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Identification by Antibody to a Synthetic Peptide of a Protein Specified by a Diploid Gene Located in the Terminal Repeats of the L Component of Herpes Simplex Virus Genome

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In the course of studies on the *a* sequences located at the termini of and at the junction between the L and S components of herpes simplex virus 1 DNA, J. Chou and B. Roizman (J. Virol. 57:629–637, 1986) noted that (i) the *a* sequence acted as a γ_1 promoter when fused to the structural sequence of the thymidine kinase gene, (ii) the *b* inverted repeat sequences located in the L component next to the *a* sequences contained an open reading frame predicted to encode the protein of 358 amino acids with a molecular weight of 37,054, and (iii) the transcription of an RNA homologous to the open reading frame initiated within the *a* sequence. The nucleotide sequence of the open reading frame predicted the presence of the triplet Ala-Thr-Pro repeated 10 times. To verify the existence of the predicted gene, designated $\gamma_1$34.5, a synthetic peptide consisting of the triplet Ala-Thr-Pro repeated 10 times was synthesized and used to raise antibodies in rabbits. The results were as follows. (i) The antiserum to the peptide reacted with a 43,500-apparent-molecular-weight protein present in lysates of cells infected with herpes simplex virus 1 but not present in mock-infected or herpes simplex virus 2-infected cells. (ii) We genetically engineered a recombinant virus containing a single copy of a truncated gene. Concordant with predictions, the antibody reacted with a faster-migrating protein in cells infected with this recombinant. (iii) The $\gamma_1$34.5 gene product was soluble, and it accumulated primarily in the cytoplasm late in infection. The overlap of the domain of the $\gamma_1$34.5 gene with the *a* sequence raises the possibility that it acts in *trans* on the *a* sequence and is associated with one of the functions currently ascribed to the *a* sequences.

We report here the identification and characterization of a protein predicted by nucleotide sequence and RNA studies to be encoded by a gene located in each of the inverted repeats flanking the L component of the herpes simplex virus 1 (HSV-1) genome. The circumstances that led to these studies were as follows.

(i) The wild-type HSV-1 genome (150 kilobase pairs [13]) consists of two components, L and S; each consists of unique sequences flanked by inverted repeats (25, 30). The repeat sequences of the L component, designated *ab* and *b'a'*, are each 9 kilobase pairs, whereas the repeat sequences of the S component, designated *a'c'* and *ca*, are each 6.5 kilobase pairs (30). The shared *a* sequence, 500-base-pairs long in HSV-1 strain F [HSV-1(F)], is present in one copy at the S component terminus and in one to several copies, in the same orientation, at the L-component terminus, and in inverted orientation at the junction between L and S components. The L and S components invert relative to each other such that the DNA extracted from virions or infected cells consists of four isomers differing solely in the orientation of the L and S components relative to each other (9). The *a* sequence appears to be a *cis*-acting site for inversions inasmuch as insertion of the *a* sequence elsewhere in the genome (4, 17–19) or deletion of the entire internal inverted repeat sequences (b'a'c') (22) leads to additional inversions or the loss of the ability of the L and S components to invert, respectively. The *a* sequence was also shown to contain the *cis*-acting sites for the circularization of the genome after infection, for cleavage of the HSV genome from concatemers, and for encapsidation of the DNA (19, 27–29).

(ii) HSV-1 genomes contain an excess of 50 genes whose expression is coordinately regulated and sequentially ordered in cascade fashion (12). The α genes are expressed first, followed by β, γ_1, and γ_2 genes. The differentiation among β, γ_1, and γ_2 genes is operationally based on the effect of inhibitors of viral DNA synthesis (6, 26). Whereas the expression of β genes is stimulated and that of γ_1 genes is only slightly reduced by inhibitors of viral DNA synthesis, the expression of γ_2 genes stringently requires viral DNA synthesis.

(iii) In the course of studies on the function of the *a* sequence, Chou and Roizman (4) noted that the chimeric structure consisting of the *a* sequence fused to the 5' transcribed, noncoding sequences of the thymidine kinase (TK) gene of HSV-1 was inducible in transfected cells and regulated as a γ_1 gene when inserted into the viral genome. This observation suggested that the terminus of the *a* sequence nearest the *b* sequence of the inverted repeats contained a promoter and the transcription initiation site of a gene whose structural sequences were located in the *b* sequences flanking the L component. Studies involving hybridization of labeled DNA probes to electrophoretically separated RNAs extracted from infected cells, and S1 nuclease analyses confirmed the existence of RNA transcripts initiating in the *a* sequence. Nucleotide sequence analyses revealed the presence of an open reading frame capable of encoding a protein 358 amino acids long (5).

