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DNA Cleavage and Packaging Proteins Encoded by Genes U_L28, U_L15, and U_L33 of Herpes Simplex Virus Type 1 Form a Complex in Infected Cells

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Previous studies have indicated that the U_L6, U_L15, U_L17, U_L28, U_L32, and U_L33 genes are required for the cleavage and packaging of herpes simplex viral DNA. To identify proteins that interact with the U_L28-encoded DNA binding protein of herpes simplex virus type 1 (HSV-1), a previously undescribed rabbit polyclonal antibody directed against the U_L28 protein fused to glutathione *S*-transferase was used to immunopurify U_L28 and the proteins with which it associated. It was found that the antibody specifically coimmunoprecipitated proteins encoded by the genes U_L28, U_L15, and U_L33 from lysates of both HEp-2 cells infected with HSV-1(F) and insect cells infected with recombinant baculoviruses expressing these three proteins. In reciprocal reactions, antibodies directed against the U_L15- or U_L33-encoded proteins also coimmunoprecipitated the U_L28 protein. The coimmunoprecipitation of the three proteins from HSV-infected cells confirms earlier reports of an association between the U_L28 and U_L15 proteins and represents the first evidence of the involvement of the U_L33 protein in this complex.

The cleavage and packaging of newly synthesized herpesvirus DNA is a highly conserved process occurring late in viral replication. During herpesvirus infection, double-stranded viral DNA genomes accumulate in cell nuclei as head-to-tail concatemers which are subsequently cleaved into single genome lengths and packaged into preformed viral capsids (reviewed in reference 19). Six genes of herpes simplex virus type 1 (HSV-1) are known to be essential for this process, namely, U_L6, U_L15, U_L17, U_L28, U_L32, and U_L33. In cells infected with viruses individually lacking these genes, capsids appear normal and are readily detected, but viral DNA is neither cleaved nor packaged (2, 3, 5, 9, 12, 25, 26, 30, 31, 35, 37, 40–42, 44).

The cleavage and packaging process of herpesviruses is believed to be similar to that employed by double-stranded DNA bacteriophages; consequently, it is useful to consider the mechanisms used by these bacteriophages as a model for the cleavage and packaging events employed by herpesviruses. Such models propose a central role for the viral "terminase," a complex of at least two proteins that (i) binds the viral DNA and links it with the empty viral capsid (HSV-1) or prohead (bacteriophages); (ii) cleaves the viral DNA at precise internal sites, resulting in the separation of unit-length genomes from concatameric DNA; and (iii) hydrolyzes ATP, providing the energy required to drive the DNA into the capsid (reviewed in reference 10). With this paradigm in mind, efforts have been expended to identify herpesvirus gene products that perform functions expected of the viral terminase, especially DNA binding, ATP hydrolysis, and at least transient association with capsids.

There is a growing body of both direct and indirect evidence suggesting that the U_L15 and U_L28 gene products comprise two subunits of the HSV-1 terminase. The U_L28 gene product has been shown to bind specifically to the HSV-1 DNA sequence *pac1*, which is found in the a sequence of the genome and is known to be essential for the generation of correct genomic termini (4, 38). The U_L15 protein has been hypothesized to hydrolyze ATP, based on limited homology with a putative nucleotide binding motif comprised of Walker boxes A and B within gp17, the larger subunit of the T4 bacteriophage terminase (15). Although the ATPase activity of the U_L15 protein has yet to be directly demonstrated, a mutation within the Walker box precludes viral DNA cleavage and packaging (46). The U_L28 and U_L15 proteins have also been shown to interact in transient-expression assays (1, 22, 23, 45) and in coimmunoprecipitation experiments using nuclear extracts of infected cells (23).

This paper describes the isolation of a protein complex consisting of the U_L28, U_L15, and U_L33 proteins by immunoprecipitation from lysates of infected cells. This suggests for the first time that the U_L33 gene product may function as a third subunit of the putative viral terminase.

