Vaccinia Virus Gene Encoding a 30-Kilodalton Subunit of the Viral DNA-Dependent RNA Polymerase†

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Antibody was raised against purified vaccinia virus RNA polymerase and used to screen a recombinant vaccinia virus-lambda gt11 library. The DNA from several immunopositive clones was shown by Southern hybridization to originate from the vaccinia virus *Hind*III E fragment. The nucleotide sequence of the RNA polymerase subunit gene predicts a polypeptide 287 amino acids in length and 30,000 daltons in mass. An early transcript with a 5' terminus just upstream of the putative initiation codon was identified by S1 nuclease protection and primer extension analyses, demonstrating that this RNA polymerase subunit is expressed as an early viral gene product. The RNA polymerase subunit was synthesized by a bacterial expression vector to demonstrate that it corresponds to the previously described 37,000-dalton RNA polymerase subunit.

Vaccinia virus, the prototypal member of the poxvirus family, is a large double-stranded-DNA virus that replicates in the cytoplasm of the host cell. The virus' autonomy from the cell nucleus is due in large part to the fact that the virus encapsidates its own DNA-dependent RNA polymerase, transcription factors, and mRNA modification enzymes (for reviews, see references 11 and 28).

The vaccinia virus RNA polymerase is a multisubunit complex of about 9 polypeptides (1, 29). All subunits of the vaccinia virus RNA polymerase appear to be encoded by the virus (17), their genes mapping to scattered loci in the genome. The nucleotide sequences for three poxvirus RNA polymerase genes have been reported (6, 30), and the predicted amino acid sequences of the two largest poxvirus subunits exhibit significant homology to the largest subunits of both eucaryotic and procaryotic RNA polymerases. The relationship of the other subunits of the poxvirus RNA polymerase, whose genes have not yet been identified, to those of cellular RNA polymerases is not known. Therefore, we have sought to identify the genes for the smaller subunits of the vaccinia virus RNA polymerase. Here we report the sequence for an RNA polymerase subunit encoded within the *Hind*III E segment of the viral genome.

MATERIALS AND METHODS

Virus preparation. Vaccinia virus strain WR was propagated on HeLa S-3 cells grown in suspension culture on modified minimal essential medium (GIBCO) supplemented with 10% calf serum, nonessential amino acids, and 0.1% Pluronic F68 (BASF Corp., Piscataway, N.J.). Virus was purified by two rounds of sucrose gradient centrifugation (16), and DNA was extracted from purified virions by treatment with 0.1% sodium dodecyl sulfate (SDS) and proteinase K (25).

Antibody preparation. Vaccinia virus RNA polymerase was extracted from virion core particles and purified by chromatography on DEAE-cellulose and single-stranded-DNA-cellulose as previously described (7). Fifty micrograms of protein mixed with Freund complete adjuvant was

used to immunize a New Zealand White rabbit subcutaneously. Two weeks later, the rabbit was boosted with an additional 20 µg of protein. The immunoglobulin G (IgG) fraction was purified from serum as described previously (24). Immunoblotting of proteins was performed by methods described previously (5, 41), with alkaline phosphatase-conjugated goat anti-rabbit IgG (Bio-Rad Laboratories, Richmond, Calif.) as the second antibody, used according to the manufacturer's instructions.

Recombinant DNA methods. The recombinant vaccinia virus-lambda gt11 library was prepared as described previously (27). Bacteriophage plaques were screened with antibodies directed against vaccinia virus RNA polymerase essentially by the method of Young and Davis (45). Nitrocellulose replicas of phage plates were exposed to antibody at a dilution of 1:1,000 in TBST solution (10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20) followed by incubation with ¹²⁵I-protein A (ICN Pharmaceuticals, Inc.) in TBST. Immunopositive phage plaques were visualized by autoradiography. All phage were subjected to three rounds of screening at a low phage density to ensure plaque purification.

Individual phage clones were grown in liquid culture and were purified by polyethylene glycol precipitation and centrifugation on glycerol gradients (22). DNA was liberated from phage particles by treatment with 0.1% SDS and proteinase K and was extracted with phenol-chloroform (1:1). Phage DNA was labeled with [α -³²P]dCTP by nick translation (32) and used to probe Southern blots (36).

For DNA sequencing, nested-set deletions of the vaccinia virus DNA cloned into the phagemid vectors pUC118 and pUC119 (42) were generated by exonuclease III digestion (14). Sequencing reactions were performed with Sequenase (U.S. Biochemical Corp., Cleveland, Ohio) or the Klenow fragment of DNA polymerase I (35). DNA sequences were assembled and analyzed with the Microgenie software program (Beckman Instruments, Palo Alto, Calif.).

