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The U_L16 Gene Product of Herpes Simplex Virus 1 Is a Virion Protein That Colocalizes with Intranuclear Capsid Proteins

DOROTHY NALWANGA,* STEPHANIE REMPEL,* BERNARD ROIZMAN,† and JOEL D. BAINES*,[1]

*Department of Microbiology and Immunology, New York State College of Veterinary Medicine, Veterinary Medical Education Center, Cornell University, Ithaca, New York 14853; and †The Marjorie Kovler Oncology Laboratories, University of Chicago, Chicago, Illinois 60637

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The U_L16 gene of herpes simplex virus maps within the intron of the U_L15 gene. This report shows the following: (i) A polyclonal antiserum directed against a bacterial fusion protein containing glutathione *S*-transferase fused to the C-terminus of the U_L16 gene reacted with an apparent M_r 40,000 protein in HSV-1 infected cell lysates. (ii) The protein encoded by U_L16 was dependent on viral DNA synthesis for accumulation to detectable levels. (iii) In immunofluorescence studies, the polyclonal U_L16/GST-specific antiserum was shown to stain the nucleus of infected cells at 18 hr after infection in areas containing high concentrations of HSV capsid proteins. These nuclear compartments have been described previously as viral assemblons (Ward *et al., J. Virol.* 70, 4623–4631, 1996) and are distinct from compartments containing replicating DNA. Localization within assemblons argues for a role of U_L16 encoded protein in capsid assembly or maturation. (iv) At 22 hr after infection, U_L16-specific immunofluorescence was present in both the nucleus and the cytoplasm. (v) Consistent with the change in localization at late times after infection, the U_L16 protein was found to be a component of purified virions.
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INTRODUCTION

The focus of this report is on the product of the U_L16 gene which maps between 0.182 and 0.222 map units (Costa *et al.*, 1985). The U_L16 gene is unusual among HSV-1 genes because it is contained within the intron of another gene, U_L15, and is transcribed antisense to the U_L15 gene. The U_L16 open reading frame (ORF) encodes 373 amino acids with a predicted M_r of 40,400 (McGeoch *et al.*, 1988). *In vitro* translation of U_L16 transcripts obtained from infected cells at 6 hr after infection yielded a 40,000 apparent M_r protein (Costa *et al.*, 1985). The U_L16 gene was shown in earlier studies to be dispensable for viral replication in cells in culture (Baines and Roizman, 1991). However, the U_L16 deletion mutant (R7210) multiplied to titers 3- to 10-fold lower than those of the wild-type parent in Vero or BHK-TK− cell lines, respectively. The goal of these studies was to characterize the protein encoded by the U_L16 gene.

Relevant to this report are the observations that most structural proteins are late or γ proteins dependent on the onset of viral DNA synthesis for their expression. The virion proteins assemble into capsids which are enveloped at the inner nuclear membrane. The three types of capsids, A, B, and C, differentiated by their protein content and electron microscopic appearance, accumulate in the nuclei of cells infected with HSV (Gibson and Roizman, 1972). Type A capsids consist of an electron-dense icosahedral shell consisting mostly of the major capsid protein ICP5. Type B capsids contain the outer shell surrounding an electron-dense inner core or scaffold that is largely composed of the protein ICP35, or VP22a (Newcomb and Brown, 1991). Type C capsids contain the outer icosahedral shell, lack the internal scaffold protein ICP35, and contain DNA (Gibson and Roizman, 1972). Type C nucleocapsids presumably leave the nucleus during the process of envelopment at the inner lamella of the nuclear membrane. Indirect immunofluorescent staining of infected cells with monoclonal antibodies directed against ICP5 and ICP35 demonstrates that these proteins accumulate to high levels in discrete regions of the nucleus late in infection. These compartments, termed assemblons, may arise as a consequence of the aggregation of ICP5 and ICP35 during capsid assembly or maturation (Ward *et al.*, 1996b).

MATERIALS AND METHODS

Viruses and cells

Vero cells were maintained in Dulbecco's medium supplemented with 5% newborn calf serum, penicillin, and streptomycin. Rabbit skin and HEp-2 cells were maintained in the same medium containing 10% newborn calf serum. Human 143TK cells were maintained in the same medium supplemented with 10% fetal bovine serum. The wild-type herpes simplex virus used by our laboratories [HSV-1(F)], the recombinant virus HSV-1(F)Δ305 from which other recombinant viruses were derived, the R7206 virus containing the *α-27tk* gene between the U_L16 and

[1] To whom correspondence and reprint requests should be addressed. Fax: (607) 253-3384. E-mail jdb11@cornell.edu.