MATERIALS AND METHODS

Cells and viruses. Vero and HEp-2 cells and transformed cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum, penicillin, and streptomycin. Sf21 (*Spodoptera frugiperda*) cells were maintained in Grace's insect cell culture medium (GibcoBRL) supplemented with 10% fetal bovine serum and gentamicin (10 μg/ml). The wild-type virus HSV-1(F) and the mutant HSV-7202 have been previously described (6, 16), and their titers were determined on Vero cell monolayers. The titer of the U_L28 deletion virus HSV-gCB was determined by propagation on the transformed cell line C1 (41). The mutant viruses lacking U_L15 and U_L33 have been described previously (5, 9).

Recombinant baculoviruses encoding the HSV-1 U_L28, U_L15, and U_L33 genes were generated by cotransfecting Sf21 cells with baculovirus (*Autographa californica* multicapsid nucleopolyhedrosis virus [Ac *M*NPV]) DNA (Invitrogen) and plasmid DNA encoding the entire open reading frame of U_L15, U_L28, or U_L33

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TABLE 1. Provenance of antibodies used in this study

Antibody directed against protein encoded by the gene U_L:	Reference or source
6	39
14	13
15	34
16	28
17	35
18	11
19	11
21	7
25	Unpublished
26.5	11
28	This paper
29 (ICP8)	36
31	33
32	Unpublished
33	32
49	24
Actin	ICN Biomedicals Inc., clone 4

under the control of the baculovirus polyhedron promoter in the pBlueBacIII vector (Invitrogen), followed by plaque purification of recombinant viruses.

Antiserum production. Plasmid pJB179, predicted to encode the full-length U_L28 protein fused to glutathione *S*-transferase (GST), was generated by ligating a 2.5-kb DNA fragment containing bases 55,682 to 58,158 (27) of HSV-1(F) to the *Eco*RI site of pGEX-4T-1 (Pharmacia). The plasmid was sequenced to confirm that the U_L28-GST junction maintained the open reading frames of the U_L28 and GST proteins (data not shown). *Escherichia coli* BL-21 cells transformed with pJB179 were cultured at 37°C for 1 h followed by induction of the fusion protein with 0.1 mM isopropyl-β-D-thiogalactopyranoside at 25°C for 3 h. The induced U_L28-GST fusion protein was isolated from the bacteria as described previously (18) with the following modifications: Triton X-100 (2% [vol/vol]) was added to the clarified bacterial lysate, and the lysate was incubated with glutathione-Sepharose beads overnight at 4°C. Bound protein was eluted from the beads by boiling in buffer containing 2% sodium dodecyl sulfate (SDS) and 5% β-mercaptoethanol, and the U_L28-GST fusion protein was separated from contaminating bacterial proteins by SDS-polyacrylamide gel electrophoresis. The separated proteins were visualized by lightly staining with Coomassie blue, and the band containing U_L28-GST was removed and minced. To emulsify the acrylamide (containing the fusion protein) for injection, sterile phosphate-buffered saline (PBS) was added to the minced gel and it was passed through successively smaller-gauge needles.

Determination of antibody specificity. Flasks (25 cm^2) of Vero cells were infected at a multiplicity of infection (MOI) of 5.0 with either HSV-1(F) or the mutant gCB virus lacking U_L28. Twenty hours postinfection the cells were harvested, pelleted, and resuspended in a buffer consisting of PBS with 0.5% Triton and 0.5% sodium deoxycholate. They were then sonicated for 5 s at low power and clarified at high speed in a microcentrifuge. Fifty microliters of the supernatant was then added to 20 μl of loading buffer, and the sample was boiled for 3 min and electrophoretically separated on an SDS-polyacrylamide gel. The proteins from the gel were transferred to nitrocellulose membranes and probed with either polyclonal rabbit antiserum (diluted in PBS plus 1% bovine serum albumin and 1% Tween 20) directed against ICP8 (36) or the antiserum directed against U_L28-GST.

