-tail concatemeric complex composed of tandem repeats of the viral genome, which is subsequently cleaved into unit-length monomers and packaged into preformed capsid precursors, with a concomitant loss of scaffolding proteins (7, 8, 10, 13). Both biochemical and genetic approaches have been exploited extensively for studies of dsDNA bacteriophage systems. Many protein components of the DNA cleavage and packaging machinery have been identified (11, 25, 27). All DNA cleavage and packaging systems identified to date contain a two-subunit terminase complex which binds and cleaves viral DNA, a translocase activity which is responsible for translocation of DNA into the capsid, with the consumption of ATP, and a scaffolding-protein-containing prohead in which portal proteins form a dodecameric ring at a portal vertex through which DNA is taken in (reviewed in reference 10).

Herpes simplex virus type 1 (HSV-1) contains a 152-kb dsDNA genome composed of two covalently linked unique regions (U_L and U_S), flanked by inverted repeats (45, 50) (Fig. 1). During DNA replication, the two unique regions U_L and U_S invert with respect to each other, presumably through a generalized recombination mechanism within the repeated regions of the genome, generating four isomeric forms of progeny viruses (14, 28, 47, 50). DNA replication is thought to result in the formation of large head-to-tail DNA concatemers (9, 29, 30), which are cleaved at specific sites within the *a* sequences into unit-length monomeric genomes and packaged into preassembled capsids, with loss of scaffolding proteins (18, 38, 54, 56).

Three types of intracellular capsids have been identified in HSV-1-infected cells by sucrose gradient sedimentation: A (empty), B (intermediate), and C (full) (16, 20, 40, 46). A and C capsids have similar protein compositions, but only C capsids contain viral DNA. B capsids were initially thought to be analogous to phage proheads in that B capsids contain a scaffolding protein (designated VP22a, encoded by the UL26.5 gene) which is lost from capsids when DNA is packaged. However, in a cell-free capsid assembly system, Newcomb and colleagues (39) recently found a spherical, unstable capsid intermediate with a protein composition similar to that of B capsids. They proposed that these less angular and more open structures rather than B capsids are authentic procapsid intermediates (39). Although B capsids may be a dead-end product of the capsid maturation process, they represent the most closely related stable structures to procapsids that can be isolated. Thus, procapsids are thought to be precursors to DNA-containing C capsids, and empty A capsids are thought to result from abortive attempts at DNA encapsidation (39, 43). Temperature-sensitive mutants whose mutations map within six HSV-1 genes, UL6, UL15, UL25, UL28, UL32, and UL33, are defective in DNA cleavage and packaging and in production of A and C capsids (1–3, 6, 44, 51, 52, 58). The involvement of at least six genes suggests that HSV DNA cleavage and packaging is a complex process.

The UL15 gene composed of two exons is one of only a few spliced genes in the HSV-1 genome and is predicted to encode a 735-amino-acid (aa) protein (15, 19). UL15 is highly conserved among the herpesvirus family, and sequence analysis

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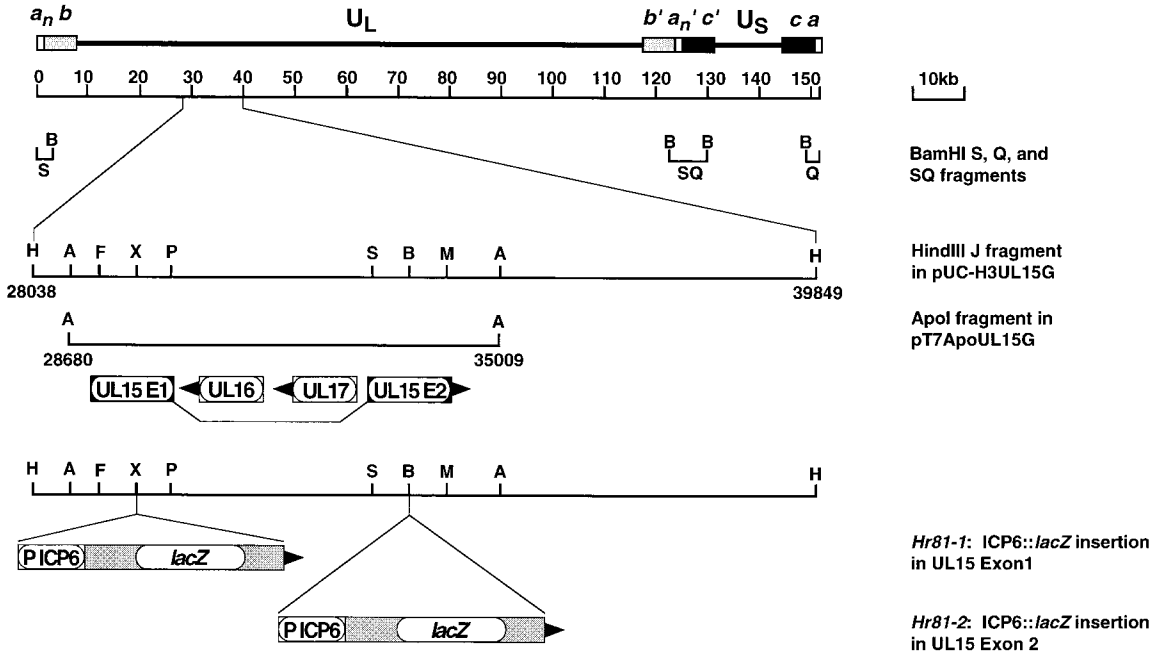

FIG. 1. Physical map of the region of the HSV-1 genome containing the UL15 gene. The HSV-1 genome and map coordinates are shown on the first two lines. On the next line are shown the locations of the terminal *Bam*HI fragments (S and Q) and the junction *Bam*HI fragment (SQ) detected by the *Bam*HI SQ probe. On the next two lines, the *Hin*dIII J and *Apo*I fragments spanning the UL15 open reading frame have been expanded to show internal cleavage sites: H, *Hin*dIII; A, *Apo*I; F, *Afl*II; X, *Xmn*I; P, *Bsp*EI; S, *Sal*I; B, *Bam*HI; M, *Mlu*I. The genomic organization of the two exons of the UL15 gene is diagrammed on the next line. UL15E1 and UL15E2 represent the first exon and the second exon of UL15, respectively. The last two lines show the positions of the ICP6::*lacZ* insertion in *hr*81-1 and *hr*81-2, respectively. Arrows represent the transcriptional orientation of each gene.

indicates a possible homology between the UL15 protein of HSV-1 and the large subunit (gp17) of the terminase complex of bacteriophage T4 (17). The most significant aspect of this homology is the presence of a putative nucleotide binding fold (Walker boxes A and B) in both proteins (19, 57), suggesting that UL15 may be able to bind and hydrolyze ATP as has been demonstrated for gp17 and other phage terminases (5, 10, 12).

A temperature-sensitive mutant (*ts*66.4) bearing a lesion in the second exon of the UL15 gene has been isolated and shown to be defective in DNA cleavage and packaging (6, 44). However, temperature-sensitive mutants are prone to problems associated with incomplete penetrance (leak), tendency to revert to wild type, and potential transdominant interference of wild-type protein function by the presence of a quasi-stable yet nonfunctional mutant protein at the nonpermissive temperature. In this report, we describe the isolation and characterization of UL15 insertional mutants in which an ICP6::*lacZ* cassette is inserted into either the first or the second exon of the UL15 gene. We show that although two UL15 mutants are able to undergo wild-type levels of DNA replication and genomic inversion, they are defective in DNA cleavage and packaging. Furthermore, we find that two proteins with molecular masses of 81 and 30 kDa are translated separately from the UL15 gene and that the 81-kDa full-length UL15 protein is required for DNA cleavage and packaging.

