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The Herpes Simplex Virus 1 U_L15 Gene Encodes Two Proteins and Is Required for Cleavage of Genomic Viral DNA

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Previous studies have shown that a *ts* mutant [herpes simplex virus 1(mP)*ts*66.4] in the U_L15 gene fails to package viral DNA into capsids (A. P. W. Poon and B. Roizman, J. Virol. 67:4497–4503, 1993) and that although the intron separating the first and second exons of the U_L15 gene contains U_L16 and U_L17 open reading frames, replacement of the first exon with a cDNA copy of the entire gene does not affect viral replication (J. D. Baines, and B. Roizman, J. Virol. 66:5621–5626, 1992). We report that (i) a polyclonal rabbit antiserum generated against a chimeric protein consisting of the bacterial maltose-binding protein fused in frame to the majority of sequences contained in the second exon of the U_L15 gene reacted with two proteins with M_r of 35,000 and 75,000, respectively, in cells infected with a virus containing the authentic gene yielding a spliced mRNA or with a virus in which the authentic U_L15 gene was replaced with a cDNA copy. (ii) Insertion of 20 additional codons into the C terminus of U_L15 exon II caused a reduction in the electrophoretic mobility of both the apparently 35,000- and 75,000-M_r proteins, unambiguously demonstrating that both share the carboxyl terminus of the U_L15 exon II. (iii) Accumulation of the 35,000-M_r protein was reduced in cells infected and maintained in the presence of phosphonoacetate, an inhibitor of viral DNA synthesis. (iv) The U_L15 proteins were localized in the perinuclear space at 6 h after infection and largely in the nucleus at 12 h after infection. (v) Viral DNA accumulating in cells infected with herpes simplex virus 1(mP)*ts*66.4 and maintained at the nonpermissive temperature was in an endless (concatemeric) form, and therefore U_L15 is required for the cleavage of mature, unit-length molecules for packaging into capsids.

The herpes simplex virus 1 (HSV-1) U_L15 gene is highly conserved among members of the family *Herpesviridae* and consists of two (for HSV-1, varicella-zoster virus, Epstein-Barr virus, and human cytomegalovirus) or three (for channel catfish herpesvirus) exons (2, 9–12, 18). Even though all U_L15 homologs consist of two or more exons, a cDNA copy of the U_L15 gene containing the first and second exons linked together can effectively support viral replication of HSV-1 (4). A potentially significant clue to the function of U_L15 came from the observation that the channel catfish herpesvirus U_L15 homolog had limited homology to the bacteriophage T4 protein gp17, a viral terminase required for cleavage and packaging of bacteriophage DNA (8, 11). A possible role for U_L15 at least in the packaging of viral DNA into capsid was deduced from the observation that in cells infected with a spontaneous temperature-sensitive (*ts*) mutant in the second exon of the U_L15 gene, both viral DNA and preformed capsids accumulated but packaging of DNA into capsids did not ensue at the nonpermissive temperature (19).

In this report we show that the U_L15 gene products consist of two polypeptides sharing sequences from the second exon, that the distribution of U_L15 protein changes during the course of infection, and that functional protein is required to cleave viral DNA from concatemers and to package the DNA into capsids.

MATERIALS AND METHODS

Cells and viruses. Vero cells (the human thymidine kinase 143^- [$143TK^-$] cells), rabbit skin cells, and HEp-2 cells were maintained as previously described (4, 6). The virus strains HSV-1(F), HSV-1(mP), and HSV-1(mP)*ts*66.4 and the genetically engineered recombinant virus R7206 containing a chimeric gene encoding thymidine kinase (*tk* gene) within the U_L15 intron just upstream of U_L16 have been described previously (3, 14, 16, 19). Viral stocks were grown and titrated in Vero cell monolayer cultures.

Enzymes. Restriction enzymes were obtained from New England Biolabs. T4 DNA polymerase was obtained from Boehringer Mannheim (Indianapolis, Ind.).

Purification and analysis of DNA. DNA probes were labeled with fluorescein-tagged nucleotides according to the directions of the manufacturer from which the nucleotides were purchased (Amersham). Viral DNA intended for transfection was purified on NaI gradients as described previously (25). For analysis of recombinant virus DNA, cytoplasmic extracts were extracted with phenol-chloroform and DNA was precipitated in ethanol as previously described (23). The conditions for agarose gel electrophoresis of viral DNA were previously described (5). Hybridization with denatured, labeled probe was done at 56°C in 30 to 50% formamide but otherwise conformed to the directions of the manufacturer of the ECL kit (Amersham). Bound probe was visualized by the addition of antifluorescein antibody followed by washing and the addition of substrate according to the directions of the manufacturer (Amersham). Fluorescent images were recorded by 5- to 30-min exposures to X-Omat radiographic film (Kodak). Alternatively, hybridization with radiolabeled probes and autoradiography were done as previously described (19).