Production of radiolabeled cell lysates. HEp-2 cell monolayers were infected at an MOI of 5.0 PFU per cell with either HSV-1(F) or the R7202 mutant virus, which is derived from HSV-1(F) but lacks the capacity to produce glycoprotein E (6). After 4 h of incubation, the medium was replaced with labeling medium containing DMEM with 10% of the normal amounts of methionine and cysteine and 50 μCi of [^{35}S]-labeled cysteine and methionine (Translabel; ICN) per ml. Twenty hours after infection, cells were washed four times with ice-cold PBS, scraped off the flask, collected, pelleted and, if necessary, stored at −80°C until use.

Immunoprecipitations. Immunoprecipitations were performed using radiolabeled lysates of infected HEp-2 cells immediately after thawing on ice (see above). Alternatively, HEp-2 cells were infected with HSV-1(F) or the gE-null mutant R7202 at an MOI of 5.0 PFU per cell and incubated at 37°C for 20 to 24 h. The cells were washed four times with ice-cold PBS, scraped off the flask, collected, pelleted and, if required, stored at −80°C until use.

Cells from the equivalent of a 12.5-cm^2 flask were sonicated for 5 s at low power in a 1-ml volume of buffer consisting of PBS containing 1% Tween 20 (PBSTw). Cells were then clarified in an Eppendorf Microfuge for 20 min at 14,000 × *g*, and the supernatants were transferred to new microcentrifuge tubes. Three microliters of antiserum was added, and the tubes were left on wet ice for 2 h. Fifty microliters of a 50% slurry of Gammabind G-Sepharose beads (Amersham Pharmacia Biotech) in PBS were washed three times in PSBTw and added to each cell lysate, and the tube was rotated at 4°C for 1 h. The beads were then washed four times in excess PBSTw and boiled in 2% SDS, and eluted proteins were separated by electrophoresis on a denaturing polyacrylamide gel. The gel was then used either for immunoblotting or fluorography. For the latter, the gel was incubated for 30 min in a 1.25 M sodium salicylate solution, vacuum dried, and placed next to scientific imaging film (X-Omat; Kodak) at −80°C for a minimum of 24 h, after which the film was developed. The method for immunoblotting has been previously described (6); the antibodies used are listed in Table 1 and detailed below in the text.

In some experiments, Sf21 cells were infected at an MOI of 5.0 PFU per cell with recombinant baculoviruses expressing the U_L15, U_L28, or U_L33 proteins. The cells were incubated at 30°C for 40 h, after which time the cells were washed four times with ice-cold PBS and stored at −80°C until use. The remainder of the immunoprecipitation was carried out as described above.

RESULTS

U_L28 and U_L15 proteins form a complex in HSV-1(F) infected cells. A polyclonal antiserum recognizing the HSV-1 protein U_L28 was produced by immunizing rabbits with full-length U_L28 fused to the gene encoding GST (see Materials and Methods). To test the specificity of the antiserum, HEp-2 cells were infected with HSV-1(F) (wild-type virus) or the U_L28-null mutant HSV-1 virus gCB (41) and harvested after 20 h. Proteins were denatured by boiling in SDS, separated on a denaturing polyacrylamide gel, transferred to a nitrocellulose membrane, and probed with antisera directed against either ICP8, a virally encoded single-stranded DNA binding protein, or the U_L28 protein. Figure 1A shows the presence of ICP8 in lysates of cells infected with either virus, indicating that the cells were infected. The antiserum directed against the U_L28 protein (Fig. 1B) recognized an 85,000 apparent M_r protein in

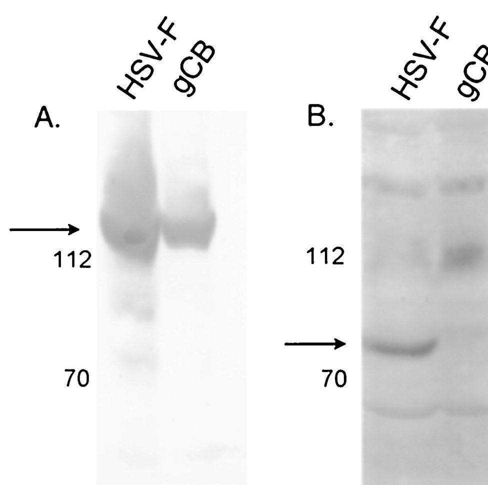

FIG. 1. Digitally scanned images of immunoblots probed with anti-ICP8 antibody (A) and anti-U_L28 antibody (B). Cells were infected with either HSV-1(F) or the U_L28 mutant virus gCB, and the proteins were separated on an 8% polyacrylamide gel before being transferred to a nitrocellulose membrane and probed with antiserum. Protein sizes are indicated on the left in thousands.

