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Use of a Bacterial Expression Vector to Identify the Gene Encoding a Major Core Protein of Vaccinia Virus

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The DNA sequence of a vaccinia virus late gene contains an open reading frame that corresponds to the 28,000-dalton (28K) polypeptide made by in vitro translation of hybrid-selected mRNA. To further characterize the protein product of this late gene, we cloned a segment of DNA containing part of the open reading frame into a bacterial expression vector. The fusion protein produced from this vector, containing 151 amino acids of the predicted vaccinia virus protein, was used to immunize rabbits. The resulting antiserum specifically bound to a major 25K structural protein that is localized in the core of vaccinia virions, as well as to a 28K protein found in infected cells. Pulse-chase experiments indicated that the 25K core protein is originally made as a 28K precursor.

Poxviruses are large double-stranded DNA viruses that replicate in the cytoplasm of infected cells (20a). Vaccinia virus, the best-characterized member of this family, has a genome of 185 kilobase pairs that codes for about 200 polypeptides. Because RNA polymerase and other enzymes are packaged within the infectious particle, de novo protein synthesis is not required for transcription of early genes, which begins almost immediately after virus entry into the cytoplasm. The expression of late genes begins about 4 h after infection but can be blocked by inhibition of early viral protein synthesis or viral DNA replication. Evidence that early and late genes are dispersed throughout the length of the genome was obtained by cell-free translation of mRNAs selected by hybridization to cloned genomic DNA fragments (2). More detailed analysis of specific regions of the genome has provided information regarding gene organization (1, 8, 9, 11, 18, 19, 23, 27, 33). Vaccinia virus genes are closely packed and contain continuous coding sequences (12, 17, 30). Functional studies have indicated that the promoter region is located upstream of the transcriptional initiation site (3, 5, 17, 24, 31) and may be only 30 to 40 base pairs (bp) long (6).

The identification of vaccinia virus genes is a complex task that requires a variety of approaches. When a selectable phenotype exists, such as for thymidine kinase (TK; 29) or DNA polymerase (14), mapping has been accomplished by marker rescue. A second method of gene localization relies on the synthesis, in a cell-free translational system, of a polypeptide with a characteristic enzyme activity. This approach was demonstrated with TK (12, 29). A third method involves the use of specific antiserum to identify a polypeptide synthesized in a cell-free system. The genes coding for the 11,000-molecular-weight (11K; 34), 62K (35), and 58K (35; J. Rosel and B. Moss, J. Virol., in press) core polypeptides were identified in this manner.

In this study, we used a fourth approach that does not depend on a phenotypic marker, enzyme activity, or the availability of specific antiserum. We inserted a vaccinia virus DNA segment, from a previously sequenced late gene encoding a 28K polypeptide (31), into an open-reading-frame bacterial expression vector (28). Antiserum, prepared against the fusion protein, was then used to identify the gene product as a major 25K component of the virus core. Formation of the latter protein involved the cleavage of a 28K precursor.

MATERIALS AND METHODS

Materials. Restriction endonucleases and other enzymes were obtained from Bethesda Research Laboratories, Inc., New England BioLabs, Inc., or Boehringer Mannheim Biochemicals. [^{35}S]methionine and ^{125}I-protein A were from Amersham Corp. Protein A-bearing *Staphylococcus aureus* was obtained from the Enzyme Center (Boston, Mass.) as IgGsorb.

Preparation of DNA. Recombinant plasmids were purified as described by Birnboim and Doly (4), and fragments were isolated from agarose gels by electrophoresis onto DEAE paper (32). Routine manipulations were performed as described by Maniatis et al. (20).

Purification of RNA, hybridization selection, and cell-free translation. Cytoplasmic RNA from vaccinia virus (strain WR)-infected HeLa cells was purified by CsCl gradient centrifugation as described previously (7). Early RNA was isolated 4 h after infection in the presence of 100 μg of cycloheximide per ml; late RNA was isolated 6 h after infection in the absence of drugs. The selection of mRNA by hybridization to plasmid DNA, immobilized on nitrocellulose filters, followed by cell-free translation has been described before (29, 31).