MATERIALS AND METHODS

Cells and viruses. African green monkey kidney cells (Vero; American Type Culture Collection, Rockville, Md.) were propagated and maintained as described previously (59). Cell lines M-3, C-2, and G-27, which are permissive for UL15 mutants (see below), were propagated as described above, but with the addition of 100 μg of the antibiotic G418 (geneticin, a neomycin analog; GIBCO Laboratories, Grand Island, N.Y.) per ml of medium. The KOS strain of HSV-1 was used as the wild-type virus as well as the parental virus for the isolation of UL15 insertion mutants *hr*81-1 and *hr*81-2. A temperature-sensitive mutant defective in the UL15 gene at the nonpermissive temperature (39.5°C), *ts*66.4, a kind gift from B. Roizman (University of Chicago), was propagated at 34°C and used to screen candidate UL15-permissive cell lines at 39.5°C.

Plasmids and bacteria. Plasmid pUC-H3UL15G was made by cloning the *Hin*dIII J fragment of HSV-1 KOS strain (nucleotides [nt] 28038 to 39849) (37) into the *Hin*dIII site of the vector pUC119. This *Hin*dIII J fragment contains the 5′-terminal portion of the UL13, UL14, the first exon of UL15, UL16, UL17, the second exon of UL15, UL18, and the promoterless 3′-terminal part of UL19 genes.

pUC-UL15E1LacZS, containing the disrupted first exon of UL15, and pUC-UL15E2LacZS, containing the disrupted second exon of UL15, were constructed as follows. pUC-UL15E1 contains the *Sac*I/*Sph*I fragment spanning the first exon of UL15 cloned into *Sac*I/*Sph*I sites of the pUC119 vector. pUC-UL15E1 was partially digested with *Xmn*I, and the linearized plasmid was gel purified. The 4.3-kb *Bam*HI fragment containing the *lacZ* gene under the control of the ICP6 promoter (ICP6::*lacZ* cassette) (23) was filled in with Klenow enzyme and ligated to the linearized plasmid pUC-UL15E1. The resultant construct, which contains the ICP6::*lacZ* cassette inserted at the *Xmn*I site (corresponding to amino acid residue 153) within the first exon of UL15, was designated pUC-UL15E1LacZS. The *Sac*I/*Sph*I fragment containing the second exon of UL15 was ligated to the *Sac*I/*Sph*I sites of pUC119 to generate pUC-UL15E2. pUC-UL15E2LacZS was made by inserting the *Bam*HI fragment of ICP6::*lacZ* cassette into the *Bam*HI site of pUC-UL15E2 at a position corresponding to amino acid residue 509 of UL15. In both pUC-UL15E1LacZS and pUC-UL15E2LacZS, the transcriptional orientation of the *lacZ* gene was same as that of the UL15 gene.

Plasmids pT7ApoUL15G, containing the UL15 genomic DNA, and pT7ApoUL15C, containing the UL15 cDNA, were constructed as follows. Plasmid pUC-H3UL15G was partially digested with *Apo*I. The *Apo*I fragment containing the UL15 locus (nt 28680 to 35009 [37]) was isolated and subcloned into the *Eco*RI site of pBluescriptIISK+ (Stratagene, La Jolla, Calif.) to generate pT7ApoUL15G. To construct the UL15 cDNA, the splice junction of UL15 exons 1 and 2 (15) was generated by PCR, using HSV-1 KOS-infected (13 h, multiplicity of infection [MOI] of 3) Vero cell RNA. Poly(A)$^+$ RNA was isolated, and 2.5 μg was used in reverse transcription-PCR, using commercial kits as described by the manufacturer (Fast Track and cDNA Cycle kits; Invitrogen, San Diego, Calif.). PCR primer oligonucleotides were 5′-CTCGTTTCCGGAC GGGTCGC-3′ in exon 1 and 5′-GGCCTCGTCGACAAAGAGCAGG-3′ in exon 2, corresponding to nt 29991 to 30010 and 33679 to 33658, respectively. These oligonucleotides contained the *Bsp*EI (nt 29992) and *Sal*I (nt 33688) sites spanning the UL15 intron. The 91-nt *Bsp*EI/*Sal*I-digested PCR product was cloned into the *Bsp*EI/*Sal*I sites to replace the intron in pT7ApoUL15G; the resultant plasmid, pT7ApoUL15C, contains only the UL15 cDNA. Nucleotide

sequence analysis of the PCR product confirmed the sequence as reported previously (37).

Plasmids pCMV-UL15C and pCMV-UL15G, which contain the UL15 cDNA or genomic DNA under the control of the constitutive cytomegalovirus immediate-early (CMV-IE) promoter, were constructed as follows. Either pT7ApoUL15C or pT7ApoUL15G was digested with *Hin*dIII/*Xba*I, and a gel-purified 2.8-kb fragment containing the UL15 cDNA from pT7ApoUL15C or a 6.4-kb fragment containing the UL15 genomic DNA from pT7ApoUL15G was ligated into the *Hin*dIII/*Xba*I sites of the expression vector pCDNAI/Amp, containing the CMV-IE promoter (Invitrogen), to construct pCMV-UL15C or pCMV-UL15G, respectively.

All recombinant plasmids were propagated in *Escherichia coli* DH5α by standard procedures (34).

Isolation of UL15 cell lines. To isolate cell lines which are permissive for UL15 mutants, 1.5×10^6 freshly trypsinized exponentially growing Vero cells were cotransfected with 2 μg of pSV2neo and 18 μg of either pT7ApoUL15G, pT7ApoUL15C, or pUC-H3UL15G as described previously (22, 24) and grown in medium containing 1 mg of G418 per ml. The G418 concentration was decreased to 0.5 mg/ml after 5 days. Two weeks after cotransfection, individual G418-resistant cells were picked and screened for the ability to support the growth of *ts*66.4 at the nonpermissive temperature (39.5°C).

Construction of UL15 insertional mutants by marker transfer. Marker transfer experiments were carried out as described previously (21). In brief, either *Xmn*I/*Hin*dIII-linearized pUC-UL15E1LacZS or pUC-UL15E2LacZS plasmid and infectious KOS DNA were used in a molar ratio of 10:1 to transfect 1.5×10^6 freshly trypsinized exponentially growing UL15-permissive cells. When maximum cytopathic effect was observed, progeny viruses were collected, titers were determined on M-3 cells, and 2,500 infectious viral particles were plated onto 50 100-mm-diameter plates containing an M-3 cell monolayer. The resultant plaques were stained with neutral red in the presence of the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal). The plaques with blue color were selected and plaque purified three times on M-3 cells before stocks were prepared.

Transient complementation assay. The transient complementation assay was carried out as described previously (36). In brief, 1.5×10^6 freshly trypsinized exponentially growing Vero cells were transfected with 8 μg of either pCDNAI/Amp, pCMV-UL15C, or pCMV-UL15G. At 24 h posttransfection, cells were superinfected with either *hr*81-1 or *hr*81-2 at an MOI of 1 PFU per cell. At 24 h postinfection, progeny viruses were harvested and assayed on the M-3 cell line for total yield.