Plasmids. A DNA oligomer, CGC GTG AAG GGC CAG AAG CCC AAC CTG CTG GAC CGC CTG CGC CAC CGC AAG AAC GGC GGG TAC CGC CAC TAA GCG C, and its complement, C GCG TTA GTG GCG GTA CCC GCC GTT CTT GCG GTG GCG CAG GCG GTC CAG GTT GGG

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CTT CTG GCC CTT CAC GCG, were heated at 90°C and allowed to anneal. The resultant mostly double-stranded oligomer incorporated a *Kpn*I site and encoded an epitope, N-KGQKPNLLDRLRHRKNGYRNH-stop, of the human cytomegalovirus glycoprotein B gene flanked by single-stranded CGCG ends (7). The cohesive ends allowed cloning of the oligomer into the *Mlu*I site at the 3′ end of the U_L15 exon II contained in plasmid pRB4125. As a consequence, the predicted amino acid sequence at the 3′ end of exon II of U_L15 is Arg-epitope-stop. The insertion of the stop codon is expected to preclude the penultimate codon (serine) of U_L15 exon II from being translated. The DNA incorporating and surrounding the inserted oligomer was verified by sequencing. The plasmid pRB4119 has been described previously and contains the N-terminal portion of a U_L15 cDNA from a *Hin*dIII site within U_L14 to a *Bam*HI site in U_L15 exon II (4). pRB4119 was cleaved with *Bam*HI, and a 1,400-bp *Bam*HI-*Bgl*II fragment from pRB4125 (map units 0.220 to 0.229) containing the epitopically tagged U_L15 exon II was inserted; the plasmid was designated pRB4203 and contained an intact C-terminal-tagged copy of the U_L15 cDNA.

In order to incorporate the tagged U_L15 cDNA into the HSV-1(F) viral genome by double recombination events, it was necessary to insert U_L16 sequences downstream of the tagged U_L15 gene. Therefore, a previously described plasmid, pRB443, containing HSV-1 DNA defined by a *Hin*dIII site and *Bgl*II site (map units 0.182 to 0.199) (4) was cut with *Sal*I, the site was blunted by T4 DNA polymerase, and the plasmid was then cut with *Sac*I. A 578-bp fragment that contained U_L16 sequences was isolated. pRB4203 was then cut with *Bsu*36I, treated with T4 DNA polymerase and nucleotides, and cleaved with *Sac*I, and a 636-bp fragment was discarded. Insertion of the 578-bp pRB443-derived fragment into the pRB4203 vector produced plasmid pRB4317 containing an epitopically tagged U_L15 gene flanked by U_L14 sequences upstream and U_L16 sequences downstream.

Production of anti-U_L15 polyclonal rabbit serum and immunoblotting. A *Hin*cII-*Xba*I fragment encoding the final 351 amino acids of the 362-codon U_L15 exon II was inserted into the vector pMal-C (New England Biolabs) cleaved with *Stu*I and *Xba*I. The resultant plasmid pRB4362 was predicted to encode the bacterial maltose-binding protein encoded by the *mal E* gene in frame with the majority of U_L15 exon II. DNA encoding the junction between *mal E* and U_L15 exon II was sequenced to verify that the two open reading frames were maintained (data not shown). Production of the fusion protein was induced by the addition of IPTG (isopropyl-β-D-thiogalactopyranoside) to the medium with *Escherichia coli* BL21 cells transformed with pRB4362, followed by affinity purification of the fusion protein from bacterial cell lysates on amylose resin according to the recommendations of the manufacturer (New England Biolabs). New Zealand White rabbits were immunized with the affinity-purified fusion protein as described previously (4, 5). The U_L15-specific antiserum was diluted 1:100 and was allowed to react overnight at 4°C with electrophoretically separated proteins that had been transferred to a nitrocellulose sheet as previously described (5).

Confocal immunofluorescence microscopy. HEp-2 cells were grown in Lab Tek (Nunc) chamber slides and were either mock infected or infected with 50 PFU of R7202 virus per cell. The effective multiplicity of infection in this cell culture system was approximately 1.0 PFU per cell. R7202, a recombinant virus derived from HSV-1(F), does not express glycoprotein E (5). This virus was used in these studies to avoid high background levels produced as a consequence of the high-affinity binding of rabbit immunoglobulin to the Fc receptor formed by glycoproteins E and I (17). At 6 and 12 h after infection, the cells were fixed in cold methanol and blocked for 30 min in phosphate-buffered saline (PBS) plus 1.0% bovine serum albumin (BSA). The cells were then reacted with the anti-U_L15 serum diluted 1:10 to 1:50 in PBS containing 0.1% BSA. The cells were then washed in excess PBS and were reacted with goat anti-rabbit immunoglobulin conjugated to Texas red. Fluorescent images were viewed and recorded with the aid of a Zeiss confocal microscope as previously described (26).

