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Retention of the Herpes Simplex Virus Type 1 (HSV-1) UL37 Protein on Single-Stranded DNA Columns Requires the HSV-1 ICP8 Protein

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The UL37 and ICP8 proteins present in herpes simplex virus type 1 (HSV-1)-infected-cell extracts produced at 24 h postinfection coeluted from single-stranded-DNA–cellulose columns. Experiments carried out with the UL37 protein expressed by a vaccinia virus recombinant (V37) revealed that the UL37 protein did not exhibit DNA-binding activity in the absence of other HSV proteins. Analysis of extracts derived from cells coinfected with V37 and an ICP8-expressing vaccinia virus recombinant (V8) and analysis of extracts prepared from cells infected with the HSV-1 ICP8 deletion mutants *d*21 and *n*10 revealed that the retention of the UL37 protein on single-stranded DNA columns required a DNA-binding-competent ICP8 protein.

The herpes simplex virus type 1 (HSV-1) genome is a linear, double-stranded DNA molecule of 160 kb (9, 15). Computer-assisted analysis of the DNA sequence has revealed at least 75 separate open reading frames (ORFs) (15). The functions of many of these genes in viral replication are at least partially understood. However, a significant fraction of HSV ORFs have not yet been characterized with regard to either the protein produced or the role that the protein plays in the viral life cycle (21). Advances in molecular techniques, along with knowledge of the precise locations of the different ORFs, now permit detailed studies on individual viral proteins with the ultimate goal directed towards the determination of individual protein function(s).

The protein product of the UL37 ORF of HSV-1 represents a viral protein for which the precise function is unknown. The UL37 gene is located in the unique long sequences between 0.527 and 0.522 map unit on the HSV-1 genome. In earlier publications, we reported on the identification and characterization of the UL37 protein in HSV-1-infected cells (1, 22). We determined that the UL37 ORF encoded a phosphorylated, nonstructural protein with an apparent molecular mass of 120 kDa. Analysis of the kinetics of production of UL37 places it in the γ1 class of HSV genes. In addition, the UL37 protein was eluted from single-stranded-DNA–agarose columns with an elution profile that completely overlapped with that of the major HSV-1 DNA-binding protein, commonly designated ICP8.

Because the UL37 protein has the same apparent molecular weight as ICP8 and comigrates with ICP8 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the presence of the UL37 protein was not detected in previous studies of HSV DNA-binding proteins (11, 12, 18–20, 23). In order to study the UL37 protein independently of other HSV-1 proteins and to examine possible interactions between the UL37 and ICP8 proteins, (i) fusions of UL37 gene sequences and the *Escherichia coli* maltose-binding protein were generated, (ii) a recombinant vaccinia virus which expressed ICP8 was constructed, and (iii) UL37- and ICP8-specific polyclonal rabbit antisera were generated. Rabbit polyclonal antisera were raised against the wild-type ICP8 protein and against an ICP8 carboxy-terminal peptide as previously described (10). Rabbit polyclonal antibodies specific for the UL37 protein were generated against (i) in vitro-translated UL37 protein, designated 487 antiserum (22); (ii) a *malE* fusion protein containing the entire UL37 protein, designated 779 antiserum; and (iii) a *malE* fusion protein containing the carboxy-terminal one-third of the UL37 protein, designated 780 antiserum (Fig. 1B). To test the specificity of each antiserum, we used the vaccinia virus recombinants V37, V8, and VSC11 as well as HSV-1-infected-cell extracts.

The construction and characterization of V37, a recombinant vaccinia virus that expresses the HSV-1 UL37 protein, and VSC11, a recombinant vaccinia virus that does not express any HSV gene and serves as a virus control, have been previously described (22). A recombinant vaccinia virus which expressed ICP8 (V8) was constructed by using the plasmid pV8 essentially as described previously (2, 13, 14). The plasmid pV8 contains the HSV-1 ICP8 gene cloned into the vaccinia virus shuttle vector pSC11 (2). A 1.6-kbp *Sal*I-*Kpn*I fragment from pNN1 (3) (obtained from Mark Challberg, National Institutes of Health) containing the 5′ end of the ICP8 ORF was cloned into pUC19, creating the plasmid pJF37. An *Rsr*II-*Hin*dIII collapse of pJF37 followed by the addition of *Bgl*II linkers created the plasmid pJF65. This placed the *Bgl*II site approximately 6 bp upstream of the ICP8 ATG translation start codon. A 4.3-kbp *Kpn*I fragment from pNN1 which contained the remainder of the ICP8 gene was then inserted at the *Kpn*I site of pJF65, creating the plasmid pJF67. The entire ORF of ICP8 was excised from pJF67 as a *Bgl*II-*Eco*RI fragment and cloned into the vaccinia virus shuttle vector pSC11, creating the plasmid pV8 (Fig. 1). The expression of ICP8 by the V8 recombinant virus was verified by immunoblot analysis of V8-infected CV-1 cell proteins by using antiserum directed against ICP8 (data not shown).