Generation of anti-UL15 (α-UL15) polyclonal antiserum. Rabbit polyclonal antiserum similar to that previously described (6) was generated against the UL15 exon 2 sequences. A *Hin*cII/*Xba*I fragment was isolated from subclone pT7ApoUL15G as described above and cloned into the *Stu*I/*Xba*I sites of the pMal-C vector (New England Biolabs, Beverly, Mass.). This clone encoded the maltose binding protein (MBP) fused to the C-terminal 351 aa of UL15. Fusion protein was expressed and purified as instructed by the vector manufacturer. Five hundred micrograms of purified antigen was mixed with complete Freund's adjuvant and inoculated intramuscularly into New Zealand White rabbits. Booster immunizations of antigen in incomplete Freund's adjuvant were performed every 4 weeks for 10 months. Serum from one rabbit, AS9, was collected by standard protocols and used as described in Results.

Western blot analysis. To analyze infected cell lysates, 3×10^6 cells in a 100-mm-diameter tissue culture dish were infected with virus stocks at an MOI of 10 PFU per cell at 37°C. Cells were collected at 14 h postinfection by centrifugation at 2,000 rpm for 10 min and rinsed with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 [pH 7.4]) twice. Cell pellets were resuspended in 150 μl of 1× sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) loading buffer (0.05 mM Tris-HCl [pH 6.8], 2% SDS, 10% glycerol, 0.1% bromophenol blue, 1.25% [vol/vol] 2-mercaptoethanol). To analyze transfected cell lysates, 10^6 freshly trypsinized exponentially growing Vero cells were transfected with 8 μg of expression plasmids as described previously (21) and plated on a 60-mm-diameter tissue culture dish for 24 h, and cell lysates were prepared and resuspended in 50 μl of 1× SDS-PAGE loading buffer as described above. Cell lysates in SDS-PAGE loading buffer were heated at 95°C for 5 min and vortexed vigorously for 30 s, and 20-μl aliquots of samples were subjected to electrophoresis on an SDS–10% polyacrylamide gel. ECL (enhanced chemiluminescence) Western blotting analysis was performed as instructed by the manufacturer (Amersham, Buckinghamshire, England). To detect the UL15 gene products, the α-UL15 rabbit polyclonal antibody was used as the primary antibody at a dilution of 1:2,000 (in 5% nonfat milk in phosphate-buffered saline containing 0.2% Tween 20) to hybridize with the membrane at 4°C overnight. Protein quantification analysis was performed as suggested by the manufacturer, using bands which were in the linear response range.

DNA isolation and analysis by Southern blotting and PFGE. Intact infectious viral genomic DNA used in marker transfer experiments was isolated as described previously (62). Total viral DNA and cellular DNA used in Southern blot analysis were isolated as described previously (61). For Southern blot analysis, cellular or viral DNA was digested with appropriate restriction endonucleases, subjected to conventional agarose gel electrophoresis or pulsed-field gel electrophoresis (PFGE), blotted to Gene Screen Plus nylon membranes (New England Nuclear Corp., Boston, Mass.), hybridized with ^{32}P-labeled specific probes, and visualized by autoradiography as instructed by the manufacturer. DNA probes were prepared by labeling desired gel-isolated restriction fragments by using a Random Primed DNA Labeling kit (Boehringer Mannheim, Indianapolis, Ind.). Preparation and analysis of encapsidated viral DNA and PFGE were carried out as described previously (35, 49).

Analysis of viral capsids. HSV-1 capsids were isolated by using a modification of a method developed by Sherman and Bachenheimer (52). Cells were infected at an MOI of 10 PFU per cell at 37°C. At 20 h postinfection, cells were collected by 4,000-rpm centrifugation at 4°C for 15 min, rinsed with Tris-buffered saline, resuspended in phosphate buffer (40 mM sodium phosphate buffer [pH 7.4], 0.15 M NaCl, 1 mm EDTA, 1 mm EGTA, 1 mm phenylmethylsulfonyl fluoride, 5 μg of pepstatin A per ml, 5 μg of leupeptin per ml) with 1% Nonidet P-40, incubated on ice for 30 min, and probe sonicated for 30 s. The cell lysate was layered onto a 15 to 50% (wt/wt in phosphate buffer) sucrose gradient and centrifuged at 35,000 rpm for 30 min at 4°C (with an SW50.1 or SW41 rotor). Capsids were visualized by light scattering upon illumination with a halogen fiber-optic lamp.

RESULTS

Isolation of UL15-containing permissive cell lines and UL15 mutants. Permissive cell lines containing the UL15 gene were isolated for the propagation of UL15 mutants. Vero cells were cotransfected with a plasmid conferring neomycin resistance (pSV2neo) and a plasmid containing either the UL15 cDNA (pT7ApoUL15C) or the UL15 genomic DNA (pT7ApoUL15G, containing the UL15, UL16, and UL17 genes, or pUC-H3UL15G, containing the 5′-terminal portion of the UL13, UL14, UL15 exon 1, UL16, UL17, UL15 exon 2, UL18, and the promoterless 3′-terminal portion of the UL19 genes) (Fig. 1). G418-resistant cells were screened for the ability to support the growth of the temperature-sensitive UL15 mutant *ts*66.4 at the nonpermissive temperature (39.5°C). Ten such cell lines were isolated, and Southern analysis revealed that the copy number of integrated UL15 genes per cell ranged from 20 to 500 (data not shown). Three cell lines were chosen for further study; M-3 cells contain the larger genomic fragment (pUC-H3UL15G), G-27 cells contain the smaller genomic fragment (pT7ApoUL15G), and C-2 cells contain the cDNA fragment (pT7ApoUL15C).

Viral mutants bearing the inactivated UL15 gene were constructed by marker transfer. The UL15-complementing cell lines, M-3, G-27, and C-2, were cotransfected with infectious KOS DNA and a mutant UL15 gene-containing plasmid, either pUC-UL15E1LacZS (exon 1 insertion) or pUC-UL15E2LacZS (exon 2 insertion). Homologous recombination between KOS DNA and these mutant plasmids would be expected to generate recombinant viruses which contain the ICP6::*lacZ* cassette inserted either in UL15 exon 1 (at residue 153) or in UL15 exon 2 (at residue 509). Progeny viruses were plated on M-3 cells, and blue plaques were isolated, purified, and propagated. Independent plaques resulting from marker transfer with pUC-UL15E1LacZS (*hr*81-1) and pUC-UL15E2LacZS (*hr*81-2) were isolated. To confirm that *hr*81-1 and *hr*81-2 contain the ICP6::*lacZ* cassette at the appropriate positions, total DNA from the cells infected with KOS, *hr*81-1 or *hr*81-2 was digested with *Xho*I and subjected to Southern blot hybridization using either the ^{32}P-labeled *Eco*RI *lacZ* fragment or the ^{32}P-labeled UL15 cDNA *Afl*II/*Mlu*I fragment as a probe as described in the legend to Fig. 2. The *lacZ* probe would be expected to detect a 5.7-kb fragment in *hr*81-1 and a 3.8-kb fragment in *hr*81-2 (Fig. 2A, lanes 3, 4, 7, and 8). When the membrane was hybridized with the UL15 cDNA probe, 4.8-, 2.8-, and 2.5-kb *Xho*I fragments would be expected in KOS DNA (Fig. 2B, lanes 2 and 6). However, since the inserted ICP6::*lacZ* cassette contains one *Xho*I recognition site, *hr*81-1 would be expected to contain 5.7- and 1.4-kb fragments instead of the 2.8-kb fragment, while *hr*81-2 was expected to contain 3.8- and 2.9-kb fragments instead of the 2.5-kb frag-