Preparation of pulse-labeled vaccinia virus-infected cell extracts. HeLa cells (500 ml; approximately 2.5×10^8 cells) were collected by centrifugation and resuspended in 10% of their original volume in Eagle suspension culture medium containing 5% horse serum. The cells were infected with a multiplicity of 30 PFU of vaccinia virus (strain WR). After 30 min at 37°C, infected cells were diluted with 450 ml of methionine-free medium containing 5% dialyzed horse serum. At 6 h postinfection, the cells were collected by centrifugation and suspended in 50 ml of methionine-free medium containing 0.5 mCi of [^{35}S]methionine. After a 20-min labeling period, the cells were sedimented and suspended in 500 ml of medium containing 10 times the normal methionine concentration of 15 mg/liter. A portion (25%) of the cell suspension was taken at this point and processed as the pulse-labeled extract. Chase extracts were prepared at 2, 6, and 18 h after labeling. To prepare extracts, cells were

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collected by centrifugation, suspended in 2.5 ml of cold 0.01 M Tris hydrochloride (pH 7.6)–0.01 M NaCl–1.5 mM $MgCl_2$, allowed to swell for 10 min, and Dounce homogenized. After centrifugation, the supernatant was frozen until use.

Immunoprecipitation. Samples for immunoprecipitation were solubilized by incubation at 37°C for 30 min in IP buffer (20 mM Tris hydrochloride [pH 8.1]–100 mM NaCl–1 mM EDTA–1% Nonidet P-40 [NP-40]–1% sodium deoxycholate [DOC]–0.1% sodium dodecyl sulfate [SDS])× 1.5. Preimmune serum was added, and the samples were mixed in a revolving tube shaker at 4°C for 6 to 12 h. Protein A-bearing *S. aureus* was added, and the incubation was continued for 30 min. After centrifugation, the supernatant was removed, and immune serum was added; the final IP concentration was 1×. The sample was incubated with immune serum for 6 to 12 h by revolving the tube at 4°C. Protein A precipitation of antigen-antibody complexes was done as with preimmune serum. The pellet was washed once with IP buffer containing 20 mg of bovine serum albumin per ml, once with IP buffer containing 1 M NaCl, twice with IP buffer, once with IP buffer without SDS or DOC, once with 0.4 M LiCl–2 M urea–10 mM Tris hydrochloride (pH 8.0), and once with IP buffer without SDS or DOC. Precipitated protein was eluted by boiling the pellet in loading buffer and run on an SDS-polyacrylamide gel (16).

Immunoblots. After SDS-polyacrylamide gel electrophoresis, proteins were electrophoretically transferred to nitrocellulose. Nitrocellulose blots were blocked as described by Johnson et al. (13), with the addition of 0.05% Tween 20 to the blocking solution, reacted with antiserum, and detected by reaction with ^{125}I-protein A.

Separation of viral envelope proteins from viral cores. Purified vaccinia virus was incubated for 30 min at 37°C in 50 mM Tris hydrochloride (pH 8.6)–0.5% NP-40–50 mM dithiothreitol as described by Katz and Moss (15). After sonication, the mixture was centrifuged, and the supernatant was removed. The pellet was resuspended in the same buffer, and the incubation followed by centrifugation was repeated. The supernatants were pooled as the NP-40 soluble fraction, and the pellet was solubilized in 100 mM Tris hydrochloride (pH 8.6)–3% SDS–5% β-mercaptoethanol.

RESULTS

Comigration of the 28K polypeptide with a late protein that is proteolytically processed. Previous studies (31) indicated that a late mRNA encoding a 28K polypeptide is initiated within the *Hin*dIII L fragment and continues into the adjacent *Hin*dIII J fragment overlapping the TK gene. Because the directions of transcription of these two genes are the same, mRNAs for both can be selected by hybridization to a single-stranded DNA segment obtained by cloning a 770-bp *Hin*dIII-*Eco*RI fragment into phage M13. The cell-free translation experiment shown in Fig. 1 demonstrated that the 28K polypeptide is the product of a stringently regulated late gene and confirmed that the TK polypeptide is the product of an early gene. Translatable mRNA for the 28K polypeptide was not present at 2 h after infection or overexpressed in the presence of cycloheximide, as was TK mRNA. Maximal amounts of translatable 28K mRNA were present at 4 to 6 h after infection. Although the majority of structural proteins are products of late genes, the 28K polypeptide did not correspond in electrophoretic mobility to any of the major polypeptides obtained by dissociation of [^{35}S]methionine-labeled virus particles (Fig. 1).

