Identification, Sequence, and Expression of the Gene Encoding a M, 35,000 Subunit of the Vaccinia Virus DNA-dependent RNA Polymerase*

Bernard Y. Amegadzie, Byung-Yoon Ahn, and Bernard Moss

From the Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby indicated this fact.

The gene rpo35, encoding a subunit of the vaccinia virus DNA-dependent RNA polymerase, was identified, and its RNA and protein products were characterized. An M, 35,000 polypeptide, which bound antibody to the purified RNA polymerase, was synthesized in reticulocyte lysates programmed with viral mRNA that hybridized to a 2,300-base pair segment of the viral genome. Determination of the sequence of the DNA segment revealed four potential protein coding regions, none of which had evident similarity to any described RNA polymerase subunit of prokaryotes or eukaryotes. One open reading frame that could encode a 35,400-Da protein was identified as rpo35 on the basis of mRNA hybridization, cell-free translation, and immunoprecipitation. The identification was confirmed by sequencing tryptic peptides of the authentic M, 35,000 RNA polymerase subunit. Antiserum to the purified recombinant protein, expressed in bacteria, reacted specifically with a M, 35,000 polypeptide that was detected starting 2 h after virus infection and that co-sedimented with RNA polymerase purified from virions. RNA analyses indicated that the 5'-end of an early transcript started 25 nucleotides upstream of rpo35, which is consistent with the location of an early promoter consensus sequence.

Most DNA viruses replicate in the cell nucleus, where they make extensive use of the host transcription apparatus. The poxviruses, of which vaccinia virus is the prototype, are exceptions in that they replicate in the cytoplasm and encode many, if not all, of the enzymes needed for DNA and RNA synthesis (reviewed in Ref. 1). Indeed, the possession of a multisubunit RNA polymerase, which we named rpo35, on the vaccinia virus (strain WR) genome (7). The 14.2-kbp BamHI insert of pA64 was digested with SalI, and the 6.0-kbp subfragment was detected starting 2 h after virus infection and that co-sedimented with RNA polymerase purified from virions. RNA analyses indicated that the 5'-end of an early transcript started 25 nucleotides upstream of rpo35, which is consistent with the location of an early promoter consensus sequence.

EXPERIMENTAL PROCEDURES

Virus and Cells—Vaccinia virus (WR strain) stocks were prepared in HeLa spinner cells that were propagated in minimum essential spinner medium (Quality Biologics Inc.) supplemented with 5% horse serum.

Plasmid Constructions—Standard methods (1) were used for isolation and construction of plasmids; restriction endonucleases and other DNA-modifying enzymes were usually from Boehringer Mannheim. Generally, the reaction conditions of the vendor were used for restriction endonuclease digestions. The pUC19-derived plasmids pA34, pA25, pA81, pA62, pA64, pA13, pA821, and pA32 contain segments derived by BamHI digestion of the HindIIIA fragment of the vaccinia virus strain WR genome (4), and analyses described here localized it to a 2.3-kbp SstI-SalI subsegment. Four complete ORFs, of which only one could encode a protein close to the size of the M, 35,000 subunit, were found upon sequencing the fragment. Further studies correlated the latter ORF with the RNA polymerase subunit gene, which we named rpo35.

* The abbreviation used are: kbp, kilobase pair; A, C, H, T, and U, adenine, cytosine; thymine; and uracil, respectively; E. coli, Escherichia coli; nuclease, endonuclease; GS, glycerol stock; BP, base pair; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; WR, Western Reserve.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) M61187.

1 The abbreviations used are: kbp, kilobase pair; AraC, cytosine arabinoside; bp, base pair; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; WR, Western Reserve.

13712
of pBA0.5s/h was digested with either SstI or Ssfl and Ssfl and then cloned into the corresponding sites of pGEM3zf (+) to produce pBA2s/sst and pBA4sst. The plasmids pBA1.4s/b, pBA6s, pBA6.6s/b, pBA2s/sst, and pBA4sst were used for hybridization and selection of viral mRNA.

For expression of rpo35 in Escherichia coli, the plasmid PET3c35 was linearized by an SphI site and the resulting chain reaction was used to isolate ND1-1 and BamHI restriction sites at the 5' and the 3'-ends of the rpo35 ORF, respectively (13), and the amplified DNA was ligated with the Ndcl- and BamHI-digested PET3c vector (14).