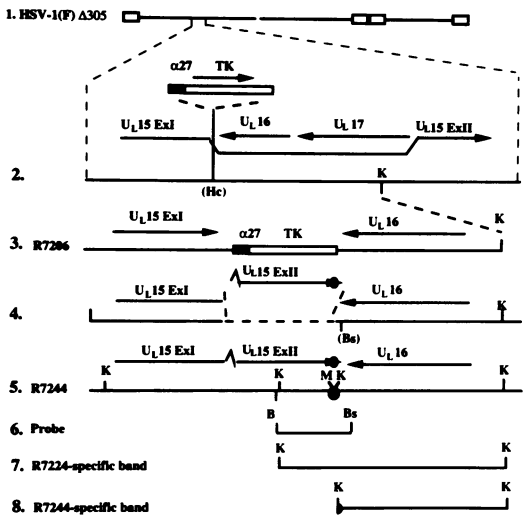

FIG. 1. Colinear schematic representations of sequence arrangements of HSV-1 wild-type and recombinant viruses relevant to these studies. Line 1, HSV-1(F)Δ305 DNA. The repeats flanking the unique sequences are indicated by open rectangles. Lines 2 and 3, insertion of chimeric *tk* gene between first exon of U_L15 and U_L16 sequences during construction of R7206 recombinant virus. The α-27 promoter is represented by a closed rectangle, and *tk* sequences are represented by an open rectangle. Lines 4 and 5, replacement of chimeric *tk* gene in R7206 DNA with second exon of U_L15 bearing an epitopic tag (closed circle) at the C terminus. R7244 viral DNA contains the cDNA of an epitopically tagged U_L15 gene inserted upstream of the U_L16 gene. Line 6, colinear schematic diagram of the probe used in Fig. 2 containing exon II sequences. Line 7, Sequences present in the R7224 specific band in Fig. 2. Line 8, sequences present in the R7244-specific band shown in Fig. 2, lane 3. The *Kpn*I site present in the inserted epitope causes the R7224-specific band to be truncated by approximately 600 bp. Abbreviations: B, *Bam*HI; Bs, *Bsu*36I; ExI, exon I; ExII, exon II, Hc, *Hin*cII; K, *Kpn*I; M, *Mlu*I.

RESULTS

Construction of HSV-1 recombinant containing an epitopically tagged carboxyl terminus of U_L15 protein. In preliminary experiments we noted that the anti-U_L15 polyclonal antibody reacted with two electrophoretically separated polypeptide bands. To verify that both react with U_L15-derived polypeptides and to relate the polypeptides to each other, we epitopically tagged the U_L15 protein at its carboxyl terminus. This was done by insertion of a DNA sequence containing a *Kpn*I site and encoding a 20-amino-acid epitope into the 3′ end of exon II of the U_L15 cDNA. Reagents at our disposal for this purpose included (i) the recombinant virus R7206 containing, between the first exon of U_L15 and U_L16, a *tk* gene driven by the HSV-1 α27 promoter (Fig. 1, lines 2 and 3) (3) and (ii) the plasmid pRB4317 containing the tagged exon II flanked by U_L15 exon I sequences upstream and U_L16 gene sequences

TABLE 1. Genotype of recombinant viruses used in these studies

Virus[a]	Genotype
HSV-1(F)	Wild type
HSV-1(F)Δ305	Δ*tk*
R7224	Δ*tk*, cDNA U_L15, second copy U_L15 exon II in native position
R7227	Δ*tk*, cDNA U_L15, α27-*tk* in second copy exon II
R7235	Δ*tk*, cDNA U_L15
R7244	Δ*tk*, cDNA U_L15 tagged at C terminus with epitope, second copy of U_L15 exon II in native position

[a] Recombinant viruses HSV-1(F)Δ305, R7224, R7227, and R7235 had been constructed previously; R7244 was constructed in these studies.

downstream (see Materials and Methods). R7206 viral DNA and pRB4317 plasmid DNA were cotransfected into rabbit skin cells. It was expected that recombination of the U_L15 exon I and U_L16 sequences within the viral DNA of R7206 and pRB4317 plasmid DNA would cause the insertion of the tagged cDNA and removal of the chimeric *tk* gene of the R7206 virus in some of the viral progeny of the cotransfection (Fig. 1, lines 4 and 5). Selection against the chimeric *tk* gene by plating the progeny of the cotransfection on 143TK$^-$ cells under a bromodeoxyuridine overlay allowed the plaque purification of a virus designated R7244.