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the first exon of the U_L15 gene, the recombinant virus R7210 lacking the majority of the U_L16 gene, and the recombinant virus R7202 containing a deletion of the gene encoding glycoprotein E have all been described previously (Post *et al.,* 1981; Ejercito *et al.,* 1968; Baines and Roizman, 1993, 1991).

The R7229 virus was derived from R7210 by cotransfection of an *Eco*RI/*Kpn*I fragment of HSV-1(F) DNA (map units 0.190–0.208) with R7210 DNA. Large plaques picked among the progeny of the cotransfection yielded several viruses that contained restored U_L16 genes by restriction enzyme analysis and confirmed by hybridization with U_L16 sequences (data not shown). One of these viruses was further plaque purified and was designated as R7229.

Enzymes

Restriction enzymes were obtained from New England Biolabs. DNA sequencing was done with T7 DNA polymerase according to the directions of the manufacturer (U.S. Biochemical).

Purification and analyses of viral DNA

Conditions for purification, analysis, and hybridization of viral DNA with radiolabeled DNA probes were as previously described (Baines and Roizman, 1993). DNA to be transfected was purified on NaI gradients as described previously (Walboomers and Ter Schagget, 1976).

Production of U_L16-specific polyclonal antiserum and immunoblotting

A 1.2-kbp *Bgl*II/*Eco*RI fragment encoding U_L16 sequences was inserted into the *Bam*HI/*Eco*RI sites of pGEX 3× (Pharmacia). This plasmid, designated pRB4591, was predicted to encode the termination and final 165 codons of the U_L16 gene fused to the gene encoding glutathione *S*-transferase (GST); DNA encoding the junction of the two genes was sequenced to ensure that they were maintained in frame (data not shown). The pRB4591 plasmid was transformed into the BL21 strain of *Escherichia coli.* Production of the fusion protein was induced by the addition of 0.3 m*M* IPTG to a log phase culture and the 49,000 apparent M_r protein was purified from inclusion bodies by electroelution from polyacrylamide gels in 0.1% SDS, 20 m*M* Tris–HCl (pH 8.0). New Zealand White rabbits were immunized with five injections of approximately 300 μg purified protein as previously described (Baines and Roizman, 1993). Immunoblotting was as previously described (Baines and Roizman, 1993) except that the antiserum directed against the U_L16/GST fusion protein was diluted 1:400 in PBS supplemented with 1% BSA and was preadsorbed against an excess of uninfected HEp-2 cells or Hep-2 cells infected with a previously described recombinant virus, R7210, lacking the majority of the U_L16 gene. Donkey alkaline phosphatase conjugate was obtained from Jackson Immunoresearch.

Confocal immunofluorescence microscopy

HEp-2 cells were grown on Lab-Tek (Nunc) dishes and were infected with recombinant virus R7202 (Baines and Roizman, 1993). The R7202 virus was used in these studies to avoid high background levels obtained as a consequence of high-affinity binding of rabbit immunoglobulins by HSV-1(F) glycoproteins E and I (Johnson *et al.,* 1988). Cells were fixed in cold methanol and reacted with preadsorbed U_L16 antiserum or mouse monoclonal antibodies directed against the ICP8, ICP5, or ICP35 proteins of HSV-1 (Goodwin Biotechnology, Inc., Plantation, FL). After extensive washing, the cells were reacted with donkey conjugated fluorescein isothiocyante (FITC) conjugated anti-mouse immunoglobulin or donkey lissamine–rhodamine conjugated anti-rabbit immunoglobulins, or both (Jackson Immunoresearch). After washing, the cells were air dried and rehydrated in 90% glycerol/10% PBS supplemented with 1 mg/ml *p*-phenyldiamine to prevent fading of fluorescein (Johnson and Araujo, 1981). Laser scanning confocal microscopy was carried out with a Zeiss Axiovert 10 Bio-Rad MRC-600 confocal microscope equipped with a krypton–argon ion laser (Ion laser Technology, Salt Lake City, UT). Fluorescent images were obtained with 488 nm and 568 nm bandpass filters for excitation of FITC and lissamine–rhodamine, respectively. Appropriate controls showed no contribution of fluorescein-specific fluorescence to the rhodamine channel. Software merging of images was carried out using the COMOS software provided. Images were recorded using a Screenstar film recorder (Presentation Technologies, Sunnyvale, CA).