Plasmid pBA0.5s/h, used for preparation of an RNA probe for nucleic S1 analysis, was constructed as follows. Two oligonucleotides, RP1 (GTCGACTTTTTGGAACGATTG) and RP2 (AAGCTT GTAGCCATTCTCTTGAGCAGCG), containing restriction endonuclease SalI and HindIII sites, respectively, were used as polymerase chain reaction primers to amplify a 500-bp fragment. The amplified DNA product was digested with SalI and HindIII restriction endonucleases and then cloned into a pGEM3zf (+) vector.

DNA Sequencing—Overlapping fragments of DNA were inserted into the phagemid vector pGEM3zf (Promega Biotech) in both orientations for complete sequencing of the two DNA strands. Single-stranded phage DNA templates were prepared and sequenced by the dideoxynucleotide chain termination method (15), using synthetic oligonucleotide primers and Sequenase kits purchased from U.S. Biochemical Corp. Microgenie (Beckman) and FASTA (16) computer programs were used for manipulations of the DNA sequence and protein homology searches, respectively.

Expression of the 35-kDa Protein in E. coli and Preparation of Antiserum—Single colonies, from fresh plates containing E. coli strain BL21 (DE3) that had been transformed with the PET3c plasmid vector (14) or with PETc35, the vector containing the ORF encoding the 35-kDa protein, were inoculated into 1 ml of L broth containing ampicillin, and after 2 h at 37°C, isopropyl-β-thiogalactoside was added. The bacteria were incubated for an additional 2 h, collected by centrifugation, resuspended in 100 μl of lysis buffer (50 mM Tris-HCl, pH 8.6, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue), and boiled for 5 min. The supernatants were then centrifuged for 5 min, and 20 μl of the supernatant proteins were resolved by SDS-PAGE on a 12% gel. For purification of the 35-kDa protein, larger preparations were made and the appropriate gel band was cut out. The protein was eluted by extracting the gel slices twice with 25 ml of sterile distilled water and then concentrated to 1 ml. The protein was injected into New Zealand White rabbits in Freund's complete adjuvant. A boost with antigen in incomplete adjuvant was given 2 weeks after the first injection.

Purification of RNA Polymerase from Vaccinia Virions—Vaccinia virions were purified twice by sucrose density gradient centrifugation, and then the RNA polymerase and one of the proteins was prepared essentially as described (3). The RNA polymerase was further purified by successive chromatography on columns of DEAE-cellulose, heparin agarose, single-strand DNA agarose, and phosphocellulose. The enzyme activity was assayed using single-stranded DNA of bacteriophage M13 mp18 as template (9). The purity of the enzyme, examined by SDS-PAGE and silver-staining of the proteins, was comparable with that previously described (3). A 10-μg sample of pure enzyme was subjected to glycerol density gradient centrifugation, and the resultant fractions were used for immunoprecipitation of the rpo35 protein, as described (9). For the preparation of enzyme used in protein sequence analysis, small volumes of heparin agarose and single-strand DNA agarose were replaced by DEAE-cellulose and Bio-Gel A-1.5m.

Protein Sequence Analysis—Purified vaccinia virus RNA polymerase (200 μg, approximately 100 μg) was resolved into subunits by SDS-PAGE. The polypeptides were transferred to nitrocellulose and visualized by staining with 0.1% Ponceau S as described by Aebersold et al. (18). The strip containing the band of approximately M, 35,000 was excised for internal protein sequencing under the direction of William Lane of the Harvard Microchemistry Facility. The excised band was subjected to in situ tryptic digestion, and the resulting peptides were separated by microbore reverse-phase high pressure liquid chromatography. The peptide-containing fractions were collected based on the UV absorption at 210 nm, and appropriate fractions were subjected to a gas-phase protein sequencer (ABI model 470A) connected with the 120A on-line phenylthiohydantoin-derivative analyzer.