The specificity of the antisera raised against UL37 and ICP8 was demonstrated by immunoblot analysis of HSV-1(F)-, V37-, V8-, and VSC11-infected-cell proteins harvested at 24 h

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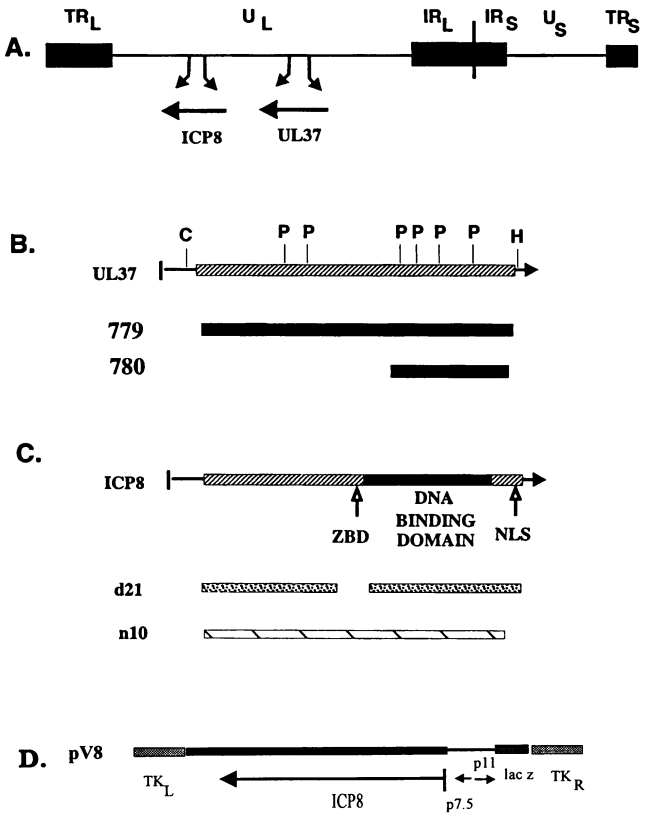

FIG. 1. (A) Sequence arrangement of the HSV-1 DNA genome, showing the locations of the unique sequences of the L and S components (U_L and U_S) and of the terminal (TR_L and TR_S) and inverted (IR_L and IR_S) repeats and the ICP8 and UL37 genes. (B) Schematic diagram of the UL37 gene. The hatched box represents the UL37-coding region. The solid boxes represent the domains of the UL37 protein in the fusions between *malE* and full-length UL37 (779) and between *malE* and the terminal 1.1 kbp of UL37 (780). C, *Cla*I; P, *Pst*I; H, *Hin*dIII. (C) Schematic diagram of the ICP8 gene, indicating the putative DNA-binding domain, potential zinc-binding domain (ZBD), and nuclear localization signal (NLS). The speckled and striped bars indicate the domains of the ICP8 protein contained in the *d*21 and *n*10 mutants, respectively. (D) Schematic diagram of the pV8 plasmid used to construct the V8 vaccinia virus recombinant as described in the text.