RESULTS

Identification of the U_L16 gene product in HSV infected cells

To identify U_L16 protein in HSV infected cells, a fusion protein containing the glutathione *S*-transferase (GST) gene fused to the C-terminus of the U_L16 protein was purified and used to immunize rabbits as described under Materials and Methods.

To characterize the specificity of the anti-U_L16 antiserum, we used the R7210 and R7229 recombinant viruses. R7210 is derived from HSV-1(F) and lacks the majority of the U_L16 ORF including the C-terminus predicted to have served as antigen for the generation of the U_L16 polyclonal antiserum. The R7229 virus, constructed for these studies and derived from R7210, contained a restored U_L16 gene (see Materials and Methods). HEp-2 cells were mock infected or were infected with 5.0 PFU per cell of HSV-1(F), R7210, or R7229. Twenty hours after infection, the cells were lysed, and proteins were electrophoretically separated on a denaturing polyacrylamide

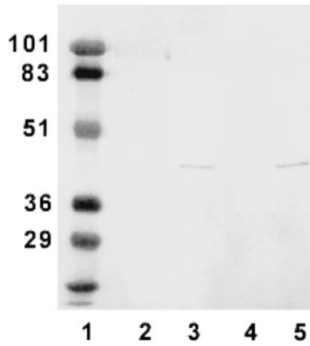

FIG. 1. Photograph of immunoblot probed with U_L16/GST-specific antiserum. HEp-2 cells were mock infected or were infected with 5.0 PFU of the indicated viruses. At 20 hr after infection, samples of cellular lysates were electrophoretically separated on a denaturing polyacrylamide gel and electrically transferred to a nitrocellulose sheet. The sheet was then probed with antiserum directed against a bacterial fusion protein containing most of UL16 fused to glutathione *S*-transferase. Bound antibody was visualized with alkaline phosphatase conjugated donkey anti-rabbit immunoglobulin followed by the addition of substrate. Lane 1, molecular weight markers. The sizes (in 1000s) of the proteins are indicated to the left of the figure. Lane 2, mock-infected cellular lysate; lane 3, cell lysate infected with HSV-1 (F); lane 4, lysate of cells infected with R7210, a deletion mutant lacking the U_L16 gene; lane 5, lysate of cells infected with R7229 derived from R7210 and containing a restored U_L16 gene.

gel and were transferred to nitrocellulose. The nitrocellulose sheet was reacted with the polyclonal antibody directed against the U_L16/GST fusion protein and bound antibody was visualized by the addition of alkaline phosphatase conjugated goat anti-rabbit immunoglobulin followed by the addition of chromogenic substrate. The results, shown in Fig. 1, were as follows:

A protein band of apparent M_r 40,000 reacted with the polyclonal antibody directed against the U_L16/GST fusion protein in lanes containing HSV-1(F) infected cell lysates (Fig. 1, lane 3). This band was not present in lanes containing mock-infected cell extracts or in lanes containing lysates of HEp-2 cells infected with the R7210 virus lacking the majority of the U_L16 gene (Fig. 1, lane 4). The 40,000 apparent M_r protein was also present in lanes containing lysates of cells infected with the R7229 virus that was derived from R7210 and that contained a restored U_L16 gene (lane 5). We conclude that the 40,000 apparent M_r protein is produced only upon infection with viruses containing wild-type U_L16 genes and that it is recognized by antisera directed against the U_L16/GST fusion protein. We therefore deduce that the 40,000 apparent M_r protein is the product of the U_L16 gene.

U_L16 is regulated as a late gene product

One objective of these studies was to characterize the temporal regulation of U_L16 protein expression in infected cells. HEp-2 cells were infected with 5 PFU of HSV-1(F) per cell. At various times after infection cell lysates were prepared and were subjected to electrophoresis in a denaturing polyacrylamide gel, transferred to a nitrocellulose sheet, and reacted with the anti-GST/U_L16 serum. The results (Fig. 2) indicate that U_L16 protein accumulated to readily detectable levels by 12 hr and accumulated to higher levels between 12 and 24 hr postinfection. U_L16 protein was not detectable at 6 hr postinfection. These data therefore suggested that U_L16 was regulated as a late (γ) gene.