Analysis of Proteins from Vaccinia Virus-Insect Cells—HeLa cells were infected with vaccinia virus at a multiplicity of 15 plaqueforming units/cell. The cells were harvested at various times after infection, resuspended in 4 volumes of 100 mM Tris-HCl, pH 8, 10 mM NaCl, 0.5% Nonidet P-40 buffer. The suspensions were mixed by vortexing and were centrifuged in a microcentrifuge. The clear supernatants were heated with SDS and mercaptoethanol and resolved by PAGE. The proteins were transferred onto a nitrocellulose membrane and incubated with antiserum, followed by 125I-labeled protein A.

RNA Analyses—RNA, from cells infected for 4 h in the presence of cycloheximide or 7 h in the presence or absence of AraC, was isolated by the RNAol (Cnna/Biotex) method. For primer extension analysis, 20 pmol of the primer was 5'-end-labeled with polynucleotide kinase and [γ-32P]ATP and annealed to 20 μg of either early or late RNA, followed by extension with avian myeloblastosis virus reverse transcriptase, as described (12). Internally labeled [α-32P]UTP complementary RNAs were synthesized and used for nucleic SI analysis, as described (34). The plasmid pBA0.5s/h was cleaved at the SalI site and transcribed using SP6 RNA polymerase in the presence of [α-32P]UTP to produce a 500-nucleotide transcript, that was used as an RNA probe for 5' end mapping. Protected segments were then analyzed by PAGE on a 6% gel.

RESULTS

Localization of the RNA Polymerase M, 35,000 Subunit Gene—The gene encoding an RNA polymerase subunit of approximately M, 35,000 was previously mapped to the 45-kbp HindIII A fragment of the vaccinia virus genome (4). The localization was achieved using antisera, raised to purified vaccinia virus RNA polymerase, for immunoprecipitation of in vitro translation products of early viral mRNAs that were selected by hybridization to immobilized, cloned genomic DNA fragments. To further localize the gene encoding this polypeptide, mRNA from cells infected with vaccinia virus in the presence of cycloheximide, a protein synthesis inhibitor that enhances ribonuclease A digestion, was hybridized to a panel of cloned restriction endonuclease fragments derived from the HindIII A segment (Fig. 1B). Following previous procedures (4), the hybridized mRNA was isolated and then translated in a micrococal nuclease-treated reticulocyte lysate containing [35S]methionine. The labeled proteins were incubated with antiserum to RNA polymerase, bound to staphylococcal A protein attached to beads, and analyzed by PAGE. As shown by the autoradiograph in Fig. 1A, an immunoprecipitable polypeptide of about M, 35,000 was made only in lysates that were programmed with mRNAs that hybridized to plasmid pA64, which contained a 14.2-kbp BamHI genome fragment.

Additional mRNA hybridizations and cell-free translations, using smaller DNA fragments, were needed to further localize the RNA polymerase gene. The 14.2-kbp BamHI fragment was subdivided by cleavage with Sall, and the estimated 6.6-kbp BamHI/Sall, 6.0-kbp Sall, and 1.4-kbp Sall/BamHI segments were cloned (Fig. 2C). The mRNAs that hybridized to these plasmids were translated in vitro, and the labeled proteins were immunoprecipitated and analyzed by SDS-PAGE (Fig. 2C). The mRNAs that hybridized to the 3.5-kbp fragment (Fig. 2A) were used to select early mRNAs. The immunoprecipitable M, 35,000 polypeptide was synthesized.
Vaccinia Virus RNA Polymerase Subunit