We considered that the 28K polypeptide might be a late nonstructural protein or the precursor to one of the structural proteins. To evaluate the latter possibility, we compared the 28K in vitro translation product with polypeptides pulse-labeled with [^{35}S]methionine 6 h after infection. We noted a prominent polypeptide that comigrated with the 28K product (Fig. 2, lanes 1 and 2). During the chase period, the 28K polypeptide greatly diminished in quantity, as did several other known structural protein precursors (e.g., P4A and P4B; Fig. 2, lanes 3 to 5). Several proteins which appeared during the chase period were smaller than 28K and consequently could be proteolytic products. Of the latter, the one closest in size to the 28K polypeptide was a 25K protein (Fig. 2, lanes 3 to 5). Furthermore, a major 25K protein was present in purified virions (Fig. 2, lane 6).

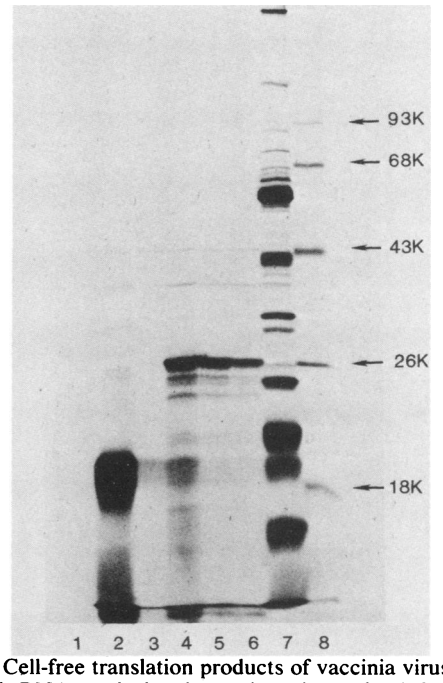

FIG. 1. Cell-free translation products of vaccinia virus mRNAs. Cytoplasmic RNA was isolated at various times after infection in the presence or absence of 100 μg of cycloheximide per ml and selected by hybridization to a cloned subfragment of *Hin*dIII-J. A 770-bp *Hin*dIII-*Eco*RI fragment at the left-hand end of the *Hin*dIII J fragment was cloned in phage M13mp8, and the single-stranded DNA was immobilized on a nitrocellulose filter and used to select vaccinia virus mRNA. Selected mRNAs were eluted and translated in a reticulocyte cell-free system containing [^{35}S]methionine. The labeled products were analyzed by SDS-polyacrylamide gel electrophoresis (16) and autoradiographed. Lanes and their translation products are as follows. Lane 1, no RNA; lane 2, 4-h RNA (cycloheximide present); lane 3, 2-h RNA; lane 4, 4-h RNA; lane 5, 6-h RNA; and lane 6, 8-h RNA. Lane 7 shows SDS-solubilized vaccinia virus, and lane 8 has molecular weight markers.

Synthesis of a fusion protein containing part of the 28K polypeptide. Standard methods of determining whether the 28K product of the late gene is related to a smaller structural protein involve purification of the 28K protein and its putative product. Because the late 28K polypeptide gene had already been sequenced (31), we considered the following alternative. Antiserum directed against either an oligopeptide of predicted sequence or a fusion protein made by a bacterial expression vector could be used to identify related proteins synthesized in vivo. We chose the latter method, because the antibodies would be directed against a wide range of epitopes likely to be present in both the precursor and product.

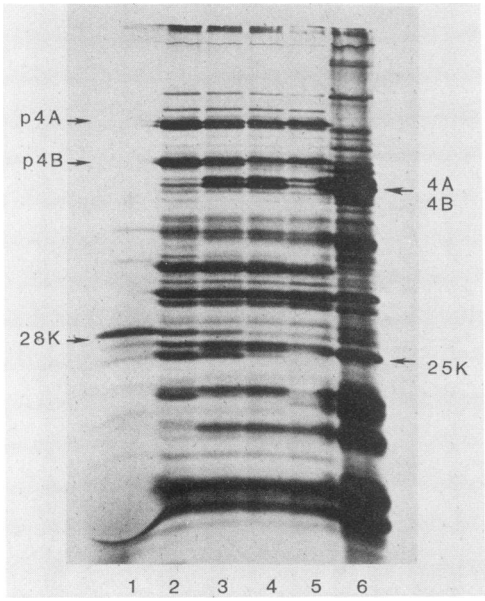

FIG. 2. Analysis of labeled vaccinia virus polypeptides at different times after pulse-labeling of infected cells. HeLa cells in methionine-free medium were pulse-labeled with [^{35}S]methionine for 20 min at 6 h after infection. Unlabeled methionine was added to 10 times the normal concentration of 15 mg/liter. Samples were taken for analysis immediately (lane 2), 2 h (lane 3), 6 h (lane 4), and 18 h (lane 5) after pulse-labeling. Lane 1 shows the cell-free translation products of vaccinia virus mRNA selected by hybridization to the left 770 bp of the *Hin*dIII J fragment. Lane 6 shows SDS-solubilized vaccinia virus.