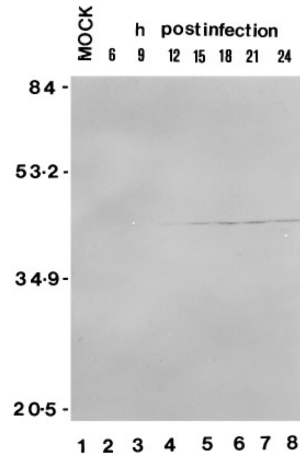

FIG. 2. Photograph of immunoblot probed with U_L16/GST-specific antiserum. HEp-2 cells were either mock infected or were infected with 5.0 PFU per cell of HSV-1(F). At the indicated times after infection cells were lysed and proteins were electrophoretically separated, transferred to nitrocellulose, and reacted with the U_L16/GST-specific antiserum as described in the legend to Fig. 1.

To test if U_L16 protein expression was dependent on viral DNA synthesis, HEp-2 cells were infected with 5 PFU per cell of HSV-1(F) or R7210 (U_L16 virus) in the presence or in the absence of phosphonoacetate (PAA; 300 μg/ml), a potent inhibitor of viral DNA synthesis. At 20 hr postinfection, infected cell proteins were electrophoretically separated, transferred to a nitrocellulose sheet, and probed with the U_L16/GST specific antiserum. The results (Fig. 3) were as follows:

In lanes containing lysates of cells infected with HSV-1(F) in the presence of PAA, the accumulation of the

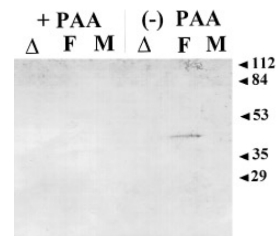

FIG. 3. Photograph of immunoblot probed with U_L16/GST-specific antiserum. HEp-2 cells were infected with 5.0 PFU of HSV-1(F) (lanes marked F), infected with R7210 lacking the majority of the U_L16 gene (lanes marked Δ), or mock infected (lanes marked M) in the presence (+) or absence (−) of phosphonoacetic acid (PAA, 300 μg per milliliter). Polypeptides were separated on a denaturing polyacrylamide gel, transferred to nitrocellulose, and probed with the U_L16/GST-specific antiserum. Sizes of molecular weight markers are indicated in thousands to the right of the figure.