The DNAs of recombinants R7244 and R7224 and of the HSV-1(F) parent werc purified from infected cells, cleaved with *Kpn*I, electrophoretically separated on a 1.0% agarose gel, transferred to a nylon membrane, and probed with fluorescein-labeled U_L15 exon II sequences. Fluorescent images were generated and recorded on radiographic film according to the procedure outlined in Materials and Methods. The recombinant R7224 served as a useful control because the R7224 and R7244 genomes are genetically identical, except that the former lacks an epitopic tag (Table 1) (4). The results (Fig. 2) were as follows. The exon II probe recognized a R7224-specific band of approximately 3.1 kbp due to the presence of a second copy of exon II within the R7224 genome. This band was not present in HSV-1(F) DNA containing a single copy of the U_L15 gene. The exon II probe recognized a R7244-specific *Kpn*I fragment of approximately 2.5 kbp. Thus, relative to the *Kpn*I fragment representing the second copy of U_L15 exon II of R7224, the corresponding band in R7244 was truncated by approximately 600 bp as a consequence of *Kpn*I cleavage within DNA encoding the epitopic tag (Fig. 2, compare bands in lanes 2 and 3; schematic representations of the DNA sequences within the R7224 and R7244-specific bands are shown in Fig. 1, lines 7 and 8, respectively). We conclude that the genome of the R7244 virus was as designed and contained a cDNA of the U_L15 gene tagged at the C terminus.

Characterization of U_L15-encoded proteins. Cells were harvested 14 h after infection with 5.0 PFU of HSV-1(F), R7224, R7227 R7235, or R7210 per cell, solubilized, subjected to electrophoresis on a denaturing polyacrylamide gel, and transferred electrically to a nylon membrane. The genotypes of R7224, R7227, R7235, and R7210 have been documented elsewhere and are described in Table 1 (3, 4). Specifically, R7224 carries two copies of U_L15 exon II, one linked to exon I as a cDNA and one in the native position; R7227 is identical to R7224 but carries an α27-*tk* gene in the native copy of exon II; R7235 is identical to R7224 but carries a single copy of U_L15 exon II linked to U_L15 exon I; R7210 carries a single

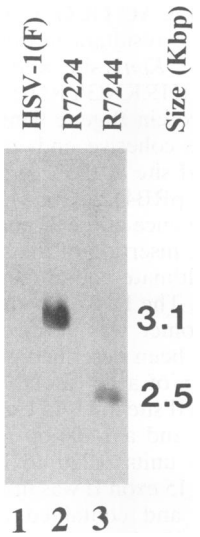

FIG. 2. Fluorographic images of *Kpn*I digests of viral DNA probed with U_L15 exon II sequences. Viral DNA was purified from cells infected with the indicated viruses and digested with *Kpn*I. The DNA fragments were electrophoretically separated, transferred to a nylon sheet, and probed with fluorescein-labeled exon II sequences. Bound probe was visualized as described in Materials and Methods. The approximate sizes of the DNA fragments hybridizing to the exon II probe are indicated on the right.

spliced copy of U_L15 but lacks the majority of the U_L16 open reading frame and therefore contains a truncated U_L15 intron.

Serum from rabbits immunized with the affinity-purified bacterial fusion protein were diluted 1:100 and reacted with the immobilized proteins. Bound antibody was visualized by the addition of alkaline phosphatase-conjugated goat anti-rabbit antibody followed by the substrate (Bio-Rad). The results (Fig. 3) were as follows. (i) The anti-U_L15 antiserum reacted with proteins with apparent M_rs of 75,000, 71,000, 66,000, and 35,000 (Fig. 3, bands 2, 3, 4, and 6, respectively) in electrophoretically separated lysates of cells infected with both spliced [HSV-1(F) and R7210] and unspliced (R7224, R7227, and R7235) U_L15 genes lacking epitopic tags. The 71,000- and 66,000-M_r bands were not detectable in all samples, suggesting that they may be degradation products of the 75,000-M_r protein. The U_L15 polypeptide bands with apparent M_rs of 75,000 and 35,000 were present in cells infected with (a) viruses carrying wild-type U_L15 genes [HSV-1(F)], (b) viruses carrying the U_L15 cDNA previously shown to be sufficient for viral replication (R7224, R7227, and R7235), (c) viruses carrying none or one copy of U_L15 exon II which was not fused to exon I (R7235 and R7224, respectively), and (d) viruses containing truncated U_L15 introns [R7210 (ΔU_L16)]. (ii) The anti-U_L15 serum reacted with polypeptides with apparent M_rs of 78,000 and 38,000 in lysates of cells infected with the R7244 virus (Fig. 3, bands 1 and 5, respectively). Inasmuch as these proteins exhibited a decreased electrophoretic mobility relative to the corresponding proteins in lysates of cells infected with other recombinant and wild-type viruses by an amount consistent with the predicted M_r of the epitope inserted into the C terminus of the U_L15 gene, we conclude that (a) the apparently 75,000- and 35,000-M_r proteins are products of the U_L15 gene and (b) the proteins share at least the carboxyl terminus of the U_L15 protein. In no instance was the 35,000-M_r protein detected in the absence of the apparently 75,000-M_r protein.