the HSV-1(F)-infected cells but not in cells infected with gCB, thus indicating recognition of the U_L28 protein.

Immunoprecipitations were performed to identify proteins that directly or indirectly associated with the U_L28 protein. Briefly, cells were infected with HSV-1(F) and infected cell proteins were radiolabeled with [^{35}S]methionine and [^{35}S]cysteine as detailed in Materials and Methods. Since the HSV-1 glycoproteins E (gE) and I form an Fc receptor and are normally present in immune complexes derived from HSV-infected cells (20), radiolabeled proteins were also immunoprecipitated from cells infected with the gE-null mutant virus R7202 (6). The infected cells were lysed in PBS supplemented with 0.5% Tween 20, briefly sonicated, and clarified extensively by centrifugation. The lysates were then reacted with anti-U_L28 serum as detailed in Materials and Methods. Immune complexes were purified, denatured in SDS, and electrophoretically separated on denaturing polyacrylamide gels, followed by fluorography.

In both the wild-type- and gE^- virus-infected cells, the anti-U_L28-GST antibody consistently immunoprecipitated two proteins of approximately 80,000 and 85,000 apparent M_r (Fig. 2). On the basis of their respective sizes, these proteins were hypothesized to be derived from U_L15 and U_L28, respectively. To address this possibility, nonradiolabeled cell lysates were harvested 24 h after infection with HSV-1(F) and immunoprecipitated with the U_L28-GST antiserum. The immunoprecipitated proteins were transferred to nitrocellulose and probed with antibodies directed against U_L28-GST or the N terminus of U_L15 fused to GST (34). As shown in Fig. 3A and B, both the U_L28 and U_L15 proteins were detected in immune complexes derived from reaction of the anti-U_L28-GST antibody with HSV-1(F)-infected cell lysates.

In order to confirm the putative interaction between U_L15 and U_L28 proteins, a reciprocal experiment was done. Cell lysates were subjected to immunoprecipitation with the antibody directed against the N terminus of the U_L15 protein, followed by immunoblotting with the anti-U_L28-GST serum. As shown in Fig. 3, the antiserum directed against the U_L15 protein immunoprecipitated both the U_L15 and U_L28 proteins (Fig. 3A and B). Control reactions containing lysates of HSV-1(F)-infected cells mixed with preimmune sera (Fig. 3) failed to immunoprecipitate either the U_L15 or U_L28 proteins. These data are therefore consistent with previously published work showing that the U_L15 and U_L28 proteins interact in HSV-1(F)-infected cells (23).

The U_L28 protein forms a complex with the U_L15 and U_L33 proteins. The U_L28 and U_L15 proteins were the most readily detected radiolabeled proteins in the above immunoprecipitations; however, it remained possible that other proteins were coimmunoprecipitated but escaped detection either because they were masked by comigrating nonspecifically immunoprecipitated radiolabeled proteins or because they did not radiolabel efficiently under the experimental conditions. Therefore, the U_L28/U_L15 complex was immunopurified, and the immunoprecipitated proteins were transferred to nitrocellulose and probed directly with antisera directed against the HSV proteins encoded by U_L6, U_L14, U_L15, U_L16, U_L17, U_L18, U_L19, U_L21, U_L25, U_L26.5, U_L28, U_L29, U_L31, U_L32, U_L33, or U_L49 or the host protein actin (see Table 1 for antibody sources). Of these proteins, only the proteins encoded by U_L15 and U_L33