The nucleotide and predicted amino acid sequence of the 28K gene are shown in Fig. 3. A bacteriophage M13 recombinant, used for sequencing the gene, contains a 450-bp DNA fragment entirely within the open reading frame of the 28K gene. This M13 clone, mp9-L4, which extends from the *Taq*I site at position 26 to the *Sal*I site at position 477 (Fig. 3), contains the DNA sequence that codes for the predicted amino acids 10 through 160. The strategy used to clone this segment of DNA into bacterial expression vector pORF1 (28) is shown in Fig. 4. Plasmid vector pORF1 contains the 5′ end of *ompF*, an *Escherichia coli* gene encoding an abundant outer membrane protein, coupled to the *lacZ* gene of *E. coli* which codes for β-galactosidase. Several restriction sites separate the two components of the vector, such that *lacZ* is out of frame with respect to *ompF*. By the insertion of a piece of DNA with an open reading frame of the correct length, all three DNA segments can become aligned in frame. The result is a *lacZ* gene that produces a tribrid protein with the translation product of the inserted DNA of interest sandwiched between that of *ompF* and *lacZ*.

The replicative form of recombinant phage mp9-L4 was digested with *Sma*I and *Pst*I, and the 3′ overhang at the *Pst*I site was removed by treatment with T4 DNA polymerase. The resultant blunt-ended fragment was purified and cloned into the *Sma*I-digested pORF1 in the two possible orientations. From the known DNA sequence of pORF1 and mp9-L4, we considered that the correctly oriented insert would produce a tribrid fusion protein with β-galactosidase activity. In agreement with this prediction, approximately half of the colonies were *lacZ*$^+$ as determined by the appearance of blue colonies on plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal).

Polyacrylamide gel electrophoresis was used to show that the plasmid produced significant amounts of the expected fusion protein. A high-molecular-weight polypeptide was produced in *E. coli* cells harboring this plasmid (Fig. 5, lane 3) which was not present in *E. coli* extracts containing pORF1 (Fig. 5, lane 1). The tribrid protein was also larger than the dibrid protein produced by fusing *ompF* in frame with the *lacZ* coding region (Fig. 5, lane 2). (The latter fusion was produced by *Bam*HI digestion of pORF1 followed by religation [28].) Because the fusion protein migrated in SDS-polyacrylamide gels in a region in which there were few other *E. coli* proteins, it was excised from a preparative polyacrylamide gel, and the crushed gel slice was used to immunize rabbits.

Identification of the 28K protein as a precursor to a viral core protein. The ability of the antiserum prepared against the tribrid fusion protein to immunoprecipitate an [^{35}S]methionine pulse-labeled protein of 28K is shown in Fig. 6. Although other viral proteins could not be completely removed by washing, the specific enrichment of the 28K polypeptide is evident by comparison with the total pulse-labeled proteins (Fig. 6). Similarly, a marked enrichment of a 25K polypeptide occurred when extracts of pulse-labeled cells that had been chased with unlabeled methionine were immunoprecipitated (Fig. 6).

An immunoblotting procedure was used to determine whether the 25K protein of purified virions reacted with the antiserum. The unlabeled virion preparation was solubilized by boiling in the presence of SDS and mercaptoethanol, and the viral proteins were resolved by polyacrylamide gel electrophoresis. After electrophoretic transfer to a nitrocellulose membrane, the immobilized polypeptides were incubated with antiserum and ^{125}I-staphylococcal A protein in succession. A single 25K polypeptide was detected by autoradiography (Fig. 7). The 25K polypeptide also was the major band obtained by immunoprecipitation of dissociated virions (Fig. 8B).

The 25K protein was further localized by analyzing [^{35}S]methionine-labeled virions that had been incubated with NP-40 and mercaptoethanol to separate the soluble envelope fraction from the insoluble core. Polyacrylamide gel electrophoresis indicated that the 25K polypeptide was associated predominantly with the core fraction (Fig. 8A).