RNA analysis. HeLa cells were infected with vaccinia virus at a multiplicity of infection of 20 PFU per cell for 6 h for late RNA and for the same period of time in the presence of 100 µg of cycloheximide per ml for early RNA. Conditions for purification of RNA and S1 nuclease protection analysis were as described previously (33). Nuclease-resistant DNA was analyzed by electrophoresis on an 8% polyacrylamide sequencing gel alongside G+A sequence markers (6). RNA

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FIG. 1. Analysis of polypeptides associated with purified vaccinia virus RNA polymerase. Vaccinia virion RNA polymerase was purified by DEAE-cellulose chromatography followed by single-stranded-DNA-cellulose chromatography (lane 1) or glycerol gradient sedimentation in 1 M NaCl (lane 2). Polypeptides were electrophoresed on an SDS-10% polyacrylamide gel and visualized by silver staining. The mobilities of molecular size standards (in kilodaltons) are shown at right, and major RNA polymerase-associated polypeptides are indicated by arrows on the left.

originating upstream of the RNA polymerase subunit gene was also analyzed by primer extension, as described by Bertholet et al. (3), by using avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim, Indianapolis, Ind.).

Protein synthesis in bacteria. The RNA polymerase subunit was synthesized in bacteria by using a bacteriophage T7 expression vector. Oligonucleotide-mediated mutagenesis (19) was used to introduce an *Nde*I restriction endonuclease cleavage site at the first initiation codon of the 30-kilodalton (kDa) open reading frame. The DNA fragment encoding the gene was then inserted into the *Nde*I site of the bacteriophage T7 expression vector pET-3a (34). This construction places the initiation codon of the 30-kDa open reading frame at the proper distance from the T7 ribosome binding site such that full-length 30-kDa polypeptide can be synthesized devoid of any fusion sequences. The plasmid was transferred into *Escherichia coli* BL21, and protein expression was induced by infection with bacteriophage λ CE6 (39).

RESULTS

Preparation of antibodies directed against vaccinia virus RNA polymerase. Vaccinia virus RNA polymerase was purified from virion core particles by sequential chromatography on DEAE-cellulose and single-stranded-DNA-cellulose (7). This purification method has been shown to yield RNA polymerase that is free of the transcription factor VETF and the capping enzyme. The subunit composition of the purified RNA polymerase was determined by SDS-polyacrylamide gel electrophoresis and silver staining (Fig. 1). Major polypeptides with molecular masses of 140, 130, 37, 34, 32, 24, 23, 22, and 19 kDa, as well as a few minor polypeptides,

FIG. 2. Immunoblotting of vaccinia virus RNA polymerase and the 30-kDa RNA polymerase subunit expressed in bacteria. Vaccinia virus RNA polymerase was purified by chromatography on DEAE-cellulose and analyzed by SDS-polyacrylamide gel electrophoresis followed by immunoblotting by using antibody directed against RNA polymerase (lane 1). Lane 2 contains total bacterial proteins from cells induced to express the 30-kDa RNA polymerase subunit with a bacteriophage T7 expression vector, and lane 3 contains proteins from identical cells not induced for expression. The mobilities of protein size standards (in kilodaltons) are shown on the right.

were detected. For comparison, RNA polymerase purified by high-ionic-strength glycerol gradient sedimentation was electrophoresed in the same gel. It can be seen that the same major polypeptides are present in both preparations of the RNA polymerase. All eight of the major polypeptides in the glycerol gradient fraction were shown previously to cosediment with the RNA polymerase in a glycerol gradient (S. Quick and S. Broyles, Virology, in press) and are therefore part of the RNA polymerase complex. The sizes of the polypeptides in the RNA polymerase purified by DEAE-cellulose and DNA-cellulose chromatography are in excellent agreement with the previously reported subunit composition of the vaccinia virus RNA polymerase (1, 29, 37). We conclude that this method of purification results in highly purified RNA polymerase.

Purified vaccinia virus RNA polymerase was used to immunize rabbits for the purpose of raising antisera. The specificity of the antisera was tested by immunoblotting of vaccinia virus RNA polymerase polypeptides. The rabbit antibodies reacted with RNA polymerase subunits of about 140, 37, 34, 32, 24, 22, and 19 kDa (Fig. 2). The sizes of these polypeptides are consistent with most of those associated with the purified RNA polymerase.