Inasmuch as U_L15-encoded proteins accumulated to detect-

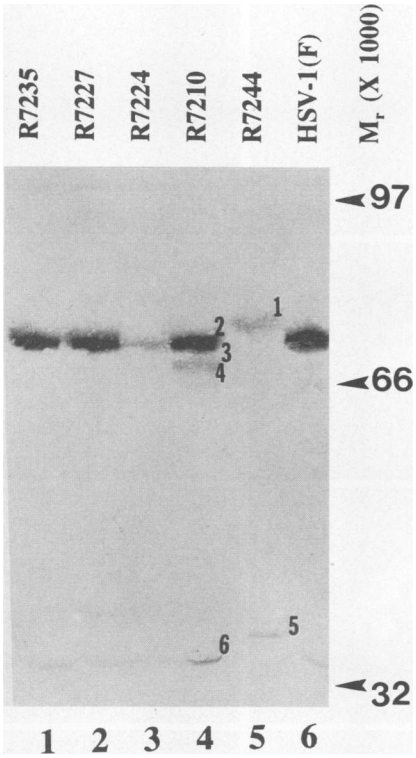

FIG. 3. Immunoblot probed with U_L15-specific antisera. HEp-2 cells were infected with 5.0 PFU of the indicated viruses. At 12 h after infection, cellular lysates were electrophoretically separated on a denaturing polyacrylamide gel and electrically transferred to a nitrocellulose sheet. The sheet was then probed with antiserum directed against a bacterial fusion protein containing U_L15 exon II linked to maltose-binding protein. The genotypes of the viruses used in this experiment are described in Table 1. The positions of the molecular weight markers and their M_rs are indicated on the right.

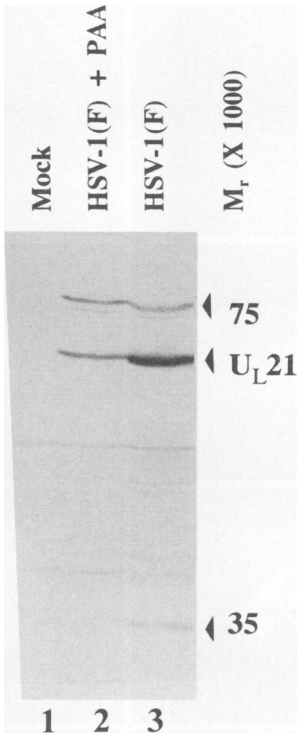

FIG. 4. Immunoblot probed with U_L15-specific antiserum. HEp-2 cells were either mock infected or infected with 5.0 PFU of HSV-1(F) per cell in the presence or absence of phosphonoacetic acid (PAA). At 12 h after infection, cells were lysed, electrophoretically separated on a denaturing polyacrylamide gel, and transferred to a nylon membrane. The proteins were then probed with the U_L15-specific antiserum. The positions of the U_L21 encoded protein and the M_rs of U_L15-encoded proteins are shown on right. The faint bands migrating between those of U_L21 and the U_L15 35,000-M_r proteins were either nonreproducibly present or they were also formed in lanes containing electrophoretically separated lysates of mock-infected cells.

able levels relatively early in infection, it was of interest to determine whether the expression of the U_L15 gene was dependent on viral DNA synthesis. Cells were infected with 5.0 PFU of HSV-1(F) per cell in the absence or presence of phosphonoacetic acid at a concentration (300 μg/ml) which precludes viral DNA synthesis. Immunoblots of infected cell lysates were reacted first with the anti-U_L15 polyclonal serum and subsequently with the antiserum directed against the U_L21 protein of HSV-1. The latter has been shown to be a γ_1 protein (6). The results (Fig. 4) indicate that the amounts of U_L21 and U_L15 35,000-M_r proteins decreased more drastically in the presence of phosphonoacetic acid than those of the U_L15 75,000-M_r protein. We conclude that the accumulation of the 35,000-M_r protein is dependent on viral DNA synthesis to a greater extent than that of 75,000-M_r protein.