**Fig. 1.** Localization of the gene encoding the M, 35,000 subunit of vaccinia virus RNA polymerase. A, cytoplasmic RNA, from HeLa cells that were infected with vaccinia virus in the presence of cycloheximide, was hybridized to plasmids containing cloned vaccinia virus DNA fragments immobilized on nitrocellulose filters. The RNA was eluted and then translated in a micrococcum nucleate-treated rabbit reticulocyte lysate in the presence of "S)methionine. The translation products were incubated successively with antiserum to purified vaccinia virus RNA polymerase and beads containing staphylococcal A protein. The bound proteins were eluted with SDS and pA81, pA62, pA64, pA13, pA821, and pA32. The p has been omitted translation products of RNA that hybridized to plasmids pA34, pA25, rabbit reticulocyte lysate in the presence of "S)methionine. The diograph is shown. Lane M contains "C-labeled protein markers with kilodaltons indicated on the left. Lane E contains the immunoprecipitated translation products of the total early RNA from the cytoplasm of infected cells. The remaining lanes contain immunoprecipitated translation products of RNA that hybridized to plasmids pA34, pA25, pA81, pA62, pA64, pA13, pA821, and pA32. The p has been omitted and 821 has been shortened to 21 to save space. ORFs and 97 and 96% identity for the 16.3- and 12.3-kDa reading frames with 99% identity for the 8.7- and 35.4-kDa frame, which was calculated to produce a polypeptide of 35.4 kDa. In addition, the SstI site interrupts an ORF of 25 kDa (data not shown). The 12.3-kDa ORF differed by 5 nucleotides from a previously identified vaccinia virus WR fusion protein gene (19). Subsequent to our collection of the above data for the WR strain of vaccinia virus, the genomic sequence of the Copenhagen strain of vaccinia virus was reported (20), allowing us to make a comparison. An alignment with the region described here indicated the same open reading frames with 99% identity for the 8.7- and 35.4-kDa ORFs and 97% identity for the 16.3- and 12.3-kDa ORFs, respectively. Capripoxvirus sequences corresponded to a part of the 35.4-kDa ORF and the complete 16.3- and 12.3-kDa ORFs (21) were 63, 50, and 37% identical with that of vaccinia WR, respectively, which is consistent with the relationships between members of different poxvirus genera.

Searches of GenBank (volume 65) revealed no similarities of polypeptides encoded within the 2.3-kbp fragment of vaccinia virus DNA with previously sequenced RNA polymerase subunits of poxviruses or eukaryotes. Based on size, however, only in the reticulocyte lysate that had been programmed with RNA that hybridized to the 2.3-kbp fragment (Fig. 2B).

**Fig. 2.** Fine mapping of the RNA polymerase subunit gene. A and B, the translation products of early vaccinia virus mRNAs that hybridized to filters containing cloned subfragments of pA64 were bound to RNA polymerase antiserum and analyzed by PAGE using procedures similar to those described in the legend to Fig. 1, except that the gel was 15% polyacrylamide. Autoradiographs are shown. M, C-labeled marker proteins in kDa; T, translation products of total early RNA from the cytoplasm of infected cells. Lanes 1-5 refer to the DNA fragment number, indicated in panel C, used to select mRNAs by hybridization. In B, + and + refer to the use of preimmune and immune antisera, respectively. C, a partial restriction map of the 14.2-kbp BamHI fragment from pA64. The numbered bars indicate the cloned fragments present in plasmids that were bound to filters and used to select mRNA by hybridization. Restriction endonuclease sites are abbreviated: B, BamHI; S, SstI, S, SalI.

The ORF that was calculated to produce a polypeptide of 35.4 kDa appeared likely to encode the M, 35,000 RNA polymerase subunit.

**Evidence That the Product of the 35.4-kDa ORF Is an RNA Polymerase Subunit**—The protein, encoded by the 35.4-kDa ORF, was expressed in bacteria in order to prepare a specific antibody that could be used to provide an alternative approach to the identification of the RNA polymerase subunit gene, as well as a reagent for expression studies. E. coli BL21(DE3) was transformed with plasmid pET3c35, which was made by inserting the 35.4-kDa ORF next to the bacteriophage T7 ph10 promoter in the vector pET3c (14). Bacteria transformed with the vector alone were also isolated to serve as a control. A prominent M, 35,000 band was resolved by SDS-PAGE and detected by Coomassie Blue staining of proteins from pET3c35- but not pET3c-transformed E. coli. After transfer to a nitrocellulose membrane, the polypeptides bound antibody raised to the RNA polymerase purified from vaccinia virions. The recombinant M, 35,000 protein was therefore purified by PAGE and used to immunize a rabbit. A preliminary assessment of the antiserum was made by its reactivity to SDS-PAGE blots of recombinant protein made by the E. coli pET3c35 transformants.

A highly purified preparation of RNA polymerase, isolated from vaccinia virions, was then sedimented through a glycerol gradient (Fig. 4A), and the proteins of individual fractions were subjected to SDS-PAGE and transferred to nitrocellulose membranes (9). The autoradiograph of a blot, incubated with antibody to the whole RNA polymerase, revealed the
large subunits that were unresolved from each other under the conditions of PAGE, as well as several small subunits (Fig. 4B). The autoradiograph of a replicate blot, incubated with antibody to the recombinant product of the 35.4-kDa ORF, specifically revealed the M, 35,000 subunit (Fig. 4C). The gradient fractions that were most reactive with these antibodies contained the peak RNA polymerase activity.