TABLE 1. Plaquing efficiencies of *hr*81-1 and *hr*81-2 on Vero, C-2, G-27, and M-3 cells

Cell	Integrated UL15 gene[a]	Copy no.	Titer (PFU/ml)		
			KOS	*hr*81-1	*hr*81-2
Vero	N/A	NA	1.5×10^8	$<5 \times 10^3$	$<5 \times 10^3$
C-2	cDNA	200	1.6×10^8	2.2×10^8	3.8×10^8
G-27	Genomic DNA[c]	60	1.2×10^8	1.2×10^8	1.5×10^8
M-3[b]	Genomic DNA[d]	20	1.0×10^8	1.9×10^8	2.7×10^8

[a] Refers to type of the UL15 gene integrated into the cell genome.
[b] Used to make virus stocks of the UL15 mutants.
[c] *Apo*I fragment containing UL15, UL16, and UL17 genes.
[d] *Hin*dIII fragment containing UL13 to UL19 genes.

ment present in KOS DNA (Fig. 2B, lanes 3, 4, 7, and 8). Figure 2 clearly demonstrates all of the expected bands, thus indicating that the ICP6::*lacZ* cassette was inserted into the expected sites in both *hr*81-1 and *hr*81-2.

Phenotypic analysis of *hr*81-1 and *hr*81-2. To determine if UL15 is essential for viral growth, *hr*81-1 and *hr*81-2 were tested for plaque-forming ability on Vero, C-2, G-27, and M-3 cell lines. Both mutants were unable to form plaques on Vero cells but were able to do so on either C-2, G-27, or M-3 cells at similar efficiencies (Table 1). Since the C-2 cell line contains only the UL15 cDNA, this result indicates that growth defects in *hr*81-1 and *hr*81-2 are within the UL15 gene and that the UL15 gene is essential for viral growth. A transient complementation assay further confirms that growth defects in *hr*81-1 and *hr*81-2 were due to loss of UL15 gene function. Vero cells were transfected with expression constructs in which either the UL15 cDNA (pCMV-UL15C) or the UL15 genome DNA (pCMV-UL15G) was under the control of a constitutive CMV-IE promoter; the transfected cells were superinfected with the UL15 mutant viruses. Table 2 shows that cells transfected with pCMV-UL15G and pCMV-UL15C could support the growth of both *hr*81-1 and *hr*81-2 whereas cells transfected with the control plasmid (pCDNAI/Amp) could not. Taken together, these results indicate that growth defects in *hr*81-1 and *hr*81-2 result from loss of the essential UL15 gene.

The abilities of *hr*81-1 and *hr*81-2 to express UL15 proteins were determined by Western blot analysis. Total cell extracts from infected cells were prepared and assessed for the presence of UL15 gene products by using an α-UL15 polyclonal antibody raised against an MBP-UL15 exon 2 fusion protein as described in Materials and Methods. The following observations were made. (i) In the KOS-infected Vero cell extract, there was a band of 81 kDa (Fig. 3A and B, lanes 2) which was absent in both *hr*81-1- and *hr*81-2-infected Vero cells (Fig. 3A and B, lanes 3 and 4). The size of this band was consistent with the predicated molecular mass of 81 kDa for the full-length UL15 protein of 735 aa. When M-3 cells were infected with either *hr*81-1 or *hr*81-2, the 81-kDa band was detected (Fig. 3A and B, lanes 7 and 8), indicating that expression of the full-length UL15 protein in the cell line could be induced by viral infection. The 81-kDa band was also detected in the extract from Vero cells transfected with expression plasmid pCMV-UL15G or pCMV-UL15C (Fig. 3A and B, lanes 10 and 11), whereas this band was absent in the extract from Vero cells transfected with the control plasmid pCDNAI/Amp (Fig. 3A and B, lanes 9). Thus, these results confirm that the 81-kDa band is the authentic full-length product of the UL15 gene. (ii) Since *hr*81-2 contains the ICP6::*lacZ* cassette inserted at amino acid residue 509, it might be expected that a 59-kDa truncated derivative of the UL15 protein lacking its 226 C-terminal amino acid residues would still be expressed (Fig. 1). In fact, in *hr*81-2-infected cells, the predicted 59-kDa band was detected (Fig. 3A and B, lanes 4 and 8). The inability of the truncated UL15 protein to support viral growth indicates that the C terminus of the full-length UL15 protein is essential. Since the ICP6::*lacZ* cassette is inserted at aa 153 in *hr*81-1, it might be expected that the 153-aa C-terminally truncated UL15 protein with the predicted molecular mass of 20 kDa would be expressed as well; however, this product would not be detected by the antibody which was raised against the second exon of

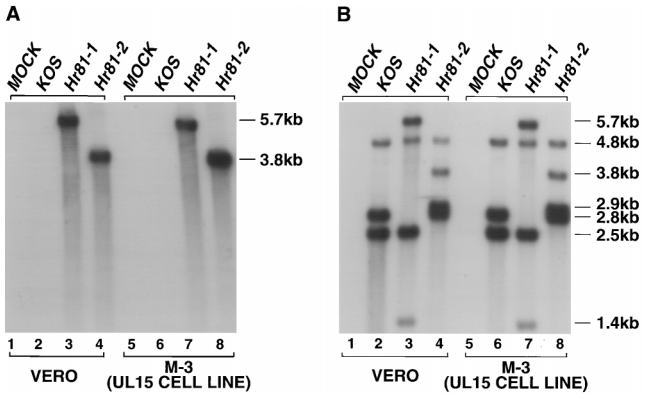

FIG. 2. The ICP6::*lacZ* cassette is inserted at the expected positions in the viral genome. Vero and M-3 cells were infected with KOS, *hr*81-1, or *hr*81-2 at an MOI of 3 PFU per cell for 12 h at 37°C. Total DNA was extracted, digested with *Xho*I, separated by conventional agarose electrophoresis, and blotted to a GeneScreen Plus membrane, which was hybridized with either the ^{32}P-labeled *Eco*RI *lacZ* fragment (A) or the ^{32}P-labeled *Afl*II/*Mlu*I UL15 cDNA fragment (B). Lanes 1 to 4 represent DNA from infected Vero cells, and lanes 5 to 8 represent DNA from infected M-3 cells. Lanes 1 and 5, mock-infected cells; lanes 2 and 6, KOS-infected cells; lanes 3 and 7, *hr*81-1-infected cells; lanes 4 and 8, *hr*81-2-infected cells. Fragment sizes are indicated.

TABLE 2. Transient complementation of *hr*81-1 and *hr*81-2 with UL15 expression plasmids[a]

Plasmid	CI[b]	
	*hr*81-1	*hr*81-2
None	1	1
pCDNAI/Amp	0.5	0.7
pCMV-UL15C	670	125
pCMV-UL15G	230	45

[a] Vero cells were transfected with the indicated plasmid and superinfected with either *hr*81-1 or *hr*81-2 as described in Materials and Methods. Progeny virus was titered on M-3 cells. This experiment was repeated three times, and similar results were obtained each time.
[b] Complementation index (CI) is calculated as PFU of progeny virus from cultures transfected with the indicated plasmid/PFU of progeny virus from mock-transfected cultures.