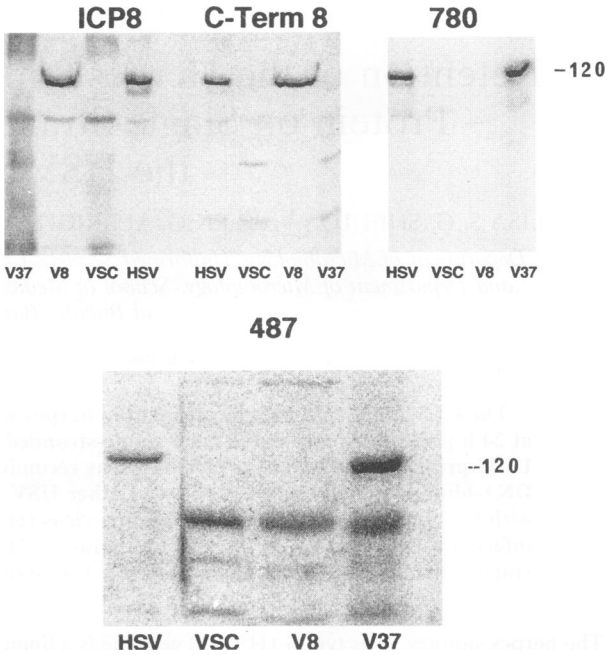

FIG. 2. Phosphorimage of immunoblots of HSV-1 (HSV)-, VSC11 (VSC)-, V37-, and V8-infected-cell proteins. Infected-cell proteins were harvested at 24 h p.i., separated on SDS–9% polyacrylamide gels, and probed with polyclonal rabbit antiserum directed against either wild-type ICP8 protein (ICP8), ICP8 carboxy-terminal peptide (C-Term 8), *malE*-1.1 UL37 fusion protein (780), or in vitro-translated UL37 (487). Antigen-antibody binding was detected with ^{125}I-labeled protein A.

postinfection (p.i.). As shown in Fig. 2, the antisera directed against ICP8 and its C-terminal peptide reacted with a 120-kDa protein from cells infected with either HSV-1 or vaccinia virus V8 but not with proteins from cells infected with vaccinia virus V37 or the VSC11 vaccinia virus control. The UL37-specific antisera 780 and 487 reacted with a 120-kDa protein from cells infected with either HSV-1 or vaccinia virus V37 but not with proteins from cells infected with vaccinia virus V8 or VSC11. Results similar to those obtained with the 780 and 487 antisera were obtained with the 779 antiserum (data not shown). These results demonstrate the specificities of the UL37 and ICP8 antibodies and show that the UL37 and ICP8 proteins comigrate in SDS-PAGE.

Previously we reported that the UL37 and ICP8 proteins from HSV-1-infected cells coeluted from single-stranded-DNA (ssDNA)–agarose columns (22). The observation that the UL37 protein from HSV-1-infected cells could be reproducibly eluted from ssDNA columns indicated that the UL37 protein either bound this DNA substrate directly or possibly interacted with other proteins, such as ICP8, which themselves bound DNA. To determine whether the UL37 protein is able to bind ssDNA in the absence of other HSV proteins, the DNA-binding capability of the UL37 protein expressed by the recombinant vaccinia virus, V37, was assessed. V37-infected-cell protein extracts were prepared from CV-1 cells at 48 h p.i. and analyzed by ssDNA-agarose chromatography as described by Shelton et al. (22). Individual fractions were analyzed by immunoblot for the presence of the UL37 protein. The vaccinia virus-expressed UL37 protein (V37 protein) was detected within the column flowthrough and wash and was absent within the gradient elution (Fig. 3A). In contrast, the ICP8 protein produced by the V8 vaccinia virus recombinant bound to ssDNA (Fig. 3B), suggesting that the lack of binding observed with the V37 protein was not caused by expression in vaccinia virus. These results demonstrated the inability of the UL37 protein to bind DNA in the absence of ICP8 and other HSV proteins. To test whether UL37 could bind DNA in the presence of ICP8, protein extracts were prepared from cells coinfected with V37 and V8 and analyzed by ssDNA-agarose chromatography. Fractions from the linear KCl elution gradient were analyzed by immunoblot for the UL37 and ICP8 proteins. As shown in Fig. 4, the full-length UL37 and ICP8 proteins coeluted in fractions 4 through 12, which encompassed KCl concentrations of 400 to 600 mM. The lower-molecular-weight bands which did not bind to the column represent a mixture of ICP8 and UL37 degradation products as well as background bands present in vaccinia virus-infected cells.

The above-described data strongly suggest that the HSV-1 ICP8 protein is required for the apparent DNA-binding prop-