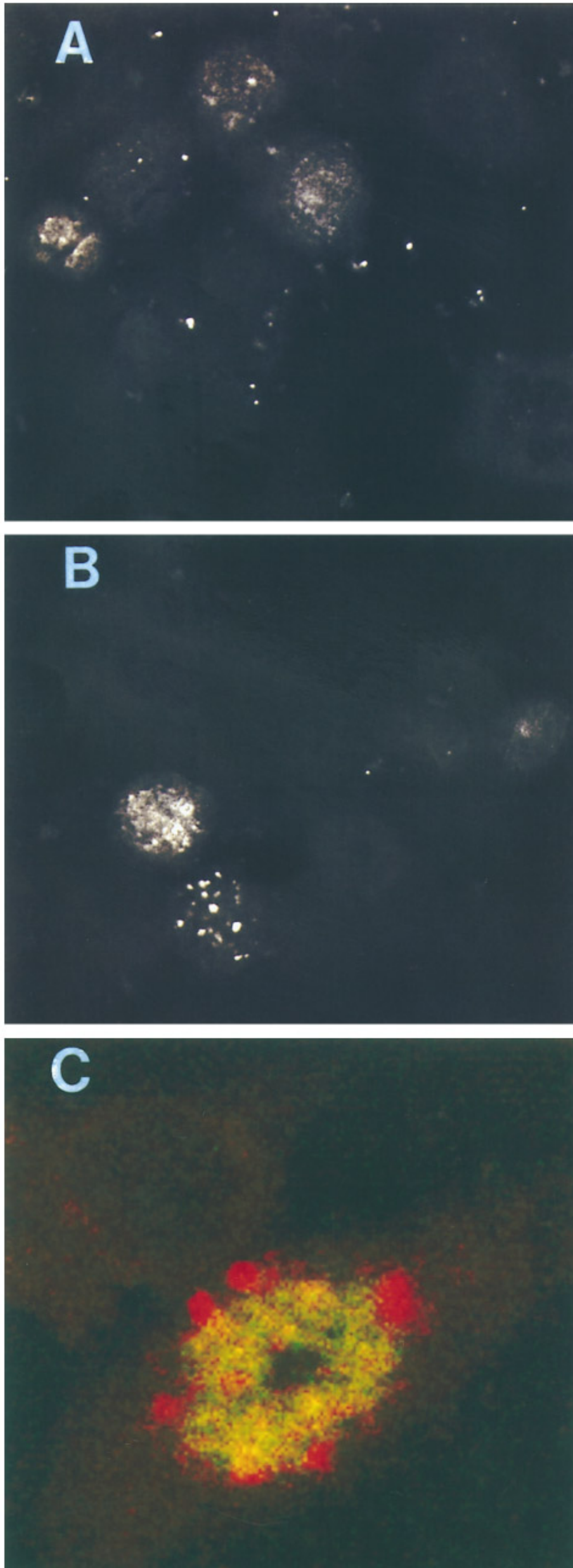

FIG. 4. Digital confocal microscopic images of infected HEp-2 cells fixed at 18 hr after infection and reacted with U_L16/GST- and ICP8-specific antibodies. (A and B) Cells were infected with the glycoprotein E negative virus R7202 at an effective multiplicity of infection of 1.0 PFU per cell. Cells were fixed in cold methanol, stained with U_L16/GST-specific antiserum, and reacted with donkey anti-rabbit immunoglobulin conjugated with lissamine–rhodamine. Cells were viewed using a Zeiss confocal microscope with laser generated light filtered appropriately for excitation of lissamine–rhodamine. Digital images were generated and recorded with Bio-Rad software. (C) Cells were infected and fixed as described in A and B. A mouse monoclonal antibody directed against ICP8 and rabbit U_L16/GST-specific antiserum were reacted with the fixed cells. Lissamine–rhodamine conjugated anti-rabbit and fluorescein conjugated anti-mouse antibodies were added and digital images were recorded from the same microscopic field in laser generated light filtered appropriately for the excitation of fluorescein or rhodamine. The image shown consists of two superimposed images; yellow areas arise as a result of superimposition of ICP8-specific immunofluorescence (green) and U_L16/GST-specific fluorescence (red).

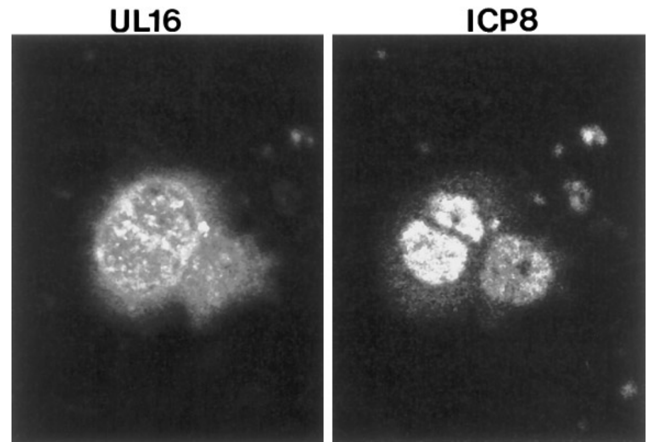

FIG. 5. Confocal microscopic images of U_L16/GST- and ICP8-specific immunofluorescence 22 hr after infection. Cells were infected, fixed, stained, and viewed as described in the legend to Fig. 6C except that the cells were fixed 22 hr after infection. The same field is shown as viewed in light filtered for the excitation of rhodamine (left, U_L16) or fluorescein (right, ICP8).

40,000 apparent M_r U_L16 protein was diminished to nondetectable levels. As expected, the 40,000 apparent M_r U_L16 encoded protein was not detectable in lanes containing lysates of cells infected with the U_L16 deletion mutant R7210. In contrast to these results, the U_L21 protein, a known γ1 gene product (Baines and Roizman, 1994), was detected at significantly reduced levels in the lysates of cells infected with U_L16 deletion virus R7210 and wild-type HSV-1(F) in the presence of PAA (data not shown). This indicated that the presence of PAA was sufficient to downregulate the expression of late gene products in this experiment.