Localization of the RNA polymerase subunit gene. Antiserum directed against the RNA polymerase was used to screen a lambda gt11 library containing vaccinia virus DNA inserts. Immunopositive clones were isolated, and their DNA was used as hybridization probes against restriction endonuclease digests of vaccinia virus DNA. Several independent isolates hybridized to vaccinia virus DNA fragments, as shown in Fig. 3A. When vaccinia virus DNA was cleaved with *Xho*I, *Sal*I, or *Hind*III, the phage DNA hybridized to DNA fragments of 28, 21, and 15 kilobase pairs (kbp), respectively. These sizes correspond to *Hind*III-E, *Sal*I-B, and *Xho*I-A, which overlap on the vaccinia virus restriction map (Fig. 3B).

FIG. 3. Localization of the RNA polymerase subunit gene in the vaccinia virus genome. (A) Vaccinia virus DNA was cleaved with *XhoI* (lane 1), *Sall* (lane 2), or *HindIII* (lane 3) and analyzed by Southern blot hybridization by using a recombinant λ gt11 phage DNA as a probe. Sizes of DNA standards (in kilobase pairs) are shown at the right. (B) Vaccinia virus restriction map, on which overlapping DNA fragments are indicated (▨).

The vaccinia virus RNA polymerase subunit gene was further localized within the *HindIII* E fragment by hybridization of phage DNA to restriction digests of a cloned *HindIII* E segment (Fig. 4). Digestion of the plasmid with *HindIII* plus *SacI*, *BamHI*, and *BglII* gave fragments of 14, 7.9, and 5.0 kbp which hybridized to the probe, indicating that the RNA polymerase subunit gene was located within the leftward half of the *HindIII* E segment. *XbaI* digestion products contained a single segment of 1.9 kbp which hybridized to the probe, narrowing the location of the RNA polymerase subunit gene to a relatively short sequence.

Nucleotide sequence of the RNA polymerase subunit gene. The 1.9-kbp *XbaI* segment derived from the *HindIII* E segment was subcloned into a phagemid vector, and its nucleotide sequence was determined (Fig. 5). Analysis of this sequence located two complete open reading frames capable of encoding a 30-kDa protein and a 20-kDa protein. The former open reading frame encodes the epitope detected by the antibody, as shown by direct sequencing of the recombinant λ gt11 DNA. This open reading frame begins at nucleotide 357 and ends at nucleotide 1133. It predicts a polypeptide 287 amino acids in length with a pI of 5.99. The predicted mass of this polypeptide is 29,829 daltons. The 30-kDa RNA polymerase subunit sequence did not reveal

FIG. 4. Localization of the RNA polymerase gene within the vaccinia virus *HindIII* E segment. A plasmid containing the vaccinia virus *HindIII* segment was cleaved with *HindIII* and *SacI* (lane 1), *PstI* (lane 2), *BamHI* (lane 3), *BglII* (lane 4), or *XbaI* (lane 5). DNA was analyzed by Southern blot hybridization as in Fig. 2. DNA size standards (in kilobase pairs) are shown in lane 6. The 2.7-kb band visible in all lanes is pUC13 DNA that hybridized to β -galactosidase sequences in the λ gt11 genome.

any significant similarity to other proteins in the National Biomedical Research Foundation protein sequence library, as determined by a computer search with the FASTA program (31), nor was the sequence found to be similar to the smaller subunits *RPC40* (23), *RPB3* (18), *RPB4* (44), or *RPB5*, *RPB6*, or *RPB8* (43) of yeast RNA polymerases. In addition, the 30-kDa vaccinia virus polymerase subunit contains no obvious sequence motifs, such as zinc fingers (2), leucine zippers (20), or glutamine-rich regions (8), that are frequently found in nucleic acid-binding proteins. A notable feature predicted by the sequence of the 30-kDa subunit gene is the prevalence of proline and acidic residues in the carboxyl end of the protein. Proline-rich (26) and acidic regions (38) of many transcription activators have been shown to function as activator domains in these proteins. It has been suggested that these regions function by contacting other proteins involved in transcription. The 30-kDa RNA polymerase subunit gene predicts that the 45 carboxyl-terminal amino acids contain 13 proline residues and 15 acidic residues.