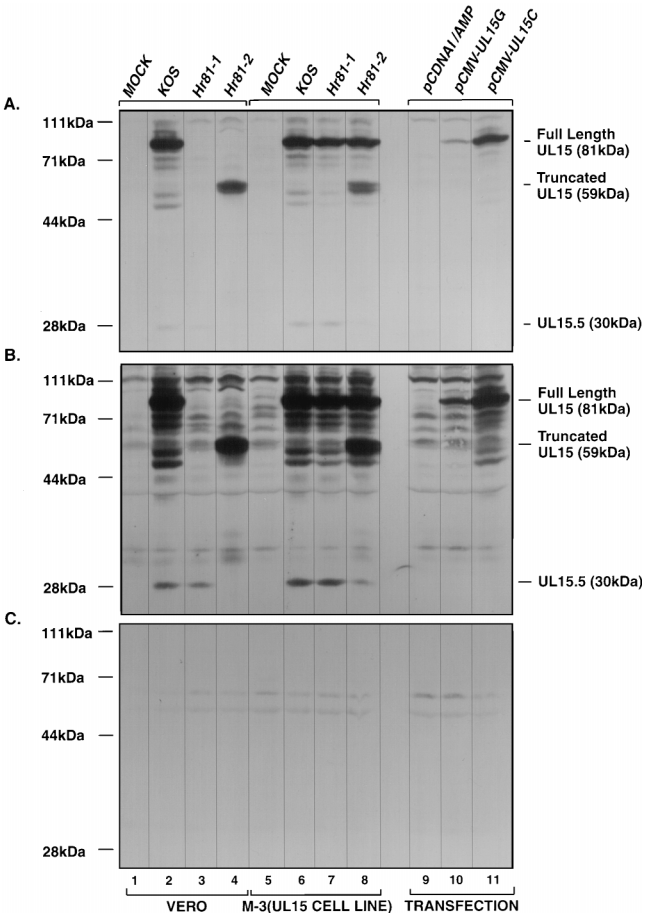

FIG. 3. Expression of UL15 gene products in infected and transfected cells. Vero and M-3 cells were infected with the indicated virus or transiently transfected with UL15 expression constructs. Lysates of 4×10^5 cells were prepared, resolved by SDS-PAGE, and immunoblotted by using an ECL protocol as described in Materials and Methods. (A) One-minute exposure using the α-UL15 polyclonal antibody as the primary antibody; (B) 20-min exposure using the α-UL15 antibody as the primary antibody (the densities of the 30-kDa bands in this panel were within the linear response range and thus could be quantified); (C) 20-min exposure using preimmune serum as the primary antibody. Lanes 1 to 4 represent infected Vero cells, lanes 5 to 8 represent infected M-3 cells, and lanes 9 to 11 represent transfected Vero cells. Lanes 1 and 5, mock-infected cells; lanes 2 and 6, KOS-infected cells; lanes 3 and 7, *hr*81-1-infected cells; lanes 4 and 8, *hr*81-2-infected cells. Lanes 9 to 11 are Vero cells transfected with control plasmid pCDNAI/Amp and UL15 expression plasmids pCMV-UL15G and pCMV-UL15C, respectively. The positions of the full-length UL15 protein, the truncated UL15 protein expressed in *hr*81-2-infected cells, and the UL15.5 protein are indicated.

UL15. (iii) The α-UL15 antibody specifically recognized a 30-kDa protein in KOS-infected cells (Fig. 3B, lanes 2 and 6), which was consistent with the observation by Baines et al. that UL15 encodes two proteins with molecular masses of 75 and 35 kDa (6). However, more interestingly, we found that *hr*81-1 was still able to express the 30-kDa protein whereas *hr*81-2 failed to express either the 81- or 30-kDa protein (Fig. 3B, lanes 3 and 4). Both mutants as well as KOS were able to express the 30-kDa UL15 protein on M-3 cells (Fig. 3B, lanes 6 to 8). Quantification of the 30-kDa protein bands indicated that the levels of 30-kDa protein expressed in *hr*81-1-infected Vero cells were comparable with those expressed by wild-type virus-infected Vero cells (data not shown). Furthermore, the absence of the 30-kDa protein in *hr*81-2-infected cells was not the result of lower overall expression from the UL15 gene

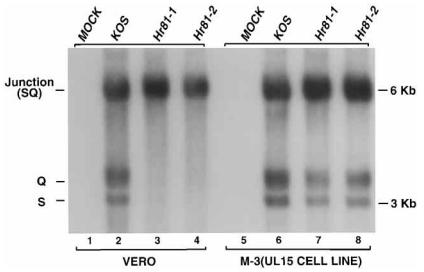

FIG. 4. UL15 mutants are defective in viral DNA cleavage. Vero or M-3 cells were infected with the indicated virus at an MOI of 3 PFU per cell at 37°C for 12 h. Five micrograms of total DNA extracts was prepared from infected cells, digested with *Bam*HI, and analyzed by Southern blot hybridization as described in the legend to Fig. 1, using the ^{32}P-labeled *Bam*HI SQ junction fragment (Fig. 1) as a probe. Lanes 1 to 4 represent infected Vero cells, and lanes 5 to 8 represent infected M-3 cells. Lanes 1 and 5, mock-infected cells; lanes 2 and 6, KOS-infected cells; lanes 3 and 7, *hr*81-1-infected cells; lanes 4 and 8, *hr*81-2-infected cells. The positions of junction (SQ) and terminal (S and Q) fragments are indicated.

since the 59-kDa truncated UL15 protein was still expressed at significant levels (Fig. 3A and B, lane 4). This observation has two implications. First, the presence of the 30-kDa version and the absence of the 81-kDa version of the UL15 protein in *hr*81-1-infected cells indicates that the 30-kDa protein is not likely to be a proteolytic breakdown product of the 81-kDa protein; it may be translated from an internal in-frame methionine downstream of residue 153 of UL15 where the ICP6::*lacZ* cassette is inserted (see Discussion). Second, the 30-kDa version of UL15 is unable to functionally substitute for the 81-kDa full-length UL15 protein, based on the growth phenotype of *hr*81-1. Figure 3C shows that the preimmune serum failed to detect either the 81- or 30-kDa band in infected or transfected cell extracts, confirming that both bands detected by the α-UL15 antibody are authentic UL15 gene products rather than nonspecific cross-reacting proteins. Although the origin of the 30-kDa protein is not clear, we tentatively designate it UL15.5 (see Discussion). In summary, we conclude that the full-length UL15 protein is essential for viral growth and that its functions cannot be substituted by the 59-kDa C-terminally truncated UL15 protein expressed by *hr*81-2 or the 30-kDa UL15.5 protein expressed by *hr*81-1.