DISCUSSION

In this report, a vaccinia virus gene that is expressed only after viral DNA replication was shown to code for one of the major core proteins of the virus. This gene was originally mapped and sequenced during an investigation into the regulation of vaccinia virus late gene expression (31). That work focused on the isolation of a representative late gene and showed that the DNA immediately upstream of the transcriptional start site contains *cis*-acting regulatory sequences. Translation of hybrid-selected mRNA revealed that the in vitro translation product of this gene was approximately 28K, but no other information regarding the identity of the encoded polypeptide was available. In preliminary efforts to identify the 28K protein, no major virion protein of corresponding size was detected. However, the in vitro-synthesized 28K protein comigrated with a pulse-labeled polypeptide from infected cells that disappeared during a chase. Because two major structural proteins, designated 4a and 4b, with molecular weights of about 62,000 and 58,000, respectively, were shown to be formed by proteolytic cleav-

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                                TAQ1
                                                                                           60                                                          90
ATG AGT CTA CTG CTA GAA AAC CTC ATC GAA GAA GAT ACC ATA TTT TTT GCA GGA AGT ATA TCT GAG TAT GAT GAT TTA CAA ATG GTT ATT
MET SER LEU LEU LEU GLU ASN LEU ILE GLU GLU ASP THR ILE PHE PHE ALA GLY SER ILE SER GLU TYR ASP ASP LEU GLN MET VAL ILE

                                    120                                     150                                     180
GCC GGC GCA AAA TCC AAA TTT CCA AGA TCT ATG CTT TCT ATT TTT AAT ATA GTA CCT AGA ACG ATG TCA AAA TAT GAG TTG GAG TTG ATT
ALA GLY ALA LYS SER LYS PHE PRO ARG SER MET LEU SER ILE PHE ASN ILE VAL PRO ARG THR MET SER LYS TYR GLU LEU GLU LEU ILE

                                    210                                     240                                     270
CAT AAC GAA AAT ATC ACA GGA GCA ATG TTT ACC ACA ATG TAT AAT ATA AGA AAC AAT TTG GGT CTA GGA GAT GAT AAA CTA ACT ATT GAA
HIS ASN GLU ASN ILE THR GLY ALA MET PHE THR THR MET TYR ASN ILE ARG ASN ASN LEU GLY LEU GLY ASP ASP LYS LEU THR ILE GLU

                                    300                                     330                                     360
GCC ATT GAA AAC TAT TTC TTG GAT CCT AAC AAT GAA GTT ATG CCT CTT ATT ATT AAT AAT ACG GAT ATG ACT GCC GTC ATT CCT AAA AAA
ALA ILE GLU ASN TYR PHE LEU ASP PRO ASN ASN GLU VAL MET PRO LEU ILE ILE ASN ASN THR ASP MET THR ALA VAL ILE PRO LYS LYS

                                    390                                     420                                     450
AGT GGT AGG AGA AAG AAT AAG AAC ATG GTT ATC TTC CGT CAA GGA TCA TCA CCT ATC TTG TGT ATT TTC CAA ACT CGT AAA AAG ATT AAT
SER GLY ARG ARG LYS ASN LYS ASN MET VAL ILE PHE ARG GLN GLY SER SER PRO ILE LEU CYS ILE PHE GLN THR ARG LYS LYS ILE ASN

                                    SAL1                                    510                                     540
ATT TAT AAA GAA AAT ATG GAA TCC GCG TCG ACT GAG TAT ACA CCT ATC GGA GAC AAC AAG GCT TTG ATA TCT AAA TAT GCG GGA ATT AAT
ILE TYR LYS GLU ASN MET GLU SER ALA SER THR GLU TYR THR PRO ILE GLY ASP ASN LYS ALA LEU ILE SER LYS TYR ALA GLY ILE ASN

                                    570                                     600                                     630
ATC CTA AAT GTG TAT TCT CCT TCC ACA TCC ATA AGA TTG AAT GCC ATT TAC GGA TTC ACC AAT AAA AAT AAA CTA GAG AAA CTT AGT ACT
ILE LEU ASN VAL TYR SER PRO SER THR SER ILE ARG LEU ASN ALA ILE TYR GLY PHE THR ASN LYS ASN LYS LEU GLU LYS LEU SER THR