(iv) Previous studies have shown that each inverted repeat of the S component contains in its entirety a gene designated

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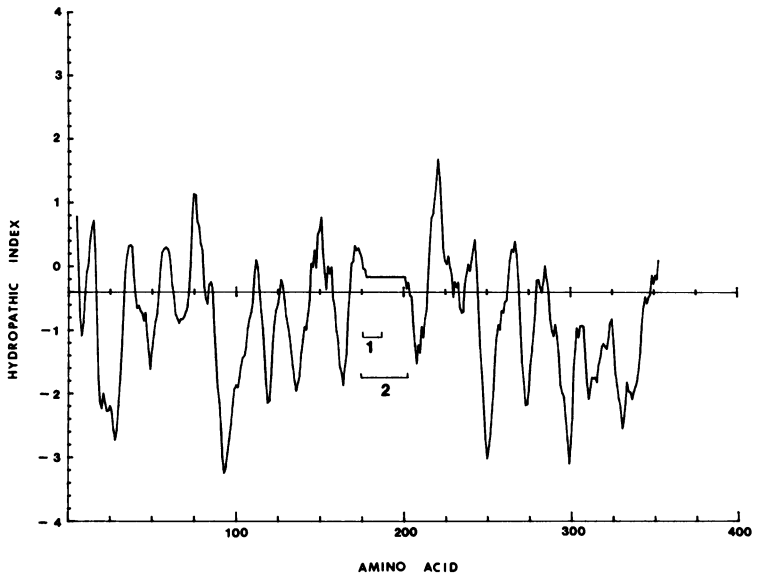

FIG. 1. Hydropathic analysis of the predicted amino acid sequence of $\gamma_1$34.5 protein. The determination of relative hydropathy as a function of position along the amino acid sequence was done by the method of Kyte and Doolittle (14) with a moving window of seven amino acids as previously described (21). The lines designated 1 and 2 identify the domain of the sequence used to synthesize the synthetic peptides.

α4, whereas each of those of the L component contains in its entirety a gene designated α0 (15, 20, 31). The putative gene identified on the basis of nucleotide sequence and analyses of RNA is also present in two copies per genome. Because of the overlap of the domain of this gene with the *a* sequence containing the *cis*-acting sites for inversion (5, 17–19), cleavage of DNA from concatemers, and packaging of the DNA (28, 29), it was of interest to identify and characterize the gene product. For this purpose, we took advantage of the observation that the nucleotide sequence predicted the presence in the protein of the amino acid triplet Ala-Thr-Pro repeated 10 times. In this work, we report that antibody to a synthetic peptide synthesized on the basis of this sequence reacted with a 43,500-apparent-molecular-weight HSV-1 protein. Some of the properties of this protein are described.

MATERIALS AND METHODS

Virus strain and cells. The properties of HSV-1(F) and HSV-2(G) (6, 7), HSV-1(HFEM) and HSV-1(HFEM)*ts*LB2 (8), and HSV-1(MP) (10) have been published. The derivation and structure of the HSV-1 recombinant virus I358 has been reported elsewhere (22). In this recombinant, the internal inverted repeat sequences located between the middle of the *Bam*HI B and promoter-regulatory domain of the α4 genes were replaced by a DNA segment containing the TK gene and a small DNA sequence derived from the left end of the L component. A TK^- derivative of I358, designated R3410 and lacking the segment inserted into the novel junction between the L and S components of I358 DNA, will be described elsewhere (R. Longnecker, M. Arsenakis, and B. Roizman, manuscript in preparation). All virus stocks were made in Vero cells maintained in medium 199-V consisting of mixture 199 supplemented with 1% calf serum.

Buffers and solutions. Disruption buffer consisted of 0.05 M Tris (pH 7.0), 8.5% (wt/vol) sucrose, 5% (vol/vol) 2-mercaptoethanol, and 2% (vol/vol) sodium dodecyl sulfate. Hypotonic buffer used for the separation of nuclei from cytoplasm (3) consisted of 1.6 mM $MgCl_2$, 6 mM KCl–10 mM Tris (pH 8), 1 mM dithiothreitol, and 0.5% (vol/vol) Nonidet P-40 (NP-40). NP-40-containing solubilization buffer consisted of phosphate-buffered saline (PBS) (8.2 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.14 M NaCl, 2.5 mM KCl) supplemented with 1% (vol/vol) NP-40, 1% sodium deoxycholate, 10^{-5} M *N*-α*p*-tosyl-L-lysine chloromethyl ketone (TLCK), and L-1-tosylamide-2-phenylmethyl chloromethyl ketone (TPCK).

Construction of recombinant plasmids. Restriction enzymes were from Bethesda Research Laboratories, Inc., Gaithersburg, Md.; Pharmacia-PL, Inc., Milwaukee, Wis.; and New England BioLabs, Inc., Beverly, Mass., and were used as recommended in the suppliers' instructions. T4 DNA ligase was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. The T4 DNA polymerase (New England BioLabs) reaction was carried out as previously described (17). After ligation, plasmid DNAs were transformed into *Escherichia coli* c600 cells. Plasmids pRB143 (18) and pRB103 (23) carried the HSV-1(F) *Bam*HI S and *Bam*HI Q fragments, respectively.

Preparation of DNA probes and hybridizations. The probes were labeled with [^{32}P]dCTP by nick translation with the aid of a kit from New England Nuclear Corp., Boston, Mass., as recommended in the manufacturer's specifications. For the hybridization tests, electrophoretically separated digests were transferred to nitrocellulose sheets and hybridized with approximately 0.5 μg of labeled DNA (5×10^6 to 20×10^6 cpm).