We conclude that U_L16 is regulated as a γ2 gene because accumulation of the U_L16 gene product to detectable levels is dependent on viral DNA synthesis.

Localization of U_L16 protein in infected cells changes during the course of infection

The purpose of the next series of experiments was to determine the site(s) of accumulation of U_L16 protein during HSV infection. Preliminary experiments suggested that U_L16 protein accumulated in both nuclear and cytoplasmic extracts late in infection. To more precisely iden-

tify intracellular sites of accumulation of U_L16 protein, HEp-2 cells were infected with the gE negative virus R7202 to preclude high background levels produced upon binding of rabbit IgG to HSV-1 encoded F_c receptors containing gE (Johnson *et al.,* 1988). At 18 and 22 hr after infection, the infected cells were fixed and stained with the rabbit anti-U_L16/GST serum preadsorbed against uninfected HEp-2 cells. After extensive washing, the cells were reacted with donkey anti-rabbit immunoglobulin conjugated to lissamine–rhodamine. Digital confocal images were generated in light appropriate for the excitation of rhodamine.

At 18 hr after infection, U_L16-specific staining was present primarily in the nuclei of infected cells (Figs. 4A and 4B). The appearance of the intranuclear staining consisted of small granules dispersed throughout the nucleus. Examining images in various Z planes indicated that the U_L16-specific fluorescence was primarily within the nucleus but a portion was associated with the nuclear periphery (see below). Uninfected cells exhibited no bright fluorescent staining and are shown in all three panels of Fig. 4 as a consequence of the relatively low multiplicity of infection of 1.0 PFU per cell. The appearance of U_L16-specific staining varied somewhat from cell to cell at 18 hr after infection. Specifically, some cells displayed a diffuse fluorescent staining whereas in others the granules displayed a more punctate appearance. A striking example of the latter staining pattern is shown in Fig. 7.

To compare the sites of accumulation of U_L16 protein with known HSV intranuclear replication compartments, HEp-2 cells were infected with R7202 and were reacted with the anti-U_L16/GST antisera and a mouse monoclonal antibody directed against the ICP8 protein of HSV, a protein that localizes within these HSV induced intranuclear compartments (Quinlan *et al.,* 1984). The cells were then washed and were reacted with donkey anti-mouse immunoglobulin conjugated to fluorescein and donkey anti-rabbit immunoglobulin conjugated to lissamine–rhodamine. The cells were examined under appropriate filters for the excitation of the individual conjugates.

The appearance of the sites of accumulation of U_L16 protein and ICP8 differed significantly in two respects:

(i) Figure 4C shows two images of cells fixed at 18 hr after infection and reacted with the U_L16-specific and ICP8-specific antibodies. Areas of convergence of the two reactivities appear yellow in the figure. Substantial amounts of yellow fluorescence are present in the nuclei of infected cells indicating some colocalization of U_L16 and ICP8 proteins. However, discrete regions of U_L16 specific fluorescence (red) and ICP8 fluorescence (green) are also present, indicating that there are also intranuclear regions that contain only U_L16 or ICP8 protein.

(ii) Figure 5 shows U_L16 specific (left) or ICP8-specific (right) of identical images of cells fixed at 22 hr after infection. Unlike ICP8-specific staining, U_L16-specific staining localized within small granules in the infected cell nucleus and largely within a cytoplasmic region surrounding the infected cell nucleus.

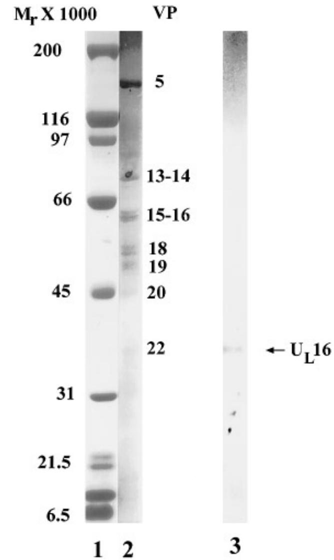

FIG. 6. Electrophoretically separated virion polypeptides stained with Coomassie blue or reacted with U_L16/GST-specific antiserum. Lanes 1 and 2, Virions were purified from infected Vero cells and virion polypeptides were denatured and electrophoretically separated on a polyacrylamide gel that was stained with Coomassie brilliant blue. Lane 1, molecular weight markers with sizes indicated to the left. Lane 2, virion polypeptides with the viral protein designation shown to the right. Lane 3, 10% of the viral protein separated in lane 2 was electrophoretically separated on the same polyacrylamide gel, transferred to nitrocellulose, and probed with the U_L16/GST-specific antiserum. An arrow indicates the position of the U_L16-specific band.