Proteins encoded by the U_L15 gene localize in the nucleus late in infection. If the function of U_L15 proteins was to cleave and package viral DNA, it would be expected that they would be localized primarily within nuclei of infected cells. To test this hypothesis, HEp-2 cells infected with R7202, as described in Materials and Methods, were fixed in methanol, reacted with various dilutions of the anti-U_L15 serum, rinsed thoroughly, reacted with goat anti-rabbit immunoglobulin conjugated to Texas red, rinsed again, and examined with a Zeiss confocal microscope. The results were as follows. (i) At 6 h after infection, the U_L15-specific signal localized within a perinuclear region and was especially prominent juxtaposed to or within the nuclear membrane (Fig. 5A). (ii) At 12 h after infection, the U_L15 antigen was localized both within a perinuclear region of the cytoplasm and as prominent well-demarcated foci within the nuclei of infected cells (Fig. 5B). Thus we conclude that the localization of U_L15 proteins changes mark-

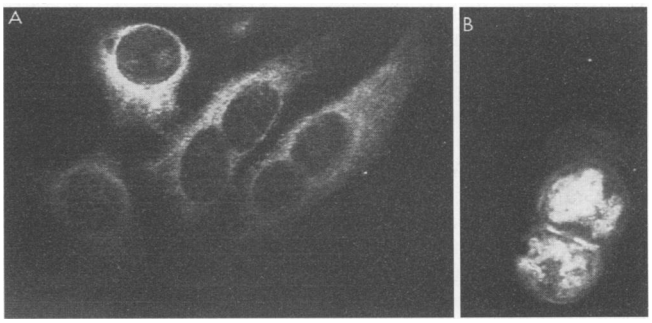

FIG. 5. Fluorescence photographs of infected HEp-2 cells reacted with anti-U_L15 rabbit polyclonal antibody and then goat anti-rabbit immunoglobulin conjugated with Texas red. Cells infected with R7202 virus at an effective concentration of 1.0 PFU per cell fixed and stained 6 (A) or 12 (B) h after infection.