Transfection and selection of recombinant viruses. The plasmid DNA carrying the α27-TK insert in the *Bam*HI S fragment was cotransfected with intact DNA of HSV-1(F) recombinant R3410 into rabbit skin cells. The TK^+ progeny were selected by plating the lysates on $143TK^-$ cells maintained in hypoxanthine-aminopterin-thymidine (HAT) medium supplemented with 6 mM thymidine, 1.7 mM hypoxanthine, and 0.2 μg of methotrexate (Lederle Laboratories, Pearl River, N.Y.) per ml. All viral DNAs analyzed in these studies were derived from cytoplasmic virions as previously described (13, 23).

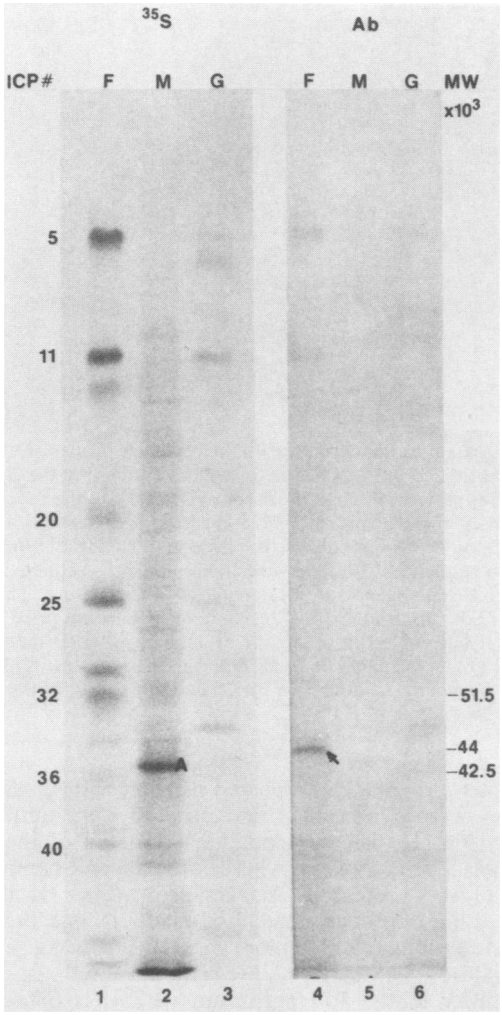

FIG. 2. Autoradiographic images and immune reactivities of polypeptides from lysates of HSV-1(F), HSV-2(G), and mock-infected cells electrophoretically separated in denaturing gels and then transferred to nitrocellulose sheets. Replicate cultures of HEp-2 cells were infected or mock infected and labeled with [^{35}S]methionine from 18 to 24 h postinfection. Cell lysates were subjected to electrophoresis in denaturing 9.3% polyacrylamide gels and transferred to nitrocellulose as described in Materials and Methods. Numbers to the left of the figure indicate the ICP designations of Honess and Roizman (11, 12) and Morse et al. (20). Numbers to the right of the figure indicate the molecular weight (in thousands). The letter A indicates the position of actin. Lanes: 1 to 3, autoradiographic images of electrophoretically separated polypeptides in lysates of infected and mock-infected cells; 4 to 6, photograph of the electrically transferred polypeptides shown in lanes 1 to 3 stained with rabbit R-4 antiserum. The arrow indicates a 43,500-apparent-molecular-weight polypeptide band of HSV-1 reacting with the antiserum. Abbreviations: F, HSV-1(F)-infected cells; G, HSV-2(G)-infected cells; M, mock-infected cells.

Infection and labeling of cells with [^{35}S]methionine. Monolayer cultures containing 4×10^6 cells were exposed to 20 PFU of virus per cell. After 1 h of adsorption, the inoculum was replaced with maintenance medium, and the cells were further incubated at 34°C. Cells were labeled with [^{35}S]methionine (New England Nuclear Corp., Boston, Mass.) at times indicated in Results. For labeling, the cells were overlaid with maintenance medium containing only 10% of the usual amount of methionine, and [^{35}S]methionine was supplemented to a final concentration of 25 μCi (1.260 Ci/mmol) per 25-cm^2 culture flask.

Preparation of labeled protein extracts. The cells were scraped out of the tissue culture flask, washed three times with PBS, and pelleted by centrifugation at 1,000 rpm for 5 min. Total cellular, nuclear, and cytoplasmic extracts were prepared as previously described (3). NP-40-soluble and -insoluble proteins were prepared by solubilization of cell lysates in NP-40 solubilization buffer. After centrifugation at 72,000 × *g* for 1 h at 4°C in an SW50.1 rotor, the supernatant fluids and pellets were solubilized in disruption buffer and subjected to electrophoresis in denaturing polyacrylamide gels.