Transcription analysis of the 30-kDa RNA polymerase subunit gene. Transcripts originating from the 30-kDa RNA polymerase subunit gene were analyzed by the S1 nuclease protection technique. Analysis of early and late RNAs

TCTAGAGTAGTAGCTCAATCATTCTCTTAAATTTTATGTATCTAGTTTCAATGCTCTCGTAAGTTTT 70
 GTGCTGCTCTTATTGCTGATTATTTCTTTTACCATTTTGGCTCTATTCTGAACTTTGCTCTTCTT 140
 ATCCATTTTATTGTTGACTCGGCATCTGATTGATCTTTCTTTTTCAGAGTAAGCTATCGTCATTTG 210
 ATCATCGGACACTGACTTTCACTAATTATAAAATTTGATCGGTATAACGCAATACAATATTAAAGATAT 280
 ATATATTCTACTTTTGTAAATCAACATAATAATGAAAAATATAAATGAACAAAGTTAATACATAAGAG 350
 TTATAAATGGAATGTATACATTAGTAGTTACTCATCAATGAACAAACATCAATGGCGGTAAACCGCTA 420
 M E N V Y I S S Y S S N E Q T S M A V T A 21
 CTGATATCCGGAATTACTATCACAATATGTTGGATGATGCCAATTGGAAGACTTAATAGAATGGGCGAT 490
 T D I R E L L S Q Y V D D A N L E D L I E W A M 45
 GGAAAAATCATCAAGTACTACATCAAGATATAGGTAATACAAAACTAATATCGAAGAACTAAATTC 560
 E K S S K Y Y I K N I G N T K S N I E E T K F 68
 GAATCAAGAATAATATTGGTATAGAACTACTCAAGGATTCCAGAAACAACTATCGTATAGAAATAAC 630
 E S K N N I G I E Y S K D S R N K L S Y R N K 91
 CGTCTATTGCCACAAATTTGGAATATAAACTACTGTGATATGTTAAGGCTACTAGCGGCACCGAAAA 700
 P S I A T N L E Y K T L C D M I K G T S G T E K 115
 AGAATTCCTTCGCTATCTCTTATTGGTATAAAATGCATTAAAGAGGAGTAGAATACAATATAGATAAA 770
 E F L R Y L L F G I K C I K K G V E Y N I D K 138
 ATAAAGGATCTGAGTACACGATTATTTTAACTGCTCGACGAGAAATACAATACCGGTCTCTTAAT 840
 I K D V S Y N D Y F N V L D E K Y N T P C P N 161
 GTAAAGTAGGAATACGCGGATGATGATTCAAACTAGACGCGCTGACGAACTCCACTAGTATAGAGA 910
 C K S R N T T P M M I Q T R A A D E P P L V R H 185
 CGCGGTAGAGATTGCAACCACTTTAAGCCTCCCAATTTAGAGCATTTCGCAATCTTAATGTTTACA 980
 A C R D C K Q H F K P P K F R A F R N L N V T 208
 ACGCAATCGATACATGAAAAAAGGAAATACAGAGATTTCTCCAGATAAATCCATCTCTCCGAAT 1050
 T Q S I H E N K E I T E I L P D N N P S P P E 231
 CTCGAGAACGAGTACCTACCTATAGATGACGGGTTAATCAGAGCTACATTGATAGAAACGACGACACC 1120
 S P E P A S P I D D G L I R A T F D R N D E P P 255
 AGAGGATGATGAATAAAAAATGATAAAATAAATTAGTTTATTACTGATTGCGTTAGTTCTCTCTAAA 1190
 E D D E 259
 AATGCTCAAAATCTATATCGACGAGCGTTCTAACGACAGATTGTGTGAGGCTATTAAACCATTTGGA 1260
 ATCGAAGAGCTACTGCTGCACAACTAATAGACAACTTAATGAGAGCGAGAGTTAATAAGCTC 1330
 TGTACGATCTTCAACGTAGTGTCTGCTACAGCTCCGACGATATTCCTCGTGGTTTATGACAAC 1400
 CGAGGCGGATAAGCCGATGCTGATGCTATGGCTGACGTACATAATAGATGATGATCGCGCAAAATCA 1470
 ATGAGAGAGGATCATAGCTCTTTGATGATGTTATTCGGCTAAAAAAATTAATTGTTGAAAGTGCTA 1540
 ACCGTGTCACCGTTAATAGTACTGCGCAAAATTAAGGAGAGATTGCTCTTTTCTGATTGAATCAGT 1610
 GGGGCTAGTAACCTCTCTACATTTTATGCTGTGTAGACATCGACGGAAGAGATTTCGATAAGCGAGAT 1680
 GGAAAACTAAACGAGATGCTAAAAATAATGCAGCTAAATGGCAGTAGATAAACTCTTGTGTTACGTCA 1750
 TCATTAGATTCTGATTCTAGTTATCAATAACAGTTAGTAGTTTATGATTAGATTGAATCATACATATTA 1820
 TTTTATTATGAGATAGATTAAAAATACAAATTTAGTACTATTAACGGGACTAGTATATTCTCTAAAG 1890
 ATGATATCTGTACAGATATTGTTAGAGCGTTTCTAGA 1928