***hr*81-1 and *hr*81-2 are defective in DNA cleavage while retaining the ability to synthesize viral DNA.** The temperature-sensitive mutant of UL15 (*ts*66.4) is defective in DNA cleavage and packaging at the nonpermissive temperature (6, 44). *hr*81-1- or *hr*81-2-infected Vero cells exhibit wild-type levels of viral DNA synthesis, as assessed by dot blot hybridization (data not shown). To confirm that UL15 is required for the viral DNA cleavage, cells infected with *hr*81-1 or *hr*81-2 were examined for the presence of free genomic termini. Total DNA from Vero or M-3 cells infected with KOS, *hr*81-1, or *hr*81-2 was digested with *Bam*HI and subjected to Southern blot hybridization with a *Bam*HI SQ-specific probe as described in the legend to Fig. 4. This probe specifically hybridizes to both terminal *Bam*HI S and Q fragments as well as junction *Bam*HI SQ fragments. The levels of SQ junction fragments present in *hr*81-1- and *hr*81-2-infected cells are similar to those in KOS-infected cells (Fig. 4, lanes 2 to 4), confirming that the UL15 mutants were capable of wild-type levels of DNA replication. However, S and Q terminal fragments were absent in both *hr*81-1- and *hr*81-2-infected Vero cells, while these terminal fragments were readily detectable in KOS-infected Vero cells as well as in *hr*81-1- or *hr*81-2-infected M-3 cells (Fig. 4, lanes 2 and 6 to 8), indicating that UL15 mutant viruses were unable

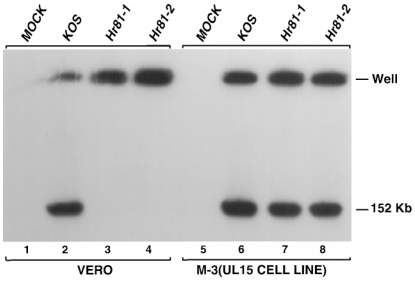

FIG. 5. UL15 mutants fail to process concatemeric replicating DNA to monomeric viral genomes. Vero or M-3 cells were infected with the indicated virus at an MOI of 5 PFU per cell at 37°C. At 18 h postinfection, 7.5×10^5 infected cells were suspended in low-melting-point agarose gel blocks, lysed in situ, subjected to PFGE as described previously (35), and analyzed by Southern blot hybridization using the ^{32}P-labeled *Afl*II/*Mlu*I UL15 cDNA fragment as a probe. Lanes 1 to 4 represent infected Vero cells, and lanes 5 to 8 represent infected M-3 cells. Lanes 1 and 5, mock-infected cells; lanes 2 and 6, KOS-infected cells; lanes 3 and 7, *hr*81-1-infected cells; lanes 4 and 8, *hr*81-2-infected cells. The positions of well (concatemeric) and 152-kb (monomeric) DNAs are indicated.

to cleave viral DNA concatemers into monomers to generate free viral genomic termini under nonpermissive conditions.

The DNA cleavage defects in *hr*81-1 and *hr*81-2 were confirmed by analyzing the structure of viral DNA which accumulates in KOS- and UL15 mutant-infected cells. Viral DNA replication results in the accumulation of concatemeric DNA intermediates, which are subsequently cleaved into mature monomeric genomes. PFGE of DNA from HSV-infected cells results in the separation of viral DNA into two bands, one which does not enter the gel and represents replicating DNA intermediates (well DNA) and one which migrates as a 152-kb genome-length DNA (35, 48, 60). Total DNA from Vero or M-3 cells infected with either KOS, *hr*81-1, or *hr*81-2 was subjected to PFGE and subsequently analyzed by Southern blot using the UL15 cDNA fragment as a probe. DNA from KOS-infected Vero cells as well as UL15 mutant- or KOS-infected M-3 cells contains both replicating well DNA and monomeric DNA (Fig. 5, lanes 2 and 6 to 8). However, DNA from *hr*81-1 and *hr*81-2-infected Vero cells contains only well DNA (Fig. 5, lanes 3 and 4). The absence of 152-kb genome-length viral DNA in *hr*81-1- and *hr*81-2-infected Vero cells indicates that inactivation of the UL15 gene results in the failure to cleave DNA concatemers into mature monomeric genomes. The comparable amounts of viral DNA detected in KOS-, *hr*81-1-, and *hr*81-2-infected Vero cells (Fig. 5, lanes 2 to 4) is again in agreement with the conclusion that UL15 is not required for viral DNA synthesis.

***hr*81-1 and *hr*81-2 are defective in DNA packaging.** To assess the ability of *hr*81-1 and *hr*81-2 to encapsidate DNA, DNase I-resistant DNA from infected cells was measured. DNase I treatment would be expected to degrade all DNA in infected cells except for encapsidated viral DNA protected by capsids. DNase I-protected DNA from Vero and M-3 cells infected with KOS or UL15 mutants was prepared as described in the legend to Fig. 6 and analyzed by Southern blot using the ^{32}P-labeled *Bam*HI SQ fragment as a probe. Figure 6 shows that DNase I-resistant S and Q terminal fragments were observed in KOS-infected Vero cells and in KOS- or UL15 mutant-infected M-3 cells, indicating that the viral monomeric DNA was encapsidated efficiently (Fig. 6, lanes 2 and 6 to 8). In contrast, DNA from *hr*81-1 and *hr*81-2-infected Vero cells was completely degraded upon DNase I treatment (Fig. 6, lanes 3 and 4), suggesting that DNA packaging is defective in these two UL15 mutants. In summary, these results suggest that the UL15 gene is essential for both cleavage and packaging of viral DNA.

***hr*81-1 and *hr*81-2 are capable of DNA inversion.** During viral DNA replication, the two unique sequences (U_L and U_S) invert relative to one another, resulting in four types of viral monomeric isomers (28). It has been suggested that genomic inversion, a result of a recombination event between the inverted repeats flanking the U_L and U_S sequences, may be stimulated by the presence of free termini generated by DNA cleavage and packaging (53). Therefore, we decided to assess genomic inversion in UL15 mutants incapable of DNA cleavage and packaging to see whether the terminal ends generated by this mechanism are essential for inversion. The inversion event can be assessed by examining the restriction pattern of replicating well DNA. For instance, as diagrammed in Fig. 7, digestion with *Spe*I, which cleaves once per monomer unit, would be expected to release bands of 118, 152, and 186 kb from the replicating intermediate had the inversion event occurred. Vero cells were infected with UL15 mutant viruses for various times, and well DNA was isolated, digested with *Spe*I, and analyzed by Southern blotting as described in the legend to Fig. 8. Using the ^{32}P-labeled *Bam*HI SQ fragment as a probe, the predicted 118-, 152-, and 186-kb bands were detected in KOS-infected as well as in *hr*81-1- and *hr*81-2-infected Vero cells as early as at 6 h postinfection (Fig. 8, lanes 4 to 6). At each of the times tested (from 3 to 15 h), the levels of three isomer bands in *hr*81-1- and *hr*81-2-infected cells were comparable to those in KOS-infected cells and increased with time. These results clearly indicate that DNA inversion occurs in UL15 mutants as efficiently as in the wild-type virus. Lamberti and Weller demonstrated that in a mutant virus defective in UL6, another gene required for DNA cleavage and packaging, DNA inversion occurs normally as well (32). Taken together, these results indicate that recombination and genomic inversion is likely linked to DNA replication rather than to DNA cleavage and packaging, which is in agreement with the model proposed by Sarisky and Weber (47).