Polyacrylamide gel electrophoresis and reaction of rabbit antiserum R-4 to electrophoretically separated polypeptides transferred to nitrocellulose sheets. The polypeptides were separated electrophoretically on 9.25% and, in some instances, 11% sodium dodecyl sulfate-polyacrylamide gels cross-linked with *N,N'*-diallyltartardiamide (20), transferred electrically to a nitrocellulose sheet, and reacted with rabbit antiserum R-4 as previously described (1, 3). Briefly, the nitrocellulose sheet was incubated for 1 h at 37°C in PBS containing 3% (wt/vol) bovine serum albumin (early studies) or 10% fetal calf serum (late studies) and then for 3 h at the same temperature with rabbit serum diluted 1:100 in 1% bovine serum albumin. The nitrocellulose sheet was then incubated in PBS containing 1% bovine serum albumin and horseradish peroxidase-coupled goat anti-rabbit immunoglobulin G (5 μl/3 ml; Miles Laboratories, Inc., Elkhart,

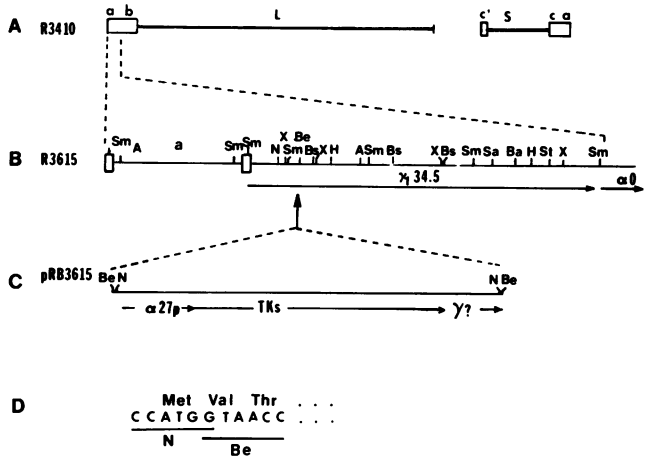

FIG. 3. Schematic diagram of the construction of the HSV-1(F) recombinant R3615 carrying a single copy of a $\gamma_1$34.5 gene truncated at the 5' terminus. Line A, Sequence arrangement of recombinant R3410. The boxes identified as *ab* and *ca* are the terminal copies of the inverted repeat sequences; all but the small fraction of the internal inverted repeats identified by a *c'* above a narrow box had been deleted. Line B, Sequence arrangement of the DNA sequence adjacent to the *a* sequence in the *b* repeat sequence of the L component. The horizontal arrows below line B indicate the transcribed domains of the $\gamma_1$34.5 gene and of the 5' portion of the α0 gene. Line C, Sequence arrangement of the α27-TK construct inserted into the unique *Bst*EII (Be) site indicated by the vertical arrow. Line D, Predicted sequence and codons at the site of fusion of the inserted fragment, between the amino acid 1 of the unidentified γ gene and amino acid 44 of $\gamma_1$34.5 protein. Abbreviations: Sm, *Sma*I; A, *Ava*II; N, *Nco*I; X, *Xma*III; Be, *Bst*EII; Bs, *Bss*HII; H, *Hin*fI; Sa, *Sac*I; Ba, *Bal*I; St, *Stu*I. The *Sma*I site at the far left of the map designates position 270 of the α0 gene domain reported by Mackem and Roizman (15).

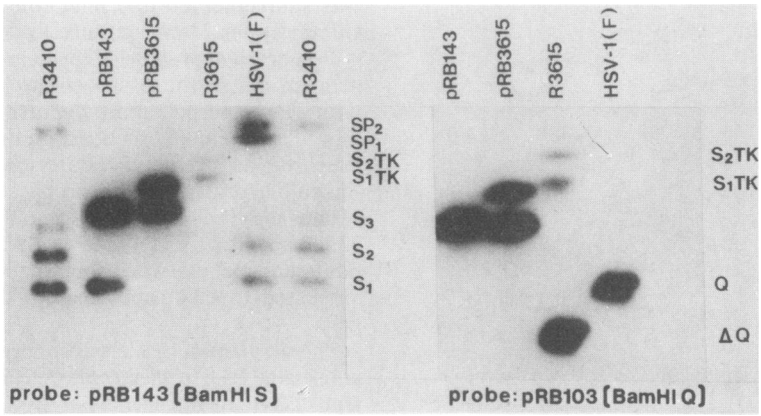

FIG. 4. Autoradiographic images of electrophoretically separated DNA fragments hybridized with ^{32}P-labeled DNA probes. Left panel, Electrophoretically separated *Bam*HI digests of viral and recombinant DNAs hybridized with ^{32}P-labeled pRB143 containing the *Bam*HI S DNA fragment from HSV-1(F). The digests shown are those of the DNAs of wild-type virus HSV-1(F), parent virus R3410, the recombinant virus R3615, plasmid pRB143 carrying the *Bam*HI S fragment in pBR322, and of the plasmid pRB3615 carrying the chimeric fragment recombined into the HSV genome in R3615. The electrophoretically separated *Bam*HI S fragments of the three viruses and the *Bam*HI SP fragment in HSV-1(F) each formed a ladder of bands differing in the number of *a* sequences. Bands containing only one *a* sequence, as the *Bam*HI S fragment cloned in pRB143, were designated S_1 or SP_1; those with two *a* sequences were designated S_2 and SP_2, etc. The R3615 bands hybridizing with ^{32}P-labeled pRB143 migrated more slowly than the *Bam*HI S bands, and the one designated S_1TK comigrated with the construct cloned as pRB3615. Right panel, Electrophoretically separated *Bam*HI digests hybridized with ^{32}P-labeled pRB103 carrying the *Bam*HI Q fragment containing the TK gene. The DNA sequences in the *Bam*HI Q DNA fragments of I358 and its derivatives, R3410 (data not shown) and R3615, lack 500 base pairs and are identified as ΔQ. The HSV-1 fragment, cloned in pRB3615 and consisting of the α27-TK chimeric gene inserted into the *Bst*EII site of *Bam*HI-S, comigrated with the S_1TK fragment of R3615.