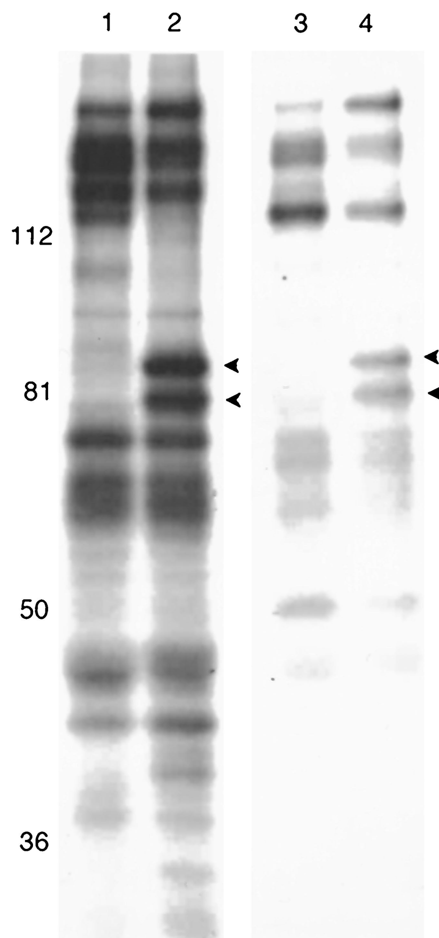

FIG. 2. Scanned digital image of fluorograph of radiolabeled proteins electrophoretically separated on an 8% polyacrylamide gel. HEp-2 cells were infected with either HSV-1(F) (lanes 1 and 2) or the gE-mutant virus strain 7202 (lanes 3 and 4) and immunoprecipitated as described in Materials and Methods by using either preimmune sera (lanes 1 and 3) or anti-U_L28 antibody (lanes 2 and 4). Protein sizes are indicated on the left in thousands. Arrowheads indicate proteins of 85,000 and 80,000 apparent M_r, which correspond to the U_L28 and U_L15 proteins, respectively.

coimmunoprecipitated with the U_L28 protein, as shown in Fig. 3B and C. To confirm that the U_L33 protein associated with the U_L28/U_L15 protein complex, antibody directed against the U_L15 protein was used to immunoprecipitate the U_L33 protein from infected cell lysates (Fig. 3C), and anti-U_L33 antibody was used successfully to immunoprecipitate both the U_L28 and U_L15 proteins (Fig. 3A and B). All experiments included two negative controls: immunoprecipitations with preimmune sera and antibody directed against the HSV-1 protein U_L14 (Fig. 3). In neither case were the U_L28, U_L15, or U_L33 proteins detected in immunoprecipitated material.

The U_L28/U_L15/U_L33 protein complex forms in lysates of recombinant baculovirus-infected insect cells. To determine if the three proteins were sufficient to interact with one another in the absence of other HSV-1-encoded proteins, Sf21 cells were infected or coinfected with recombinant baculoviruses expressing the U_L28, U_L15, and U_L33 proteins. In the first experiment, insect cells were coinfected with recombinant