                                    660                                     690                                     720
AAT AAG GAA CTA GAA TCG TAT AGT TCT AGC CCT CTT CAA GAA CCC ATT AGG TTA AAT GAT TTT CTG GGA CTA TTG GAA TGT GTT AAA AAG
ASN LYS GLU LEU GLU SER TYR SER SER SER PRO LEU GLN GLU PRO ILE ARG LEU ASN ASP PHE LEU GLY LEU LEU GLU CYS VAL LYS LYS

                                    750
AAT ATT CCT CTA ACA GAT ATT CCG ACA AAG GAT TGA
ASN ILE PRO LEU THR ASP ILE PRO THR LYS ASP END
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FIG. 3. Predicted amino acid sequence of the 28K gene. The location of the *Taq*I and *Sal*I sites, which form the boundary of the open-reading DNA segment used to make clone mp9-L4, are shown.

age (21), we considered the possibility that the 28K polypeptide is a precursor to another protein formed in the same manner. The putative proteolytic cleavage products that increased in amount during the chase included one of 25K.

To identify the processed 28K gene product, we prepared a specific antiserum. This was accomplished by expressing the gene in *E. coli* and using the recombinant-derived protein as an immunogen. A bacterial expression vector, pORF1

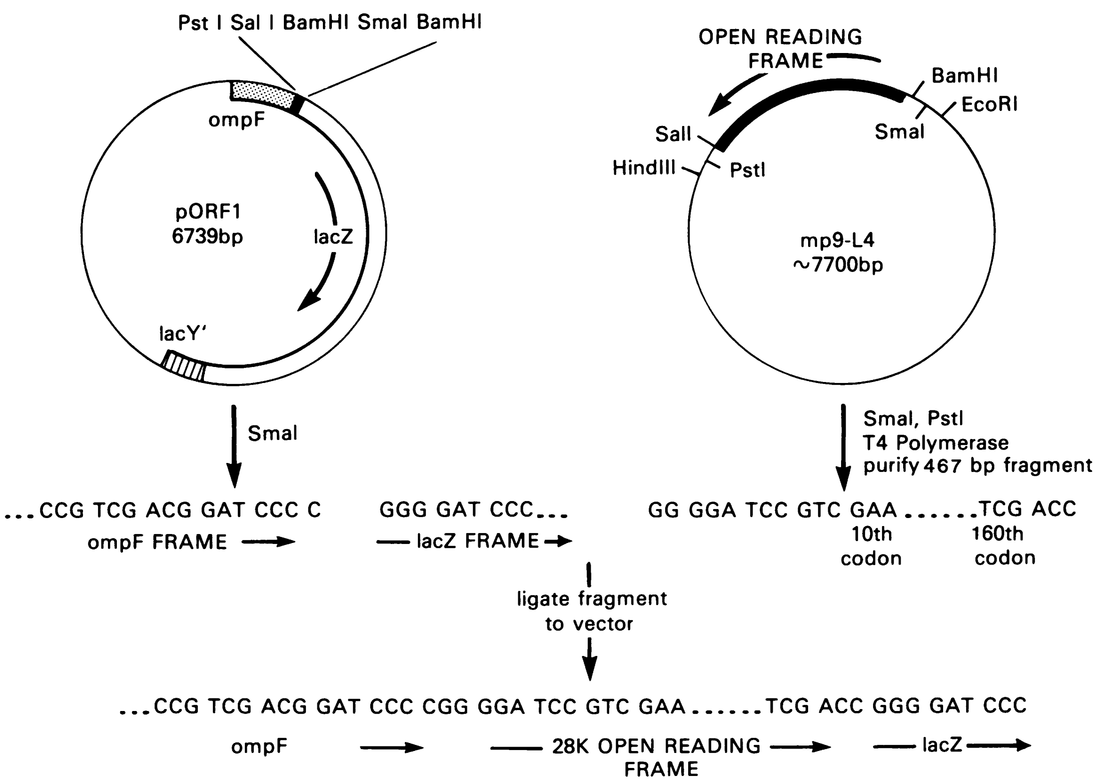

FIG. 4. Construction of a vector that expresses part of the vaccinia virus 28K protein in *E. coli*. *E. coli* plasmid expression vector pORF1 (28) was used to construct a tribrid fusion protein containing 151 amino acids of the 28K protein. Clone mp9-L4, containing 453 bp of the open reading frame for the 28K gene, was digested with *Sma*I and *Pst*I. The *Pst*I overhang was filled in with nucleotides by using T4 DNA polymerase, and the 467-bp fragment was cloned into pORF1 cleaved with *Sma*I.