Ind.). After being washed with PBS, the nitrocellulose sheet was transferred to a substrate solution containing 0.01% (wt/vol) 4-chloro-1-naphthol (Aldrich Chemical Co., Inc., Milwaukee, Wis.) and 0.015% H_2O_2. Autoradiographic images of the labeled bands were made on Cronex X-ray film (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). The fetal calf serum was more effective than was bovine serum albumin in blocking nonspecific reactivity of the R-4 antiserum with polypeptide bands.

Peptide synthesis. The synthetic peptides described in the text were synthesized, cleaved from the resin, and conjugated to keyhole limpet hemocyanin (Calbiochem-Behring, La Jolla, Calif.) by the method of Merrifield (16).

Immunization of rabbits. New Zealand White rabbits were injected intracutaneously once every 2 weeks a total of eight times each with a mixture consisting of 150 μg of peptide conjugated to keyhole limpet hemocyanin in 1 ml of PBS mixed with an equal volume of Freund complete adjuvant (Difco Laboratories, Detroit, Mich.) for the first immunization and with an equal volume of Freund incomplete adjuvant for the subsequent immunizations. Two weeks after the eighth immunization, rabbits were bled, and the immune and preimmune sera were tested for reaction with infected-cell lysates as described in the text and previously (2).

RESULTS

Selection of amino acid sequence for peptide synthesis. The hydropathic analysis (14) of the amino acid sequence predicted by the open reading frame in the inverted repeats of the L component adjacent to the *a* sequence indicated that the protein should be highly hydrophilic (Fig. 1), and several domains of the protein would have been suitable candidates for synthesis of synthetic peptides for the purpose of inducing antibody. At the time these studies were begun, only a portion of the sequence of the gene was known with any reasonable degree of confidence. The striking feature of the known sequence predicted the presence within the protein of the triplet Pro-Ala-Thr repeated 10 times. Two synthetic peptides were synthesized and used to raise antibody in rabbits (Fig. 1). The first peptide consisted of the amino acids $(Ala\text{-}Thr\text{-}Pro)_5$-Cys, whereas the second peptide consisted of the sequence $(Ala\text{-}Thr\text{-}Pro)_{10}$-Cys. Of the two antisera, the one designated R-4, made against the longer synthetic peptide, reacted more strongly and was used in the studies described below.

Reactivity of the R-4 antiserum with electrophoretically separated lysates of infected and mock-infected cells. In this series of experiments, replicate cultures of HEp-2 cells were infected with HSV-1(F) or HSV-2(G) or were mock infected and then labeled with [^{35}S]methionine from 18 until 24 h postinfection. The cells were then harvested, solubilized in disruption buffer, and sonicated, and the lysates were subjected to electrophoresis in 9.3% polyacrylamide gel and electrically transferred to a nitrocellulose sheet. The nitrocellulose sheet was then reacted with R-4 rabbit antiserum. The R-4 antiserum reacted most strongly with an HSV-1(F) polypeptide whose electrophoretic mobility in denaturing gels corresponded to that of a protein with an apparent molecular weight of 43,500 (Fig. 2). Although the antiserum faintly illuminated several bands in lysates of HSV-1- and HSV-2-infected cells, the reactivity of these bands with the rabbit antiserum was considerably weaker than that of the HSV-1(F) protein with the apparent molecular weight of 43,500. The protein was not previously detected among electrophoretically separated proteins of HSV-1 because the protein contains a single N-terminal methionine residue (5) and overlaps in part with the bands formed by the infected-cell protein 35 (ICP35). On the basis of its electrophoretic mobility, it was designated $\gamma_1$34.5.

Genetic engineering of a truncated $\gamma_1$34.5. The experiments described above indicated that an infected-cell protein with an apparent molecular weight of 43,500 reacted with the R-4 rabbit antiserum. Although the R-4 antiserum was raised in rabbits immunized with a synthetic peptide whose sequence