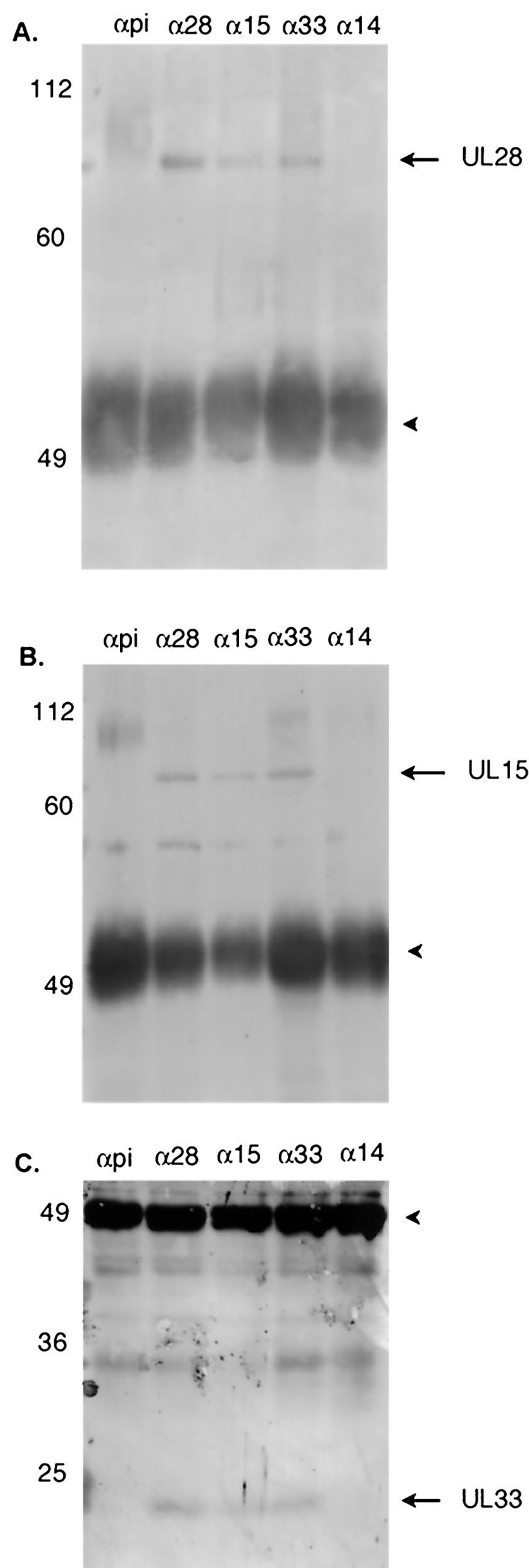

FIG. 3. Scanned digital images of immunoblots probed with antisera directed against the U_L28 (A), U_L15 (B), or U_L33 (C) proteins. Cell lysates were immunoprecipitated with preimmune serum (αpi) or antibody directed against U_L28 (α28), U_L15 (α15), U_L33 (α33), or U_L14 (α14), as described in the text. The position and size of molecular weight standards are indicated on the left in thousands. The heavy chain of the immunoprecipitating antibody can be seen as a dark band around 49,000 (arrowhead). (A and B) 8% polyacrylamide gels; (C) 15% polyacrylamide gel.

baculoviruses expressing all three proteins, and lysates of the infected cells were reacted with antibody directed against either the U_L28, U_L15, or U_L33 protein. Immunoprecipitated material was then denatured and analyzed on immunoblots probed with antibodies directed against U_L28-encoded protein. It was found that all three antibodies immunoprecipitated the U_L28 protein, thus showing that the three proteins can form a complex in the absence of other HSV proteins when coexpressed in insect cells (Fig. 4).

To identify which proteins within the $U_L28/U_L15/U_L33$ protein complex directly interact, insect cells were coinfected with recombinant baculoviruses expressing either (i) U_L28 and U_L33, (ii) U_L15 and U_L33, or (iii) U_L28 and U_L15. Lysates of the coinfected insect cells were then subjected to immunoprecipitation with antisera as described in Materials and Methods. As had been reported previously (1), U_L15 and U_L28 were coimmunoprecipitated by the anti-U_L28 antibody in the absence of U_L33 (Fig. 5C). The anti-U_L33 antibody was also found to coimmunoprecipitate the U_L28 protein in the absence of U_L15 and the U_L15 protein in the absence of U_L28 protein (Fig. 5A and B). The addition of anti-U_L33 antibody to Sf21 cells infected with only the U_L28- and U_L15-expressing baculoviruses did not result in the coimmunoprecipitation of either protein in the absence of the U_L33 protein (data not shown). Further controls included in these experiments were (i) immunoprecipitations using preimmune sera followed by immunoblotting with antisera directed against the U_L28, U_L15, and U_L33 proteins, and (ii) immunoprecipitations from lysates of Sf21 cells infected with wild-type baculovirus followed by immunoblotting with the respective antisera. These samples were uniformly negative, confirming that U_L28, U_L15, and U_L33 form a specific complex when coexpressed by infection of Sf21 cells with recombinant baculoviruses.