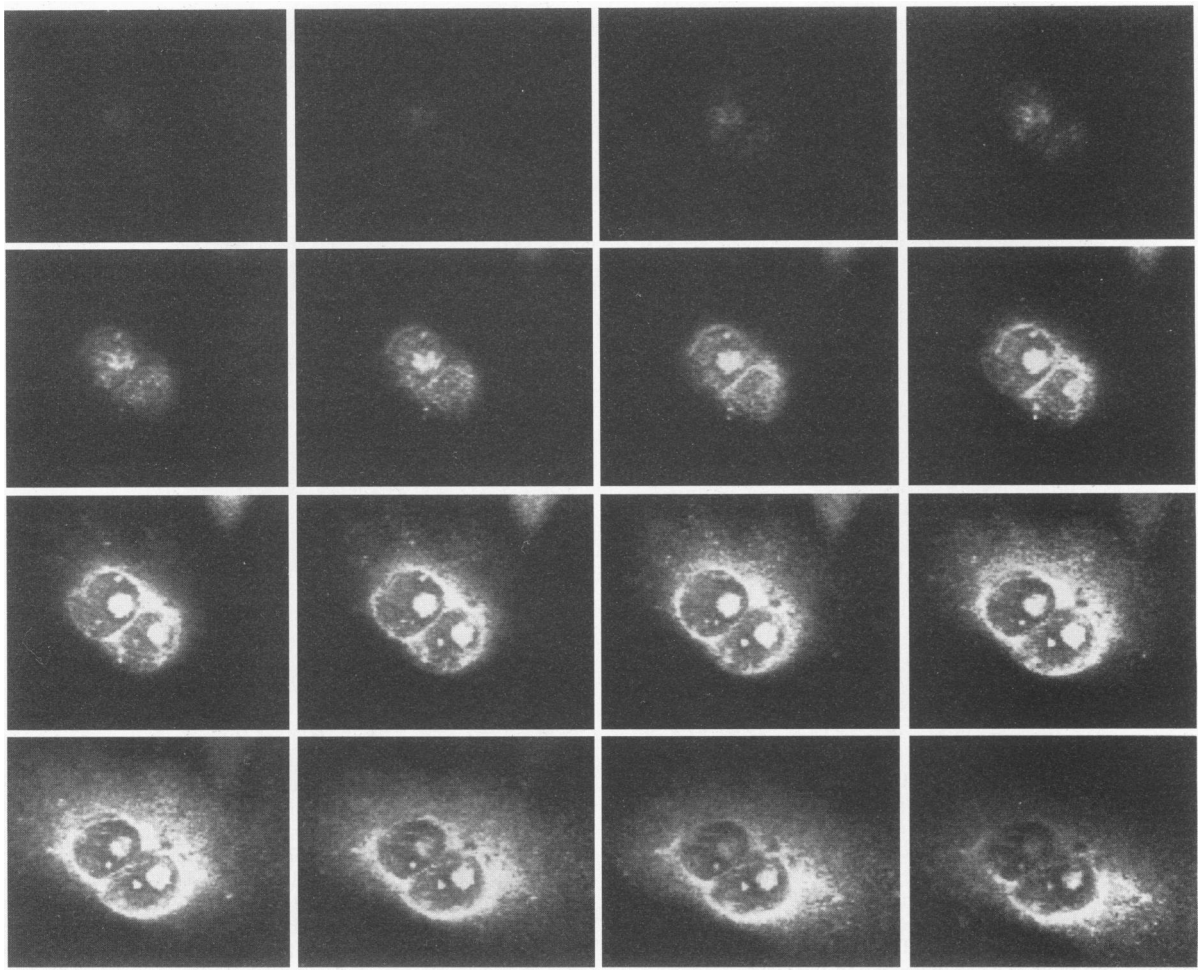

FIG. 6. Fluorescence photograph of HEp-2 cells infected with R7202 virus and stained with U_L15-specific antiserum. Digital 500-nm sections starting from the basolateral surface (top left image) and moving towards the apical surface (bottom right image) are shown.

edly during the course of infection from a predominantly perinuclear cytoplasmic distribution to well-demarcated regions within the infected cell nucleus.

In order to more fully characterize the intranuclear regions associated with U_L15-specific immunofluorescence, 500-nm digital sections starting from the basolateral surface (Fig. 6, upper left image) and moving towards the apical surface (lower right image) were recorded. The images of two cells shown in Fig. 6 indicate that the intranuclear U_L15 protein localized in a structure elongated along the *z*-axis of the infected cell whereas the shape in cross section along the *x-y* axes varied considerably from mostly circular, as in Fig. 6, to thin and ribbon-like (data not shown).

The U_L15 protein is required for both cleavage and packaging of viral DNA. Earlier studies have shown that both viral DNA and empty capsids accumulated but packaging of DNA did not ensue in cells infected and maintained at the nonpermissive temperature with HSV-1(mP)*ts*66.4, a spontaneous mutant mapped to exon II of the U_L15 gene (19). The following experiment was done to determine whether viral DNA accumulating at the nonpermissive temperature was maintained in an endless (concatemeric) configuration or was cleaved to mature, unit-length molecules. Replicate Vero cell cultures were infected with 10 PFU of parent [HSV-1(mP)] or mutant [HSV-1(mP)*ts*66.4] virus per cell and maintained at 34 or 39.5°C. Cells were harvested at 16 h after infection, and purified viral DNA was digested with *Bam*HI, subjected to electrophoresis in a 0.7% agarose gel, transferred to a zeta-probe blotting membrane, and probed with ^{32}P-labeled terminal fragment *Bam*HI S. The results shown in Fig. 7 indicate that (i) at the permissive temperature, cells infected with both parent and mutant viruses accumulated fragments containing the junction fragment *Bam*HI SP and a ladder of more rapidly migrating *Bam*HI S fragments. Earlier studies have shown that the ladder of *Bam*HI S fragments reflects variability in the number of terminal *a* sequences at the L-component terminus of the HSV-1 DNA (24). (ii) The parent virus HSV-1(mP) multiplies at 39.5°C, and consistent with this observation, digests of viral DNA accumulating in infected cells maintained at this temperature show both the *Bam*HI SP and S fragments. (iii) Digests of the mutant DNA extracted from cells infected and maintained at the nonpermissive temperature exhibit the *Bam*HI SP fragment but not the terminal *Bam*HI S fragments. (iv) A curious observation not previously recorded is that the amounts of parent viral DNA represented by the *Bam*HI SP fragment were higher in cells maintained at 39.5°C than at 34°C.