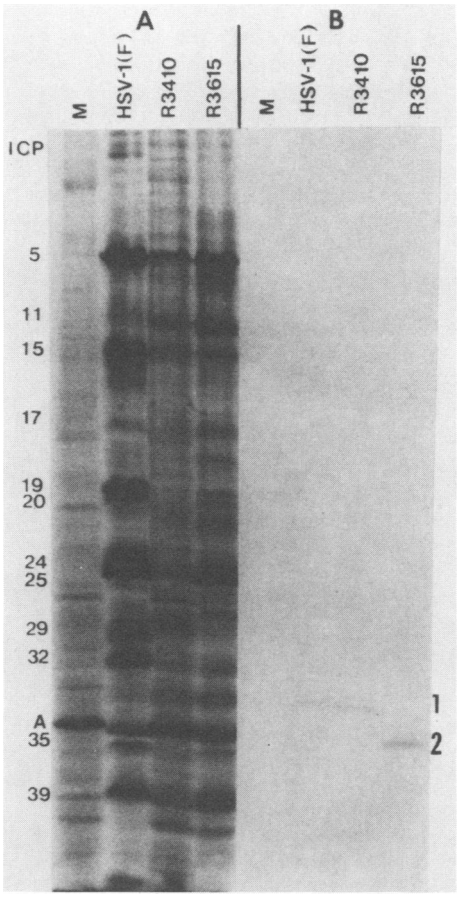

FIG. 5. Autoradiographic images (A) and immune reactivities with R-4 antiserum (B) of electrophoretically separated polypeptides from lysates of HSV-1(F), R3410, R3615, and mock-infected cells. The infected cells were labeled with [^{35}S]methionine from 20 to 24 h postinfection, harvested, solubilized in disruption buffer, electrophoretically separated in denaturing polyacrylamide gels, and transferred electrically to a nitrocellulose sheet. The $\gamma_1$34.5 protein of R3615 migrated more rapidly, as predicted from Fig. 3, than the wild-type protein made by the parent, R3410 recombinant virus. Numbers to the left of the figure indicate the ICP designation. The numbers to the right of the figure indicate HSV-1 (1) and HSV-2 (2).

was predicted by the nucleotide sequence of the gene, we could not exclude the possibility that the R-4 antibody reacted with another viral protein containing an Ala-Thr-Pro repeat. Definitive evidence that R-4 antibody indeed reacted with the product of the $\gamma_1$34.5 gene was obtained by genetically engineering a virus carrying only a single gene specifying a truncated $\gamma_1$34.5 and by showing that the gene product migrated faster than the wild-type protein in denaturing polyacrylamide gels.

The genetic engineering of the virus carrying a truncated $\gamma_1$34.5 gene was done as follows: The α-TK chimeric gene, consisting of the α27 promoter-regulatory domain fused to the structural sequences of the TK gene as described by Mackem and Roizman (15), was cleaved with the *Nco*I restriction endonuclease. The 1.7-kilobase-pair *Nco*I fragment containing the α27-TK chimera was then inserted by blunt-end ligation into the cloned *Bam*HI S fragment (pRB143) at the *Bst*EII site after T4 polymerase reaction. The orientation of the insertion was such that the chimeric TK gene was in the same transcriptional orientation as the $\gamma_1$34.5 gene. The cloned fragment containing the insertion (Fig. 3) was cotransfected with R3410 DNA into rabbit skin cells. The progeny of transfection were plated under HAT medium on human 143TK$^-$ cells, and TK$^+$ progeny were selected. A viable virus (R3615) was selected and was shown to contain the α27-TK insert at the *Bst*EII site in the gene encoding $\gamma_1$34.5 (Fig. 4). The *Nco*I cleavage site located at the 3′ terminus of the TK gene is within the domain of the translation initiation site of a late γ HSV-1 gene whose product has not been identified (24). Fusion of the *Nco*I fragment to the *Bst*EII site in the $\gamma_1$34.5 gene would result in the frame linkage of the first amino acid (Met) of the unidentified γ gene fused to amino acid 44 of the $\gamma_1$34.5 gene (Fig. 3D). Recombinant R3615 would be expected to contain a single copy of the $\gamma_1$34.5 gene specifying a protein truncated at its N terminus but containing the site of binding of the R-4 antibody. The band reacting with R-4 antibody in lysates of R3615-infected cells migrated faster in denaturing polyacrylamide gels than the corresponding protein of the parent R3410 virus (Fig. 5). The phenotypic properties of the R3615 recombinant and of other mutants in the $\gamma_1$34.5 gene will be reported elsewhere (J. Chou and B. Roizman, manuscript in preparation).

Compartmentalization of $\gamma_1$34.5 protein. In this series of experiments, HEp-2 cells labeled from 18 until 24 h postinfection were harvested at 24 h postinfection and fractionated into nuclear and cytoplasmic fractions. The 43,500-apparent-molecular-weight protein accumulated largely in the cytoplasm (Fig. 6).

Solubility of the 43,500-apparent-molecular-weight protein. Because many HSV proteins are not soluble in physiologic solutions, it was of interest to determine whether the 43,500-apparent-molecular-weight protein reactive with the R-4 antiserum was soluble. In this study, HEp-2 cells labeled with [^{35}S]methionine from 10 until 24 h postinfection with HSV-1 were harvested at 24 h postinfection and solubilized in a solution containing PBS and NP-40 nonionic detergent as described in Materials and Methods. The solubilized lysate was centrifuged for 1 h at 72,000 $\times$ *g*. In contrast to the many HSV-1 proteins studied to date (1, 3, 11), the bulk of the protein reactive with the R-4 antiserum was recovered from the supernatant fluid (Fig. 7).

Reactivity of the R-4 antiserum with polypeptides specified by other HSV-1 strains. The objective of these experiments was to determine whether R-4 antiserum reacts with polypeptides specified by other strains of HSV-1. It seemed likely, although data on this point are not available, that the number of reiterations of the amino acid triplet Pro-Ala-Thr could vary from one virus strain to another. Variation in the number of reiterations could change the conformation and the reactivity of the protein with the R-4 antiserum. The R-4 antiserum did not react with electrophoretically separated polypeptides from lysates of HEp-2 cells infected with HSV-1(HFEM) and HSV-1(MP) (Fig. 8).