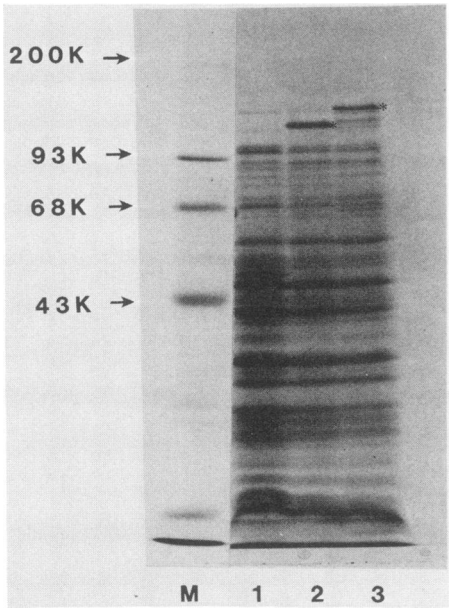

FIG. 5. Expression of the vaccinia virus 28K protein as a fusion protein. Cells were grown in L broth at 30°C to an A_{600} of 0.2, and the temperature was then raised to 40°C for 2 h. Whole-cell extracts were subjected to SDS-polyacrylamide gel electrophoresis and stained with Coomassie blue. Protein molecular weight standards are shown in lane M. Lane 1, Extract of cells containing pORF1; lane 2, extract of cells containing a pORF1 modified such that *ompF* and *lacZ* are in frame; lane 3, extract of cells containing the vector that expresses the tribrid 28K fusion protein. The fusion proteins are indicated by asterisks.

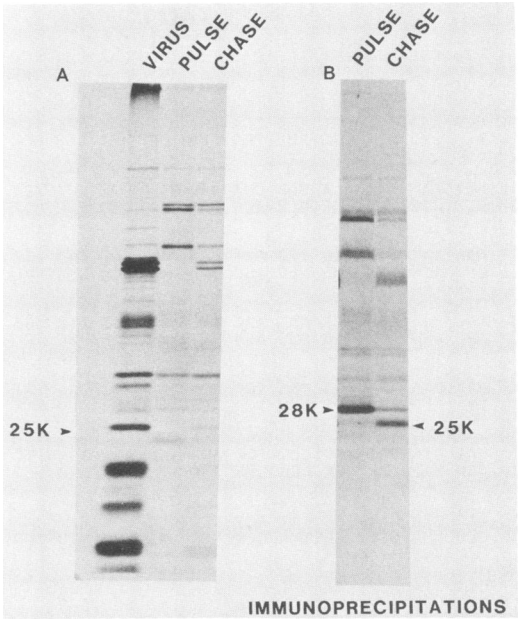

FIG. 6. Immunoprecipitation of precursor and product proteins with fusion protein antisera. Vaccinia virus-infected cells were pulse-labeled with [^{35}S]methionine and chased with unlabeled methionine as described in the legend to Fig. 2. Total (panel A) and immunoprecipitated (panel B) extracts were electrophoresed on a 15% SDS-polyacrylamide gel. [^{35}S]methionine-labeled virion polypeptides are on the extreme left. An autoradiograph is shown.

(28), was used. This vector expresses an open reading frame sandwiched between *ompF* and the gene coding for β-galactosidase as a tribrid fusion protein. Previous work with a fragment from the herpes simplex virus TK gene showed that antibodies raised to such a tribrid protein would precipitate TK from virus-infected cells (28). Open-reading-frame vectors also have been used to construct expression libraries with randomly digested DNA. In the latter case, the libraries were screened with antibodies to known proteins. This method has been used to map the varicella-zoster virus glycoprotein C gene (10). In our case, the reverse strategy was used, i.e., the identification of the product of a cloned gene for which no antiserum was available.

The segment of the 28K gene inserted into pORF1 contained a 151-amino-acid open reading frame. Expression of the fusion protein was demonstrated by polyacrylamide gel electrophoresis, and crushed gel slices were used to immunize rabbits. As anticipated, the antiserum immunoprecipitated a pulse-labeled polypeptide of 28K. Significantly, however, a 25K polypeptide that formed during the chase also was immunoprecipitated. Moreover, this 25K polypeptide was the only one labeled in immunoblots of purified virions.