FIG. 5. Nucleotide sequence of the 30-kDa RNA polymerase subunit gene. The nucleotide sequence of the 1.9-kb *Xba*I fragment derived from the *Hind*III E segment is shown. The deduced amino acid sequence of the RNA polymerase subunit is shown below the gene sequence. The initiation and termination codons for a putative gene for a 20-kDa protein are underscored at nucleotides 1192 and 1762, respectively. The 5' end of the mRNA for the RNA polymerase subunit is below the asterisk.

indicated that only early RNA gave an S1 nuclease protection product mapping to the vicinity of the 5' end of the gene (Fig. 6). No protection product was detected with late RNA. The size of the protection product obtained with early RNA places the 5' end of the mRNA at nucleotide 331 of Fig. 5, 24 nucleotides upstream of the putative initiation codon for the gene.

Transcripts arising from the RNA polymerase subunit gene were also analyzed by the primer extension technique. An end-labeled restriction fragment derived from the interior of the open reading frame was used as a primer for reverse transcriptase reactions (Fig. 7). Again, a major transcript was detected in early RNA, originating at nucleotide 361 of Fig. 5. No RNA of significant abundance was detected in late RNA, in agreement with the S1 nuclease protection experiment. In addition to the major transcript originating upstream of the open reading frame, a less abundant early transcript was detected, originating at nucleotide 382 of Fig. 5. This is 25 nucleotides downstream of the putative initiation codon for the 30-kDa RNA polymerase subunit gene. There are several possible ATG initiation codons just downstream of this site. The first, at nucleotide 391, is not part of

FIG. 6. S1 nuclease protection analysis of RNA transcripts originating from the RNA polymerase subunit gene. The hybridization probe consisted of nucleotides 1 through 491 of Fig. 5, labeled at the 5' end of the *Nco*I site. RNAs hybridized to the probe were: 5 μ g of early RNA (lane 1), 10 μ g of early RNA (lane 2), 20 μ g of early RNA (lane 3), 10 μ g of late RNA (lane 4), and 20 μ g of late RNA (lane 5). Lane M is a G+A sequence ladder. The sequence around the site of the primary protection product (denoted by an asterisk) is shown at right.

a significant open reading frame. The second, at nucleotide 404, is in frame with the 30-kDa protein gene.

Synthesis of the 30-kDa RNA polymerase subunit in bacteria. On the basis of the nucleotide sequence of the gene, the RNA polymerase subunit is predicted to be 29,829 Da. This size is similar to that of several vaccinia virus RNA polymerase subunits reported: 147, 138, 37, 34, 32, 24, 22, 20, and 17 kDa (1, 29). In order to assign the gene reported here to one of the RNA polymerase subunits, the RNA polymerase gene product was synthesized in bacteria by using a bacteriophage T7 expression vector. The vector was constructed such that the full-length open reading frame of the RNA polymerase gene with no fusion sequences would be expressed. When cells were induced to synthesize the RNA polymerase subunit, an abundant new polypeptide of 37 kDa was observed (Fig. 8). This same polypeptide was recognized by the antibody directed against vaccinia virus RNA polymerase and has an electrophoretic mobility that is identical to that of the 37-kDa subunit of the viral RNA polymerase when subjected to immunoblotting (Fig. 2). This suggests that the 30-kDa RNA polymerase subunit has an anomalous mobility on SDS-polyacrylamide gels. We therefore suggest that this polypeptide is the previously described 37-kDa subunit of the RNA polymerase.