DISCUSSION

In an earlier work, Chou and Roizman (5) reported the probable presence of a hitherto unsuspected gene within the inverted repeats of the L component of the HSV-1 genome. The putative gene was identified on the basis of the mapping of RNA transcripts, identification of a regulatory-promoter region, and demonstration that the nucleotide sequence of the domain of the putative gene contained an open reading frame capable of encoding a protein 358 amino acids in length with a molecular weight of 37,054. In this work, we report that an antibody to an oligomer synthesized on the

basis of the predicted amino acid sequence reacted with a protein with an apparent molecular weight of 43,500. This is consistent with the observation that the electrophoretic mobility of the HSV-1 proteins rich in proline is that of proteins with molecular weights 10 to 25% greater than that predicted by the nucleotide sequence. $\gamma_1$34.5 protein contains 75 residues of proline, or 20.8% of the total. Relevant to the discovery of $\gamma_1$34.5 are the following.

(i) To date, only α genes 0 and 4 have been reported to map within inverted repeat sequences flanking the L and S components, and these genes are present in two copies per genome (15, 20, 31). The gene specifying $\gamma_1$34.5 is the first non-α gene shown to map within the inverted repeats and to

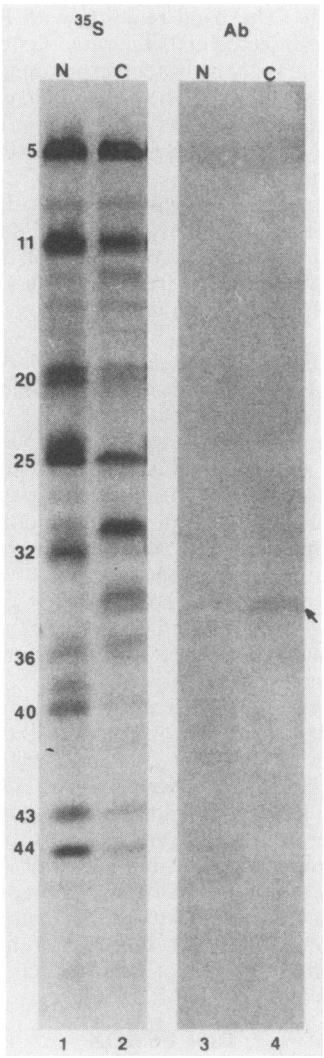

FIG. 6. Autoradiographic images and immune reactivities of electrophoretically separated polypeptides from nuclei and cytoplasm of HEp-2 cells infected with HSV-1(F) and incubated in medium containing [^{35}S]methionine (^{35}S) from 18 to 24 h postinfection. Numbers to the left of the figure indicate the ICP designations. Lanes: 1 and 2, autoradiographic images; 3 and 4, photographs of the electrophoretically separated, transferred proteins stained with the R-4 antiserum. The arrow indicates the HSV-1(F) polypeptide band reacting with the R-4 antiserum. Lanes 1 and 3 contain the nuclear (N) fractions, whereas lanes 2 and 4 contain polypeptides present in the cytoplasmic (C) fractions.

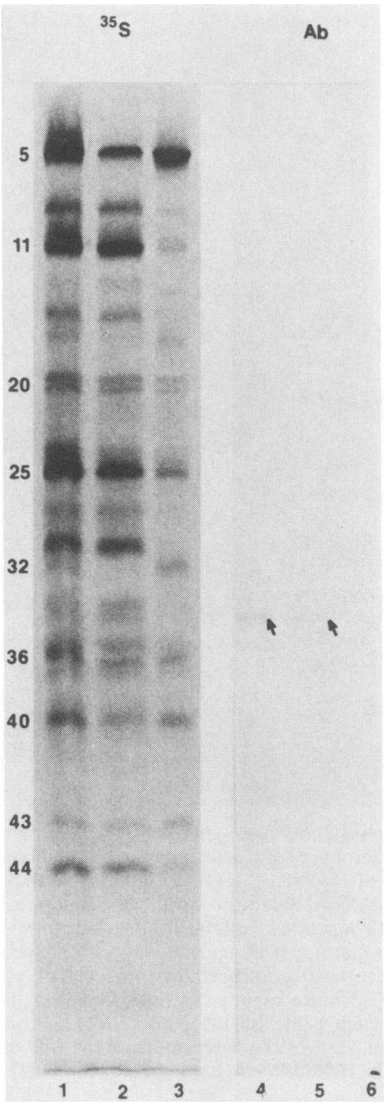

FIG. 7. Autoradiographic images and immune reactivities of electrophoretically separated proteins in lysates of HSV-1(F)-infected HEp-2 cells after fractionation by centrifugation. HEp-2 cells were incubated from 10 to 24 h postinfection with HSV-1(F) in medium containing [^{35}S]methionine (^{35}S). At 24 h postinfection, the cells were harvested and solubilized in a solution containing PBS and NP-40 nonionic detergent and centrifuged as described in Results. The polypeptides contained in the various fractions were solubilized in disruption buffer and subjected to electrophoresis in denaturing polyacrylamide gels, transferred to a nitrocellulose sheet, and reacted with the R-4 rabbit antiserum. Numbers to the left of the figure indicate ICP designations. Lanes: 1 to 3, autoradiographic images; 4 to 6, reactivities of the electrophoretically separated polypeptides shown in lanes 1 to 3 with the R-4 antiserum. The polypeptide bands reacting with the antiserum are indicated by arrows. Lane 1, Total cell lysates; lane 2, supernatant fluid obtained after centrifugation of total cell lysates; lane 3, pellet obtained after centrifugation of the total cell lysates; lane 4, reactivity of polypeptides in lane 1 with the R-4 antiserum; lane 5, reactivity of polypeptides in lane 2 with R-4 antiserum; lane 6, immune reactivity of the electrophoretically separated polypeptides shown in lane 3.