Taken together, we conclude that the pattern of U_L16-specific protein staining changes during the course of infection from a primarily nuclear location at 18 hr to one of nuclear and cytoplasmic fluorescence later in infection. We also conclude that intranuclear U_L16 protein is not present exclusively within viral replication compartments.

U_L16 encoded protein is a component of virions

Inasmuch as the U_L16 protein appeared in perinuclear regions of the cytoplasm late in infection and infectious virions often accumulate in a perinuclear region prior to their transport toward the extracellular space, we viewed it as likely that the U_L16 protein was incorporated into virions. To test this possibility, virions were purified from HSV-1(F) infected Vero cells as described previously (Spear and Roizman, 1972; Baines and Roizman, 1993). Virion polypeptides were separated on a denaturing polyacrylamide gel and lanes containing virion proteins were either stained with Coomassie blue or electrically transferred to nitrocellulose and reacted with the U_L16-specific antiserum. The results (Fig. 6) indicate that the U_L16-specific protein band of apparent M_r 40,000 is readily detectable in immunoblots of purified virion preparations

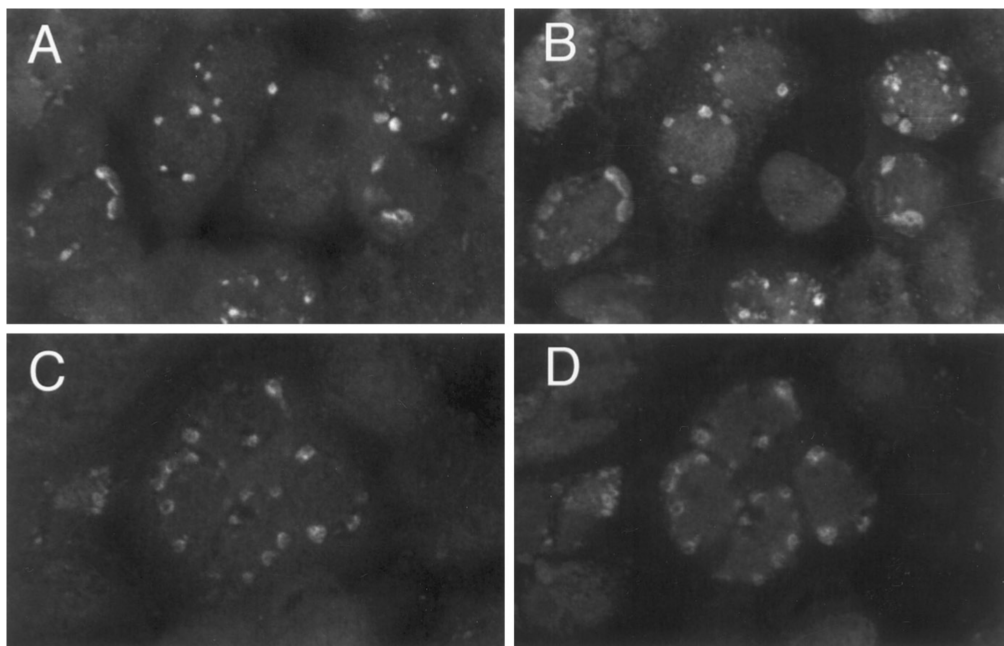

FIG. 7. Digital confocal images of U_L16 protein and HSV capsid proteins ICP5 and ICP35. HEp-2 cells were fixed at 18 hr after infection with R7202 and were reacted with U_L16/GST-specific rabbit polyclonal and (i) ICP5-specific monoclonal antibody or (ii) ICP35 specific monoclonal antibody. Cells were then reacted with appropriate conjugates and viewed as described in the legend to Fig. 6. (A and C) U_L16/GST-specific immunofluorescence. (B) Fluorescein generated (ICP5 specific) fluorescence of the same field as A. (D) The same field as C but viewed in light appropriate for excitation of fluorescein (ICP35 specific).

reacted with the U_L16-specific antiserum. This band was electrophoretically indistinguishable from the U_L16 protein band in lysates of infected cells (data not shown). The U_L16-specific band comigrated with denatured VP22, an abundant virion protein, and is therefore not visible as a separate band in Coomassie stained virion preparations.