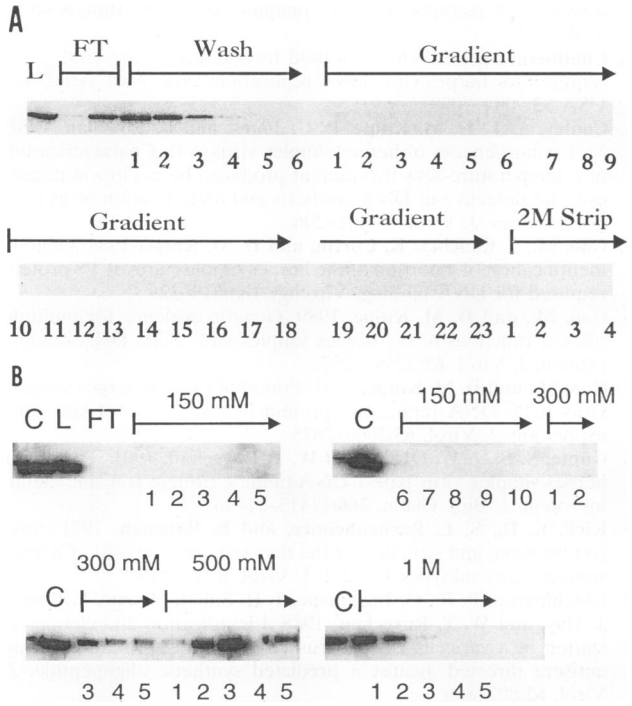

FIG. 3. Phosphorimage of immunoblots of fractions from ssDNA-agarose columns. (A) V37-infected-cell proteins were harvested at 47 h p.i. and separated on ssDNA-agarose columns. Individual fractions were separated on SDS–9% polyacrylamide gels and probed with 780 (UL37) antiserum. (B) V8-infected-cell proteins were harvested at 48 h p.i., separated on ssDNA-agarose columns by using step elutions of 150, 300, 500, and 1000 mM KCl, and probed with ICP8 antiserum. Antigen-antibody binding was detected with ^{125}I-labeled protein A. C, control; L, load; FT, flowthrough.

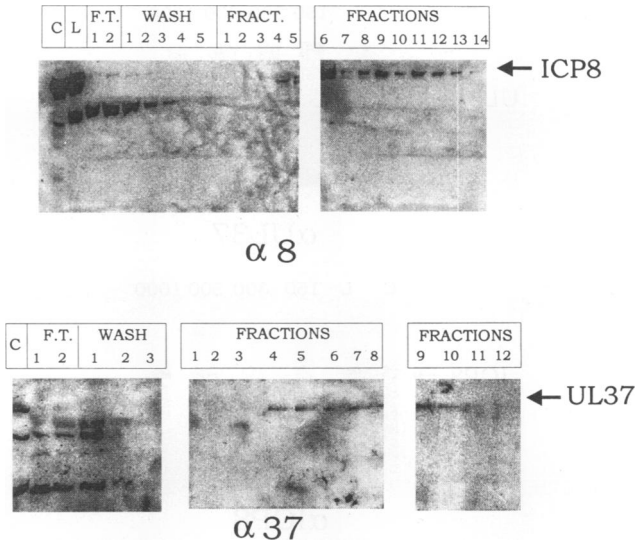

FIG. 4. Phosphorimage of immunoblots of fractions from ssDNA-agarose columns. CV-1 cells were coinfected with V37 and V8, and protein extracts were harvested at 48 h p.i. and separated on an ssDNA-agarose column by using a linear KCl gradient. Individual fractions were separated on SDS–9% polyacrylamide gels and probed with either ICP8 (α8) or 487 (α37) antiserum. Antigen-antibody binding was detected with ^{125}I-labeled protein A. C, control; L, load; F.T., flowthrough fractions.

erties of UL37. To investigate whether a DNA-binding-competent ICP8 was required for UL37 to bind DNA in HSV-1-infected-cell extracts, the ICP8 deletion mutants *d*21 and *n*10 were obtained from Priscilla Schaffer (Harvard University) and David Knipe (Harvard University), respectively. The *d*21 mutant has 260 internal amino acids deleted from the ICP8 gene, including the entire potential zinc-binding domain (5, 8) and 23 amino acids into the putative DNA-binding region (6, 7, 24) (Fig. 1C). The *n*10 mutant has 36 amino acids deleted from the carboxy terminus of the ICP8 gene, which results in the removal of the nuclear localization signal (Fig. 1C) (6). When these mutants are grown in noncomplementing cells, an HSV DNA-negative phenotype results, and the ICP8 molecules remain in the cytoplasm (6, 17).

Since a functional ICP8 molecule is essential for HSV DNA replication (3, 4, 16, 18, 25, 26) and since UL37 demonstrates γ1 kinetics, which requires DNA replication for full expression, we first determined whether *d*21 and *n*10 infection of noncomplementing cells would result in detectable UL37 expression, thus allowing us to assess correlations between a functional ICP8 and the DNA-binding properties of UL37. Immunoblot analysis of *d*21-infected CV-1 (noncomplementing) and U-47 (complementing) cells was performed with UL37- and ICP8-specific antisera. U-47 is an ICP8-complementing cell line which contains three copies of the ICP8 gene per haploid genome (17). The UL37 protein was detected in both CV-1 and U-47 cells infected with *d*21 or *n*10, with a diminished but clearly detectable level of expression found in the noncomplementing (CV-1) cells. In the CV-1 cells, only the faster-migrating ICP8 species encoded by *d*21 was detected, while in the U-47 cells, both the shorter ICP8 protein encoded by the *d*21 mutant and the full-length wild-type ICP8 protein expressed by the cell line were observed. Similar results were obtained with the *n*10 mutant (data not shown).