DISCUSSION

This paper describes the immunopurification of a complex of proteins containing the U_L28, U_L15, and U_L33 gene products. These data support the conclusions of others that the U_L28 and U_L15 proteins interact (1, 22, 23, 45), and they represent the first report to show a specific interaction between the U_L33 protein and other cleavage and packaging proteins. These results are supported by previously reported immunofluorescence assays that detailed a similar distribution of all three proteins in both the cytoplasm and replication compartments of the nucleus in infected cells (8, 23, 32, 45).

The U_L33 protein is the smallest of the seven cleavage and packaging proteins, with an apparent M_r of 19,000. Since it contains only two methionine and two cysteine codons in its open reading frame, the U_L33 protein does not radiolabel to high specific activities under the conditions used in these studies and is therefore difficult to detect by fluorography. To overcome this problem, immunoprecipitated material was subjected to immunoblotting with the U_L33-GST antisera. Using this method, the U_L33 protein was readily detected in lysates

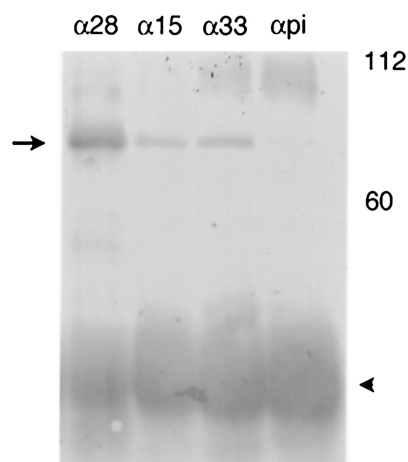

FIG. 4. Digitally scanned image of an immunoblot showing the detection of the $U_L28/U_L15/U_L33$ protein complex from baculovirus-infected Sf21 cells. Cell lysates were immunoprecipitated as described in Materials and Methods with antibody directed against the U_L28 (α28), U_L15 (α15), or U_L33 (α33) proteins or preimmune serum (αpi). The position of the U_L28 protein is indicated with an arrow. The heavy chain of the antibody can be seen as a dark band at approximately 50,000 (arrowhead). Molecular weight standards are marked at the right in thousands.

of infected cells immunoprecipitated with antiserum directed against the U_L28, U_L15, or U_L33 proteins (Fig. 3C).

The U_L33 protein is expressed as a late gene product and, using indirect immunofluorescence, it can be identified in the cytoplasm as well as nuclear replication compartments late in infection (32). Viruses lacking a functional U_L33 gene are unable to carry out cleavage and packaging of the HSV genome (5), but until recently no evidence revealing a specific role for the protein in DNA cleavage and packaging reactions had been reported. However, the interaction with the U_L28 and U_L15 proteins described in this paper suggests that the U_L33 protein may serve in some way that is relevant to terminase function. Although the precise function of U_L33 protein remains unknown, some possibilities include (i) ensuring that the terminase is correctly folded or assembled, (ii) ensuring that the terminase complex is correctly transported from the cytoplasm into the nucleus, and (iii) a role as part of the terminase holoenzyme. In light of the small size of U_L33, it is intriguing that HSV proteins of 21,000 and 22,000 apparent M_r have been shown to bind the a sequence of HSV-1 DNA (14). Although it is not known if these proteins are encoded by U_L33, the discovery that the U_L33 protein binds to the U_L28 and U_L15 proteins indicates that further work into its potential role as a member of the HSV terminase, possibly as a DNA-binding protein, is warranted.

The presence of a third protein in the U_L28/U_L15 protein complex reveals that, if this tripartite complex does comprise the terminase, it is structurally more complex than its counterpart in bacteriophage λ, where the C-terminal end of gpNu1 simply interacts with the N terminus of gpA (17). The full extent of the HSV terminase may in fact be larger still. Sixteen HSV proteins and one host protein were screened in the present work for inclusion in the putative terminase complex (Table 1). This work represents a thorough search of likely interacting candidates, including all of the cleavage-packaging proteins; however, it is not an exhaustive search of all HSV proteins. It is also possible that the antibodies directed against the U_L28 protein in the immunoprecipitation reactions could disrupt interactions with additional proteins, or that the experimental technique is not sensitive enough to detect the presence of additional proteins. Finally, there remains the possibility that a host protein may be included in the complex, as suggested by previous workers (21).