In this experiment, well DNA was also analyzed for the presence of free termini. It has been reported that wild-type well DNA contains only one type of free genomic termini corresponding to the U_L portion of the HSV-1 genome (35, 60). Furthermore, the U_L termini can be released as either an

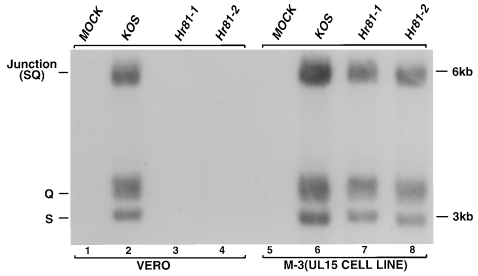

FIG. 6. UL15 mutants fail to encapsidate viral DNA. Vero and M-3 cells were infected with the indicated virus at an MOI of 3 PFU per cell at 37°C for 12 h. Cells were lysed and treated with 50 μg of DNase I per ml for 2 h. Samples were resuspended in 0.6% SDS–10 mM EDTA–10 mM Tris (pH 7.4)–100 μg of proteinase K per ml for 4 h to inactivate DNase I and to remove protein. Samples were subsequently incubated with 100 μg of RNase per ml for 30 min to remove RNA. All enzyme treatments were carried out at 37°C. After phenol extraction, 5 μg of DNase I-resistant DNA was digested with *Bam*HI and analyzed by Southern blot hybridization as described in the legend to Fig. 5. Lanes 1 to 4 represent infected Vero cells, and lanes 5 to 8 represent infected M-3 cells. Lanes 1 and 5, mock-infected cells; lanes 2 and 6, KOS-infected cells; lanes 3 and 7, *hr*81-1-infected cells; lanes 4 and 8, *hr*81-2-infected cells. The positions of *Bam*HI junction (SQ) and terminal (S and Q) fragments are indicated.

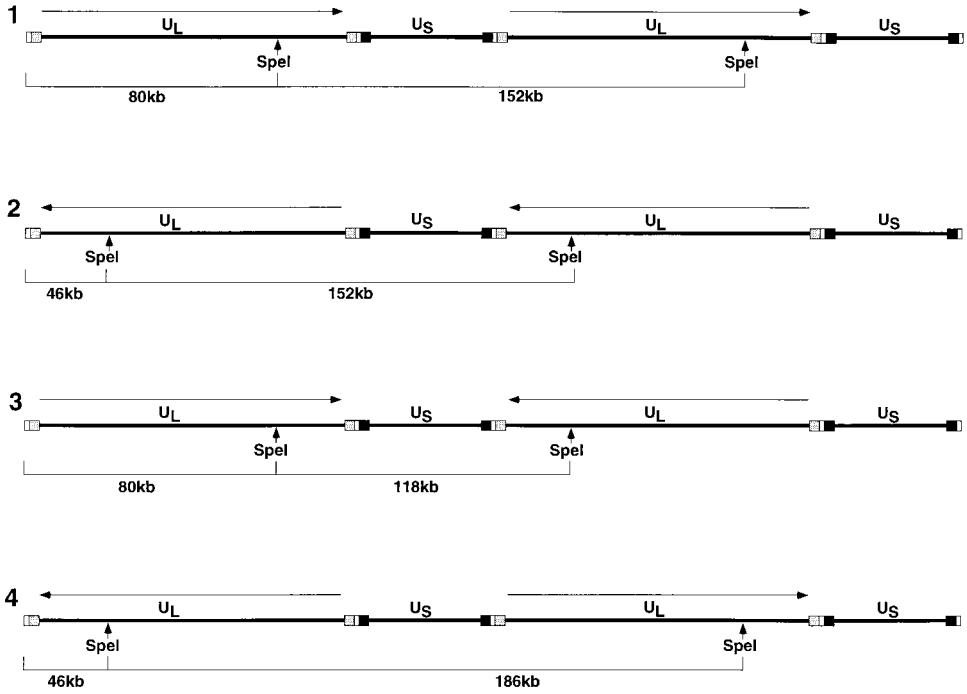

FIG. 7. Schematic diagram of replicating HSV-1 DNA showing expected fragments released by *Spe*I digestion. Four different relative orientations of neighboring U_L arms due to genomic inversion (lines 1 to 4) give rise to three discrete isomeric forms of the linear HSV-1 genome with sizes of 118, 152, and 186 kb upon *Spe*I digestion. The expected 46-kb (lanes 2 and 4) and 80-kb (lanes 1 and 3) U_L terminal fragments released by *Spe*I digestion are also indicated, depending on the orientation of the terminal U_L arm. Hypothetical U_S terminal fragments of 72 or 106 kb released by *Spe*I digestion are not shown.

80-kb or a 46-kb band upon *Spe*I digestion, depending on the orientation of the terminal U_L arm as diagrammed in Fig. 7, and these two bands were found to hybridize with a U_L terminus-specific probe but not with a U_S-specific probe in Southern blot analysis (32, 60). In Fig. 8, two extra bands, corresponding to the 80- and 46-kb *Spe*I U_L-terminal fragments, were detected in the *Spe*I-treated wild-type well DNA as early as at 9 h postinfection as reported previously, while they were absent in the *Spe*I-treated well DNA from either *hr*81-1- or *hr*81-2-infected cells at all times tested. The presence of U_L termini in wild-type replicating DNA led Zhang et al. to propose that these free termini may result either from the initiation and termination of DNA replication or from the cleavage/packaging process (60). The absence of these two bands in the *Spe*I-treated well DNA from *hr*81-1- or *hr*81-2-infected cells reported in this paper, together with the observation by Lamberti and Weller that U_L termini were also absent in *Spe*I-treated well DNA from the UL6 mutant (32), suggests that these free U_L termini present in the replicating concatemeric DNA intermediates are products of DNA cleavage and packaging rather than DNA replication.

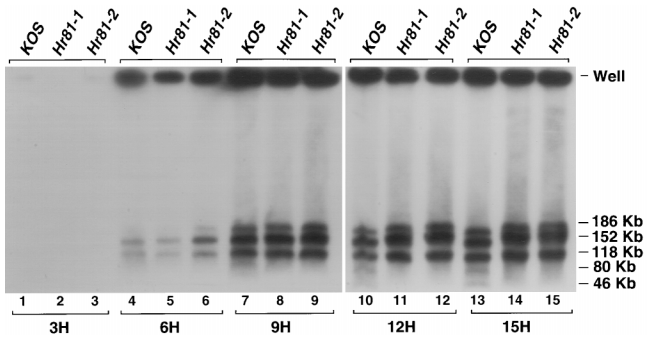

FIG. 8. UL15 mutants retain the ability to undergo DNA inversion. A total of 7.5×10^5 cells were infected with the indicated virus at an MOI of 5 PFU per cell at 37°C for 3 h (lanes 1 to 3), 6 h (lanes 4 to 6), 9 h (lanes 7 to 9), 12 h (lanes 10 to 12), and 15 h (lanes 13 to 15). Well DNA was twice purified away from monomeric DNA as described previously (35), digested with *Spe*I in situ, subjected to PFGE, and analyzed by Southern blot hybridization using the ^{32}P-labeled *Bam*HI SQ junction fragment as a probe. Lanes 1, 4, 7, 10, and 13, KOS-infected cells; lanes 2, 5, 8, 11, and 14, *hr*81-1-infected cells; lanes 3, 6, 9, 12, and 15, *hr*81-2-infected cells. Bands corresponding to positions of well, 46-kb, 80-kb, 118-kb, 152-kb, and 186-kb DNAs are indicated.