CV-1 cells infected with *d*21 or *n*10 were harvested at 24 h p.i., and high-salt extracts were prepared and analyzed by ssDNA-agarose chromatography. The mutant ICP8 protein

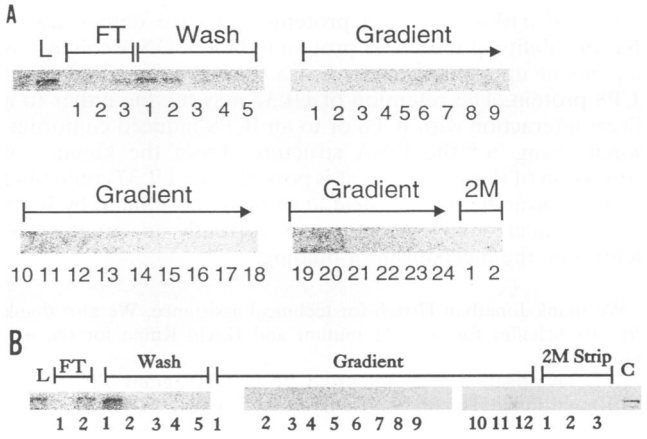

FIG. 5. Phosphorimage of immunoblots of fractions from ssDNA-agarose columns. *d*21-infected-cell proteins were harvested at 24 h p.i. and separated on ssDNA-agarose columns. Individual fractions were separated on SDS–9% polyacrylamide gels and probed with ICP8 (A) or 780 (UL37) (B) antiserum. Panels A and B represent separate experiments. Antigen-antibody binding was detected with ^{125}I-labeled protein A. L, load; FT, flowthrough; C, control.

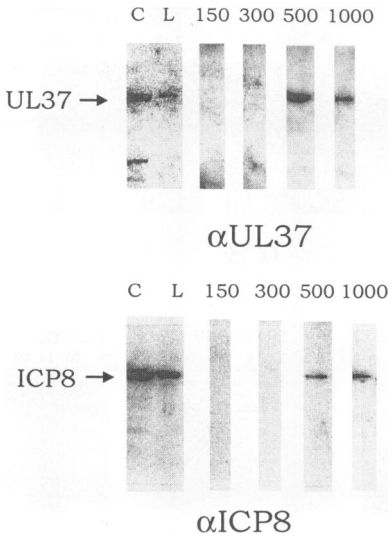

FIG. 6. Phosphorimage of immunoblots of fractions from ssDNA-agarose column. *n*10-infected cell proteins were harvested at 24 h p.i. and separated on an ssDNA column by using a step gradient of 150, 300, 500, and 1,000 mM KCl. Individual fractions were separated on SDS–9% polyacrylamide gels and probed with either 487 (αUL37) or ICP8 (αICP8) antiserum. Antigen-antibody binding was detected with ^{125}I-labeled protein A. For detection with the 487 antiserum, individual fractions were concentrated prior to loading. C, control; L, load; numbers indicate KCl concentration (millimolar).

encoded by the *d*21 virus was unable to bind to ssDNA (Fig. 5A), as was predicted by Orberg and Schaffer (17). Immunoblot analysis of the *d*21 fractions showed the presence of the UL37 protein in the column wash but not within the elution gradient (Fig. 5B), indicating the inability of UL37 (in the absence of a DNA-binding-competent ICP8 protein) to bind ssDNA. In contrast to the results obtained with the *d*21 mutant, the ICP8 protein expressed by the *n*10 mutant was able to bind ssDNA, which agreed with previous results of Gao and Knipe (6), and the UL37 protein cofractionated with the *n*10 ICP8 in 500 and 1,000 mM KCl step elutions (Fig. 6).

In conclusion, by comparative studies using HSV-1-, V37-, *d*21-, and *n*10-infected-cell proteins, we have demonstrated that the ability of the UL37 protein to bind ssDNA columns is dependent upon the presence of a DNA binding-competent ICP8 protein. The retention of UL37 may be due either to a direct interaction with ICP8 or to an ICP8-induced conformational change of the DNA structure. From the kinetics of expression of these proteins, it is possible that UL37 could bind to single-stranded nucleic acid at or near sites bound by ICP8 in the infected cell. Studies are currently in progress to determine the mechanism of binding.

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