A recent report has suggested that the U_L33 protein of HSV-2 is transported into the nuclei of cells transiently coexpressing the U_L14 and U_L33 proteins, implying that the proteins interact (43). Such a conclusion was not corroborated in this study. The results of immunoprecipitation with antibody directed against the U_L14 protein followed by immunoblotting with monospecific antisera failed to detect either the U_L28, U_L15, or U_L33 proteins in immunoprecipitated material (Fig. 3). In addition, the U_L14 protein was not detected when material immunoprecipitated by the U_L28 antibody was probed with U_L14-specific antiserum, whereas the U_L33 protein was readily detected in this assay.

The anti-U_L28 antibody immunoprecipitated all three proteins from lysates of Sf21 cells coinfected with baculoviruses expressing U_L28, U_L15, and U_L33, thus mirroring the results obtained in lysates of HEp-2 cells infected with HSV-1(F). This indicated that other HSV proteins were not necessary for the interactions of the tripartite complex. When Sf21 cells were infected with subsets of the recombinant baculoviruses, it was revealed that none of the possible interactions were dependent

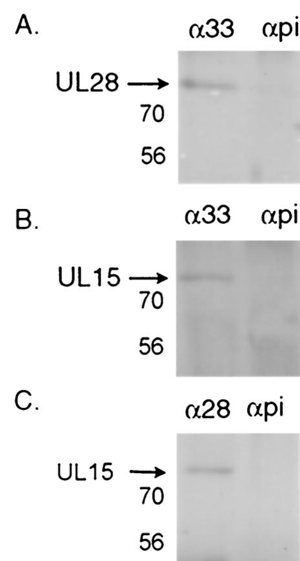

FIG. 5. Digitally scanned images of Western blots showing the proteins immunoprecipitated from dual-infected Sf21 cells. Cells were infected with baculoviruses expressing U_L28 and U_L33 (A), U_L15 and U_L33 (B), or U_L28 and U_L15 (C) and processed as described in Materials and Methods. Proteins were immunoprecipitated with antibody directed against the U_L33 protein (α33) or the U_L28 protein (α28), or with preimmune serum (αpi). An equivalent amount of lysate was used in each lane. Membranes were blotted with antibody directed against the U_L28 protein (A) or U_L15 protein (B and C). Protein sizes are indicated on the left in thousands.

on the presence of the third protein in the complex, implying direct interactions between all three proteins.

The relatively mild conditions used to produce the HSV-1(F)-infected cell lysates in these studies suggest that these lysates contain predominantly cytoplasmic rather than nuclear proteins. It is therefore hypothesized that the $U_L28/U_L15/U_L33$ complex forms in the cytoplasm of infected cells and is subsequently imported into the nucleus, where all three proteins are present late in infection (8, 23, 32). Further studies are necessary to address this possibility. The data also suggest that the putative terminase complex forms independently from capsids, since none of the major or minor capsid proteins, including the putative portal vertex protein encoded by U_L6 (29), were detected within the complex. Assembly of the terminase as a distinct process from assembly of the capsid might serve to (i) promote proper assembly of the terminase prior to import into the nucleus where DNA cleavage and packaging takes place, (ii) promote diffusion of the terminase within the nucleoplasm to increase the likelihood that genomic ends are engaged, or (iii) regulate the initiation of DNA cleavage and packaging.

Although the identification of the genes required for HSV-1 DNA cleavage and packaging was completed a number of years ago, elucidating the exact functions of these proteins has proved challenging. While the models of bacteriophage cleavage and packaging have been useful for heuristic purposes, this and other studies suggest that the analogous processes of herpesviruses are likely to be more complex.

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