Although the above experiment demonstrated that highly purified RNA polymerase retained detectable amounts of M, 35,000 polypeptide, the possibility remained that only a small percentage of the total virion-derived polypeptide was associated with polymerase. To investigate this question, the soluble extract of vaccinia virions was applied directly to a glycerol gradient, and the fractions obtained after sedimentation were analyzed by immunoblotting. Immunoreactive 35-kDa polypeptide was detected only in association with polymerase. To investigate this question, the soluble extract of vaccinia virions was applied directly to a glycerol gradient, and the fractions obtained after sedimentation were analyzed by immunoblotting. Immunoreactive 35-kDa polypeptide was detected only in association with polymerase. To investigate this question, the soluble extract of vaccinia virions was applied directly to a glycerol gradient, and the fractions obtained after sedimentation were analyzed by immunoblotting. Immunoreactive 35-kDa polypeptide was detected only in association with polymerase.

Amino Acid Sequence of Tryptic Peptides—The electrophoretic mobility of the RNA polymerase subunit that reacted with the antibody to the rpo35 ORF was similar to that of the previously described rpo30 subunit, which maps to the HindIIIIE fragment of the vaccinia virus genome and migrates anomalously slowly on PAGE (9, 11). It seemed likely that the two different subunits were simply unresolved by SDS-PAGE. Another possibility, however, was that there is only one subunit to which the different antibodies cross-reacted. Proof that both polypeptides are subunits was obtained by nuclease S1 protection and primer extension techniques. For the former analysis, a 500-nucleotide [a-32P]UTP uniformly labeled RNA probe made in vitro using bacteriophage SP6 RNA polymerase was hybridized to the three sets of RNAs. The unhybridized, single-stranded RNA probe was digested with nuclease S1, and the resistant material was resolved by PAGE.

Regulation of Expression of rpo35—We used the specific antisera prepared to the rpo35 product to study the time of synthesis of the RNA polymerase subunit in vaccinia virus-infected cells. Cytoplasmic proteins, obtained at various times after infection, were resolved by SDS-PAGE and transferred to a nitrocellulose membrane that was incubated with the antibody. An immunoreactive 35-kDa polypeptide was first detected at 2 h after infection, increased markedly between 4 and 6 h, and continued to accumulate for several more hours (Fig. 7). This time course was consistent with expression from an early class promoter or a compound promoter containing both early and late promoter elements.

Transcriptional Analysis of the rpo35 Gene—Early RNA was obtained from cells infected with vaccinia virus in the presence of cycloheximide or AraC, inhibitors of protein synthesis and DNA replication, respectively. Late RNA was obtained at 7 h after infection in the absence of drugs. The 5'-end of the rpo35 transcript was analyzed by both nuclease S1 protection and primer extension techniques. For the former analysis, a 500-nucleotide [a-32P]UTP uniformly labeled RNA probe made in vitro using bacteriophage SP6 RNA polymerase was hybridized to the three sets of RNAs. The unhybridized, single-stranded RNA probe was digested with nuclease S1, and the resistant material was resolved by PAGE.
Vaccinia Virus RNA Polymerase Subunit

FIG. 4. Co-sedimentation of the product of the 35.4-kDa ORF with purified vaccinia virus RNA polymerase. Highly purified RNA polymerase (RNA pol) from vaccinia virions was applied to a 15–35% linear glycerol gradient and sedimented as described (9). The fraction numbers are in increasing order from the bottom to the top of the tube. A, RNA polymerase activity was measured in alternate fractions. Arrowheads indicate the positions of three protein markers (thyroglobulin, M, 690,000; bovine serum albumin, M, 66,000; carbonic anhydrase, M, 29,000) sedimented in a parallel gradient. B, proteins in alternate fractions were treated with SDS, resolved by PAGE on a 15% gel, transferred to nitrocellulose, and incubated with antiserum to vaccinia virus RNA polymerase and 125I-staphylococcal protein A. An autoradiograph is shown. C, autoradiograph of a replica blot incubated with antiserum to the product of the 35.4-kDa ORF made in E. coli. The numbers on the right indicate the positions of protein markers in kilodaltons.