We conclude that in the absence of functional U_L15 proteins viral DNA accumulates in an endless, concatemeric configu-

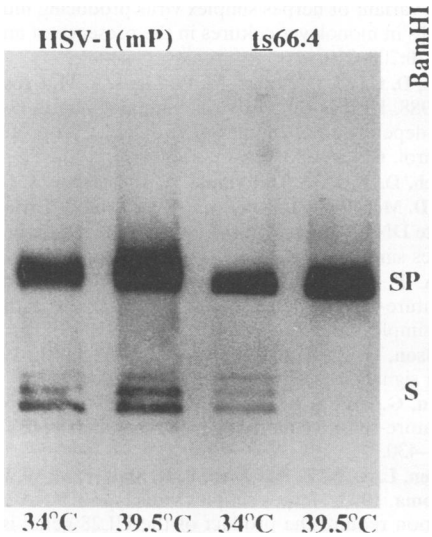

FIG. 7. Autoradiographic images of *Bam*HI-digested viral DNA probed with radiolabeled *Bam*HI S fragment representing the junction of the L and S components. Viral DNA was purified from total lysates of cells infected with HSV-1(mP) or HSV-1(mP)*ts*66.4 and held at the indicated temperatures. The DNA was cleaved with *Bam*HI, and DNA fragments were separated on a 0.7% agarose gel, transferred to a Zeta probe sheet, and probed with the ^{32}P-labeled *Bam*HI S fragment. Bound probe was visualized by autoradiography.

ration and is not cleaved into mature, genomic full-length molecules.

DISCUSSION

The salient features of our results were as follows.

(i) The γ_1 U_L15 mRNA isolated and translated in vitro by Costa et al. (10) yielded two polypeptide bands with apparent M_rs ranging from 77,000 to 80,000. Our studies indicate that the anti-U_L15 serum produced by us reacted with a polypeptide with an M_r of 75,000, in good agreement with the earlier results. Inasmuch as the electrophoretic mobility of this protein was reduced in cells infected with recombinant viruses carrying an epitopically tagged U_L15 gene, our results unambiguously demonstrate that this protein is a product of the U_L15 gene.

(ii) The anti-U_L15 serum also reacted with a polypeptide with an apparent M_r of 35,000. Studies of proteins encoded by the epitopically tagged viruses unambiguously indicate that the 35,000-M_r protein shares at least the carboxyl terminus of the amino sequence encoded by exon II of the U_L15 gene with the 75,000-M_r protein. The 35,000-M_r protein is largely dependent on viral DNA replication for accumulation of normal amounts of protein whereas the 75,000-apparent-M_r protein is less so dependent.

The origin of the 35,000-M_r protein is currently unclear. It may be a consequence of translation of a novel mRNA that is regulated differently from the mRNA encoding the 75,000-apparent-M_r protein, or it may be derived by proteolytic cleavage of the 75,000-M_r protein; given the latter scenario, the protease responsible for the cleavage would be expected to be dependent on viral DNA replication for its synthesis or for optimal activity.

(iii) The localization of the U_L15 protein changed during the course of infection from the perinuclear space at 6 h after infection to distinct intranuclear structures at 12 h after infection. The U_L15 open reading frame predicts the sequence PPKKRAKV (first exon, amino acids 183 to 190), which resembles documented nuclear localization signals (20). If this sequence acts as a nuclear localization signal, then the change in cellular distribution from cytoplasm to nucleus suggests the possibility that intranuclear transport of U_L15 proteins is specifically delayed until after the onset of viral DNA synthesis. Thus U_L15 localization may be the consequence of a conformational change exposing this putative nuclear localization signal; alternatively, the U_L15 protein may be recruited into the nucleus as a consequence of phosphorylation, dephosphorylation, or interaction with other proteins destined to reside in the nucleus (15). Although preliminary experiments suggest that the 75,000-M_r protein accumulating in nuclei is electrophoretically distinct from that accumulating in the cytoplasm (work in progress), additional studies will be required to determine whether the proteins retained in these compartments differ with respect to posttranslational modifications. Parenthetically, distribution of the HSV-1 U_L3 gene product also changes from cytoplasmic to nuclear during the course of infection, but this protein presumably has a function other than DNA packaging since the gene is dispensable for growth in cultured cells (3, 28).

(iv) The sites of U_L15-specific intranuclear fluorescence were elongated along the z axis of infected cells. These sites superficially resembled the column-like intranuclear distribution of DNA replication compartments described recently and may arise as a consequence of occlusion from other regions of the nucleus by chromatin or other intranuclear structures (13). Additional studies will be required to establish the relationship between the accumulation of U_L15 proteins and viral DNA synthesis.

(v) Extension of the studies of the HSV-1(mP)*ts*66.4 mutant described earlier indicate that in cells infected with this mutant and maintained at the nonpermissive temperature, viral DNA is not processed to mature, genomic-length molecules. We cannot differentiate between the possibility that the U_L15 protein is required solely for cleavage of viral DNA and the hypothesis that the protein is required for both cleavage and packaging. To date, however, all HSV-1 proteins required for viral DNA packaging were found to be required also for cleavage of viral DNA (1, 21, 22, 27). If this trend continues, the processes of genomic DNA cleavage and packaging may well be operationally linked.

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