Because the previously described vaccinia virus proteins that are proteolytically processed are within the core structure (21), the location of the 28K protein was examined. As previously noted (22, 25), about five major polypeptides presumed to be envelope components were solublized when labeled virions were incubated with NP-40 and dithiothreitol. The insoluble proteins included at least six major species, of which 4a and 4b, with molecular weights of about 62,000 and 58,000, were most prominent. The genes coding for the precursors of these two core polypeptides have

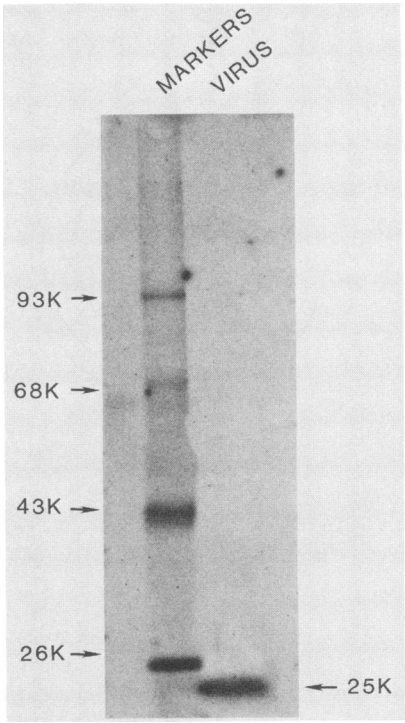

FIG. 7. Immunoblot detection of the 28K gene product in SDS-solubilized vaccinia virus. ^{14}C-labeled protein markers were run alongside purified vaccinia virus, dissociated by boiling in gel loading buffer (16), and run on a 15% SDS-polyacrylamide gel. Proteins were transferred to nitrocellulose and reacted with antiserum made to the 28K fusion polypeptide. An autoradiograph is shown.

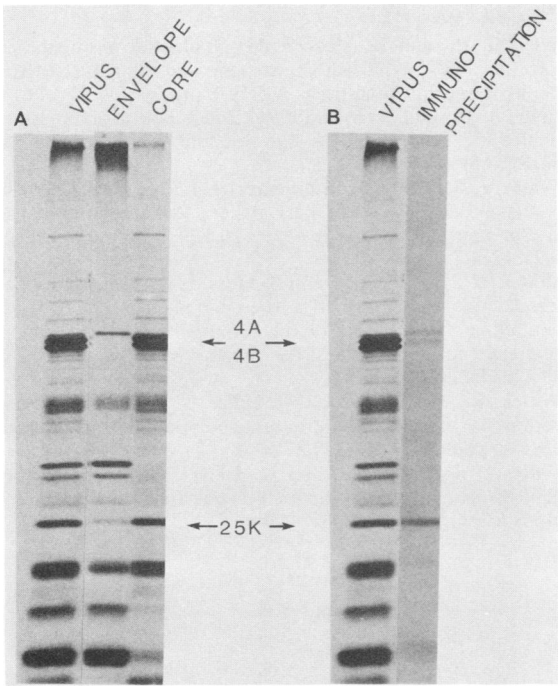

FIG. 8. Identification of the 28K gene product as a component of the viral core. Purified [^{35}S]methionine-labeled vaccinia virus was separated into envelope and core fractions by extraction with NP-40 and dithiothreitol. After SDS treatment, the polypeptides were run on a 15% SDS-polyacrylamide gel (panel A). Immunoprecipitation of dissociated viral proteins with the antiserum to the 28K fusion protein is shown in panel B. Autoradiographs are shown.

recently been mapped within the 50-kb *Hin*dIII A fragment (35; Rosel and Moss, in press). The 25K protein also was assigned to the core fraction based on detergent extraction. The present mapping of the gene encoding the 25K protein precursor within the *Hin*dIII L fragment indicates that the core polypeptide genes are not closely linked.

Proteolytic processing of vaccinia virus proteins appears to be coupled to virion maturation, because drugs that prevent assembly, such as rifampin, also prevent proteolysis (21). Further evidence for coordination of these events has been obtained with temperature-sensitive mutants of vaccinia virus (26). The availability of the nucleotide sequence for the genes encoding precursors to two core proteins (31; Rosel and Moss, in press) may make it possible to determine the signals for processing.

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