With early RNA, made with either cycloheximide or AraC, a band of approximately 185 nucleotides was resolved. This size corresponded to a transcript that starts 25 bp upstream of the translation initiation codon of the 35.4-kDa ORF. With late RNA, the 185 nucleotide band was not detected, but instead, there was a prominent 360 nucleotide band. The latter corresponded to a transcript that starts near the conserved late promoter TAAAT motif of a short 77-amino acid (8.7-kDa) ORF that maps upstream of and partially overlaps rpo35 (Fig. 3, not translated). A 360 nucleotide band was barely detected in cells infected in the presence of AraC and not at all when cycloheximide was used to block late transcription (Fig. 8A).

The location of the 5′-end of the early rpo35 transcript, as determined by primer extension analysis (Fig. 8B), was consistent with that obtained by the nuclease S1 method. Analyzing the early transcript, it extended 25 nucleotides downstream of the translation initiation codon.
analyzed by SDS-PAGE. The resolved polypeptides were transferred onto a nitrocellulose membrane and incubated with antiserum to the 35,000 markers in kilodaltons are indicated on the presence of cycloheximide, the resistant material was resolved by PAGE in 7 to a 500-nucleotide [n-:PRUTP internally labeled complementary RNA preparations, from cells at 4 h after vaccinia virus infection in the vaccinia virus genome used to make the complementary RNA probe. The possible initiation codon of the end of the early transcript as reasons for the insensitivity include the heterogeneity of 5'-poly(A) leaders (22-25) and competition for primer binding by anti-sense RNA (26, 27). The significance of this negative result is not obvious to any known nonpoxvirus gene, nor does it have any recognizable sequence motifs. In contrast, the two large subunits of vaccinia virus and cowpox virus RNA polymerases are homologous to the corresponding prokaryotic and eukaryotic subunits (5-7), and rpo30 is homologous to a eukaryotic transcription elongation factor (9).

All of the vaccinia virus RNA polymerase genes identified thus far have early promoters and are expressed prior to DNA replication. Some of them also have late promoter elements providing a mechanism for their continued synthesis throughout the life cycle of the virus. rpo35 is transcribed under conditions that block intermediate and late gene expression, indicating that it has an early promoter. The 5'-end of the mRNA was mapped 25 bp upstream of the translation initiation codon, and the sequence AAAAGTCTGAAAAA from -13 to -26 relative to the RNA start site closely resembles in sequence and location the early promoter consensus AAAATGAAAAAAA (28). Two forms, TTTTTTT and TTTTNTT, of the vaccinia virus early transcriptional termination motif, TTTTNTT (29), occur 18 and 51 bp past the translation termination codon of rpo35. Consistent with this feature, the 3'-end of the RNA maps about 60 bp downstream of the stop codon. The presence of two tandem transcription termination motifs is unusual but not unprecedented; it also occurs in the vaccinia virus growth factor gene (30). Immunoblotting studies indicated that synthesis of the M, 35,000 RNA polymerase subunit begins within 2 h after infection, which also is consistent with early regulation of the gene. The polypeptide increases in amount between 2 and 6 h after infection but continues to accumulate for at least several more hours. Just upstream of the rpo35 gene is a 77-amino acid ORF that is preceded by a conserved TAAAT late promoter motif (31, 32) and is transcribed at late times after infection. Since this late RNA does not terminate before rpo35, it might serve as a polycistronic message for continued synthesis of the M, 35,000 polypeptide.

In conclusion, the discovery of rpo35 increases the number of reported vaccinia virus RNA polymerase subunit genes to six. An additional subunit gene, rpo19, is located within another region of the HindIII A fragment. Thus, the complexity of the vaccinia virus RNA polymerase is approaching that of eukaryotic RNA polymerase II, for which 10 subunits have been identified (33).
Acknowledgments—We thank Elaine V. Jones for providing data on the localization of rpo35 to the 14.2-kbp BamHI fragment of the vaccinia virus genome, Jerry Sisler for assistance with sequencing and preparing oligonucleotides, and Norman Cooper for cells and virus.

REFERENCES