U_L16 colocalizes with HSV capsid proteins

The sites of intranuclear accumulation of U_L16 protein had an appearance similar to those of cells fixed late in infection and stained with antibodies directed against HSV capsid proteins (Ward *et al.,* 1996a). To test the possibility that U_L16 protein colocalized with HSV capsid proteins, cells were stained with rabbit antibodies directed against U_L16 and ICP5, or ICP35. The results, shown in Fig. 7, indicated that the U_L16-specific immunofluorescence (Figs. 7A and 7C) and identical fields stained with ICP5- and ICP35-specific antibodies (Figs. 7B and 7D, respectively) colocalized in discrete, brightly staining regions of the infected cell nucleus. We conclude that U_L16 protein, like ICP5 and ICP35, localizes within assemblons in the infected cell nucleus.

DISCUSSION

We have shown that the U_L16 protein accumulates in HSV infected cells as a 40,000 apparent M_r protein late in the course of HSV infection and that its accumulation is largely dependent on DNA replication. As expected, the U_L16 encoded protein was not produced in cells infected with a virus lacking the U_L16 gene. In agreement with these results, Costa *et al.* previously mapped a β/γ ($\gamma1$) mRNA encoding U_L16 protein and showed that it produced a 40,000 apparent M_r protein in *in vitro* translation reactions (Costa *et al.,* 1985).

The arrangement of the U_L15 and U_L16 genes of HSV-1 indicates that the U_L15 intron is transcribed antisense to the U_L16 gene. Working under the hypothesis that U_L15 intron expression might downregulate U_L16 protein expression by an antisense mechanism, we examined expression of U_L16 protein in cells infected with viruses containing and lacking U_L15 introns (Baines and Roizman, 1992). No discernible differences in the amounts of U_L16 protein accumulation could be detected (data not shown). These data and the observation that U_L15 gene splicing is dispensable for virus replication (Baines and Roizman, 1992) make the significance of the U_L15 splicing event unclear at this time.

The U_L16 encoded protein first appears in infected cell nuclei in sites that are not coincident with previously documented intranuclear replication compartments (Quinlan *et al.,* 1984). Much of the U_L16-specific staining was located within discrete regions of the infected cell nucleus that also contained HSV capsid proteins (Ward *et al.,* 1996a). It has been suggested that such sites, termed assemblons, may be areas in which capsid assembly or DNA cleavage/packaging may occur in the infected cell nucleus (Ward *et al.,* 1996b). The observation that U_L16 protein localizes within assemblons coinci-

dent with high concentrations of HSV capsid proteins argues for a role for U_L16 in capsid or virion assembly or maturation. Although attempts to detect U_L16 encoded protein in purified B capsids were not successful (data not shown), we cannot rule out the possibility that the U_L16 protein is a rare component of B capsids. Preliminary results indicate that the electron microscopic appearance of Vero cells infected with the U_L16 deletion mutant do not differ significantly when compared to cells infected with wild-type virus (data not shown), raising the possibility that the function of U_L16 may be cell specific.

Between 18 and 22 hr after infection, the localization of U_L16 protein largely changes to perinuclear sites within the cytoplasm of infected cells. This may be due at least in part to incorporation of U_L16 protein into virions which accumulate in the cytoplasm after nucleocapsid envelopment. Similar to these results, the human cytomegalovirus homolog of the U_L16 protein, U_L94, has been shown to be expressed late in infection and is associated with purified virions (Wing *et al.*, 1996).

The apparent movement of U_L16 protein from intranuclear assemblons into virions argues that (i) assemblons contain some proteins such as ICP5 and U_L16 encoded protein destined to become incorporated into type C capsids and virions and (ii) U_L16 protein likely becomes incorporated into virions as components of type C intranuclear capsids that are subsequently enveloped at the inner lamella of the nuclear membrane.

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