Analysis of capsid formation in *hr*81-1 and *hr*81-2. Herpesvirus-infected cells contain three types of capsid structures (A, B, and C), as identified by sucrose gradient velocity centrifugation. It was reported that only B-type capsids were found in Vero cells infected with mutants defective in cleavage and packaging (1–3, 32, 42, 52, 55). The absence of A- and C-type capsids suggests that DNA packaging is not even attempted. In contrast, infection of Vero cells with a mutant defective for viral alkaline nuclease (UL12) results in the appearance of B capsids, an increased abundance of A capsids, and few C capsids. This result suggests that packaging was attempted but was either interrupted or aborted (49). In this study, lysates of Vero cells infected with KOS, *hr*81-1, or *hr*81-2 were subjected to 15 to 50% sucrose gradient centrifugation as described in Materials and Methods. In KOS-infected Vero cells, all three capsid bands, A, B, and C, were clearly observed (Fig. 9, lane 1). However, in both *hr*81-1- and *hr*81-2-infected cells, only B-type capsids were seen (Fig. 9, lanes 2 and 3). The absence of A and C capsids in *hr*81-1- and *hr*81-2-infected Vero cells suggests that, unlike the UL12 mutant, in which DNA encapsidation is attempted but aborted, both UL15 mutants were defective at

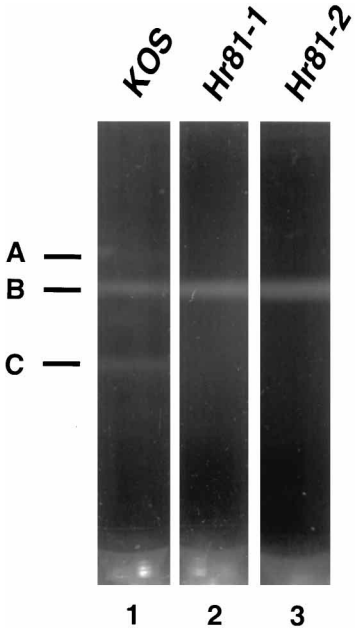

FIG. 9. UL15 mutants fail to make A and C capsids. A total of 1.5×10^7 Vero cells were infected with KOS, *hr*81-1, or *hr*81-2 at an MOI of 10 PFU per cell at 37°C. At 20 h postinfection, the cells were lysed in 1% Nonidet P40-containing lysis buffer by sonication and subjected to 15 to 50% sucrose gradient centrifugation as described in Materials and Methods. Gradients 1, 2, and 3 represent the capsid profiles of lysates from KOS-, *hr*81-1-, and *hr*81-2-infected Vero cells, respectively. The positions of A, B, and C capsid bands of wild-type HSV-1 are indicated.

an earlier stage, in which DNA encapsidation has not even been attempted.

DISCUSSION

In this study, permissive cells were constructed in order to isolate and propagate HSV-1 mutants *hr*81-1 and *hr*81-2 containing the disrupted UL15 gene in which the ICP6::*lacZ* mutagenic cassette was inserted in exon 1 and exon 2, respectively. *hr*81-1 and *hr*81-2 can grow and form plaques on UL15-permissive cells but not on Vero cells, confirming that UL15 is an essential gene for viral growth. Both mutants are defective in DNA cleavage and packaging, and they accumulate only B-type capsids although they still retain the ability to synthesize wild-type levels of DNA. Only cells containing the UL15 gene either stably as in permissive cell lines or transiently as in the transient complementation assay are able to support the growth of *hr*81-1 and *hr*81-2, indicating that the phenotypes of UL15 mutant viruses are due to loss of UL15 function. Our results are consistent with those obtained from studies of the UL15 temperature-sensitive mutant *ts*66.4 (6, 44).

The wild-type virus synthesizes two proteins with molecular masses of 81 and 30 kDa, detected by Western blot analysis using a polyclonal antibody raised against the UL15 exon 2 sequence. These results confirm those of Baines et al. for assays using an independently isolated UL15 antibody (6). Furthermore, a C-terminally truncated derivative of the UL15 protein expressed in *hr*81-2-infected cells was not able to support viral growth, indicating that the C-terminal 226 aa are essential for UL15 function. Interestingly, *hr*81-1 was able to synthesize the 30-kDa protein but not the 81-kDa UL15 protein. This result indicates that the 30-kDa protein is a separate translation product rather than a degradation product of the full-length 81-kDa UL15 protein. Analysis of the UL15 protein sequence reveals five internal methionines situated at amino acid residues 370, 418, 443, 460, and 500, which, if used for initiation, would encode proteins with predicted molecular masses of 40, 34, 32, 30, and 25 kDa, respectively; methionine residues 370 and 443 appear to be in a more optimal context for translation than the other three (31). Costa et al. have identified a 2.7-kb mRNA in the UL15 region which presumably encodes the 81-kDa protein; however, two smaller mRNAs which may have been generated either from independent promoters or by alternative splicing events were observed (15). It is possible that one of these smaller mRNAs encodes the 30-kDa protein; alternatively, the protein may be translated from the 2.7-kb mRNA, using an internal methionine as a start codon. In this paper, we have tentatively designated this 30-kDa protein as UL15.5.

Although our results indicate that UL15 is essential for viral growth and for DNA cleavage and packaging, its precise role in these processes is still unknown. It has been speculated that the UL15 protein may have a terminase function, based on its homology with the large subunit of T4 terminase complex (gp17) (17). For most dsDNA bacteriophages, the terminase is composed of two subunits, a 10- to 25-kDa low-molecular-mass protein and a 60- to 75-kDa high-molecular-mass protein, for instance, gp16 and gp17 of T4, gp18 and gp19 of T3, and gpA and gpNu1 of lambda. Large subunits of the terminase usually possess prohead binding and DNA-dependent ATPase activities while the small subunits exhibit sequence-specific DNA binding activities (10, 26). Furthermore, in these dsDNA phages, genes for two subunits are often situated adjacent to each other in the genome. In the HSV-1 UL15 gene region, all five possible start codons for translation of UL15.5 are situated in UL15 exon 2 and downstream of the potential ATP binding site (GKT [amino acid residues 263 to 265] and DE [amino acid residues 356 to 357]), suggesting that the UL15.5 protein may not have ATP binding activity. It is possible that the UL15.5 protein acts as the small subunit of the terminase. Alternatively, it is possible that another viral protein such as one of the other cleavage and packaging proteins performs this function or that the subunit structure of the HSV terminase is unlike that of the dsDNA bacteriophages.

Since DNA cleavage and packaging are biologically linked with capsid formation, it is expected that some DNA cleavage/packaging proteins may interact with capsids in vivo. It has been reported that UL6 is present stably in all three types of capsids and mature virion particles and that UL25 is associated with purified HSV-1 virions as well (4, 33, 41). Preliminary immunoblot analysis reveals that the full-length 81-kDa UL15 protein expressed in KOS-infected cells is associated with B capsids (data not shown). Thus, at least three minor-capsid proteins, UL6, UL15, and UL25, have also been implicated in cleavage and packaging, although their roles are not clear. In dsDNA bacteriophage systems, terminase has been found to interact with proheads. The finding of UL15 in B capsids is consistent with its putative function as the HSV-1 terminase. In addition, by analogy to dsDNA bacteriophage systems, if there is a unique portal vertex, it is intriguing to speculate that some of the minor-capsid components may be part of this structure. Alternatively, herpesviruses may utilize a different DNA packaging mechanism which does not require the presence of a unique portal.

The reagents generated in this study will facilitate the further functional analysis of the UL15 gene. The availability of UL15-permissive cell lines and ICP6::*lacZ* insertional mutants which are capable of expressing blue color in the presence of X-Gal will facilitate the isolation of viruses bearing subtle

site-specific mutations. The null background of UL15 insertional mutants will allow us to develop biochemical assays in vitro and to carry out in vivo complementation assays for the detailed assessment of the functions of individual domains within the UL15 gene.

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