DISCUSSION

The vaccinia virus RNA polymerase subunit gene identified here predicts a polypeptide of 30 kDa. Synthesis of this

FIG. 7. Primer extension analysis of RNA originating from the RNA polymerase subunit gene. A DNA fragment encoding the RNA polymerase gene was labeled on the 5' end at the *Nco*I site at nucleotide 487 of Fig. 5 and cut with *Bst*EII (nucleotide 410) to liberate a 77-base-pair fragment. The fragment was used as a primer for DNA synthesis by reverse transcriptase on early RNA (lane E) or late RNA (lane L) templates. The DNA products corresponding to the major and minor transcripts are indicated by a large and small arrow, respectively, on the left. Sequence ladders (lanes G, A, T, and C) were produced by chain termination sequencing using the same primer.

subunit in bacteria demonstrated that it migrates through an SDS-polyacrylamide gel as though it were 37 kDa, consistent with the size of one of the polypeptides associated with purified RNA polymerase (1, 29, 37). It is also consistent with the size of one subunit whose gene was previously mapped to the *Hind*III E fragment of the vaccinia virus genome. Jones et al. showed that RNA polymerase subunits of 37 kDa and 34 kDa were encoded within this DNA segment (17). It is likely that the gene described in this report is identical to that which encodes the larger of those two subunits. It is possible that the 34-kDa RNA polymerase subunit is also encoded within the open reading frame described here since a minor transcript originates within the 30-kDa open reading frame. The anomalous electrophoretic mobility of the 30-kDa polypeptide in SDS-polyacrylamide gels makes the assignment of the translation product to the minor transcript difficult. It should be noted that the arrangement of initiation codons near the 5' end of this transcript is much less than favorable for translation; the first ATG initiation codon is not part of an open reading frame of significant length and therefore would probably interfere with translation initiation from downstream initiation codons. Further experiments are necessary to determine

FIG. 8. Synthesis of the 30-kDa RNA polymerase subunit in bacteria. The 30-kDa RNA polymerase subunit gene was placed under the control of a bacteriophage T7 promoter. Total bacterial proteins were electrophoresed on an SDS-polyacrylamide gel, and proteins were visualized by staining with Coomassie blue. Lanes: 1, uninduced cells; 2, cells induced to produce the T7 RNA polymerase and thereby activate the RNA polymerase subunit gene. The mobilities of protein size standards (in kilodaltons) are indicated on the right.

whether the 34-kDa RNA subunit is encoded by this gene or another gene.

Analysis of RNA transcripts originating from the RNA polymerase subunit gene has identified an early RNA whose 5' end is just upstream of the initiation codon. No transcripts could be identified in vaccinia virus late RNA, suggesting that this RNA polymerase subunit gene is transcribed exclusively as an early gene. The nucleotide sequence upstream of the 5' end of the transcript identified here compares well with the promoters of other poxvirus RNA polymerase subunit genes (for a review, see reference 9). The sequence TAATAATGAAAAAATA is found at nucleotides -25 to -10 relative to the transcription start site. This is very similar in sequence and distance from the start site to the sequence AAAAATTGAAAAACTA, determined by Davison and Moss to be optimal for the activity of the 7.5-kDa early promoter (9). Because this sequence is important for both promoter strength and initiation site selection, it appears to function in a manner analogous to that of the eucaryotic TATA promoter element (4). All of the vaccinia virus RNA polymerase subunit genes studied to date appear to be transcribed as early genes. Early viral RNA will support translation of the subunits in vitro, whereas late RNA will not (17). It is worth noting that the sequence TAAATG surrounds the initiation codon for the 30-kDa RNA polymerase subunit gene. This sequence is found at the initiation codon of most vaccinia virus late genes (13, 33) and is generally regarded as the hallmark of a late gene. Despite the presence of this sequence at the start of the RNA polymerase subunit gene, we were unable to detect a late RNA transcript originating there. We cannot rule out the possibility, however, that late transcripts are produced from this gene at levels too low for us to detect them.

The nucleotide sequence surrounding the RNA polymerase subunit gene does not encode the canonical early transcription signal TTTTNT, where N is any nucleotide (46).

This sequence is not found within or near the 3' end of the open reading frame. The first termination signal encountered after the end of the RNA polymerase gene is located at the end of the distal 20-kDa open reading frame, suggesting that the RNAs for these two genes may be 3' coterminal. Northern (RNA) blotting of early RNA with an RNA polymerase gene probe supports this notion (data not shown). Transcripts of 2,100 and 1,700 nucleotides were detected, explainable on the basis of a termination event after the 20-kDa gene and another beyond our sequence information. This type of coterminal message has been observed on several vaccinia virus genes (12, 21).

The 22- and 147-kDa subunits of the vaccinia virus RNA polymerase are known to be essential for virus growth because of temperature-sensitive mutant versions of these proteins (15). At present, we do not know whether the 30-kDa RNA polymerase subunit is essential; however, several conditional-lethal mutations map to the general vicinity of the 30-kDa open reading frame (10, 40). The identification of the gene for this subunit will permit assignment of mutations therein.

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