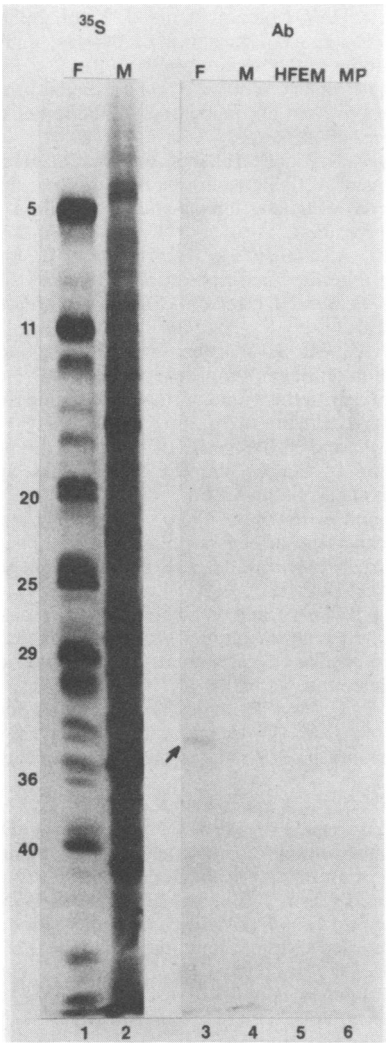

FIG. 8. Autoradiographic images and immune reactivities of electrophoretically separated polypeptides from lysates of cells infected with HSV-1(F), HSV-1(HFEM), and HSV-1(MP) and from mock-infected cells with the R-4 rabbit antiserum. Replicate HEp-2 cell cultures were infected with HSV-1(F) or mock infected, incubated in a medium containing [^{35}S]methionine at 18 h, and harvested at 24 h postinfection. The cell lysates were subjected to electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, transferred to nitrocellulose, and reacted with the R-4 antiserum. Lanes: 1 and 2, autoradiographic images of ^{35}S-labeled HSV-1(F) and mock (M)-infected cell lysates; 3 to 7, immune reactivity of electrophoretically separated polypeptides in lysates of infected and mock-infected cells (Ab). Lane 3, HSV-1(F)-infected cells; lane 4, mock-infected cells; lane 5, HSV-1(HFEM)-infected cells; lane 6, HSV-1(MP)-infected cells. Numbers to the left of the figure indicate ICP designations. The arrow indicates the polypeptide bands reactive with the R-4 antiserum.

be present in two copies per genome. To date, most γ genes whose function has been identified appear to specify structural components of the virion. It remains to be seen whether this is also the case for $\gamma_1$34.5.

(ii) We immunized rabbits with oligomers consisting of the three repeating amino acids repeated 5 and 10 times. Only the 10-mer induced antibody which reacted with a protein specified by HSV-1(F). That the antibody to the 10-mer reacted with a more rapidly migrating protein in cells infected with a virus mutant whose single copy of the gene was truncated by insertional mutagenesis is conclusive evidence that the open reading frame and its transcript detected previously specify a protein and that the antibody to the 10-mer reacts specifically with this protein. The failure of the antibody to react with the protein specified by HSV-1(MP) and HSV-1(HFEM) was unexpected but not surprising. The polyclonality of the antibody to three amino acids is likely to be somewhat limited. Inasmuch as the antisera made by two rabbits against the 5-mer failed to react with HSV-1(F) cell lysates, it is conceivable that the antisera to the 10-mer failed to react with the proteins of the two other HSV-1 viruses either because the number of the triplet repeats was smaller or because of an amino acid substitution.

(iii) $\gamma_1$34.5 is of special interest because its domain overlaps in part with the *a* sequence. As noted in the Introduction, the *a* sequence contains the *cis*-acting sites for cleavage and packaging of the genome and for inversion of L and S components of HSV DNA (4, 18, 27–29), and therefore the question arises whether $\gamma_1$34.5 acts on the *a* sequence as is the case for genes involved in site-specific recombination in many diverse systems. The studies described in this report indicate that $\gamma_1$34.5 is soluble and accumulates in both nucleus and cytoplasm late in infection. The results, therefore, support the conclusion that the open reading frame predicted by DNA sequencing analysis is expressed but is not sufficient to deduce the function of the product. Some understanding of the function of the $\gamma_1$34.5 gene may emerge from studies of recombinant R3615 and of other mutants in the $\gamma_1$34.5 gene (Chou and Roizman, in preparation).

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