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Biochemistry

Early transcription factor subunits are encoded by vaccinia virus late genes

(mRNA/DNA-dependent RNA polymerase/gene regulation/DNA-affinity magnetic beads/*Escherichia coli* expression)

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ABSTRACT **The vaccinia virus early transcription factor (VETF) was shown to be a virus-encoded heterodimer. The gene for the 82-kDa subunit was identified as open reading frame (ORF) *A8L*, based on the N-terminal sequence of factor purified by using DNA-affinity magnetic beads. The 70-kDa subunit of VETF was refractory to N-terminal analysis, and so N-terminal sequences were obtained for three internal tryptic peptides. All three peptides matched sequences within ORF *D6R*. ORFs *A8L* and *D6R* are located within the central region of the vaccinia virus genome and are separated by about 13,600 base pairs. Proteins corresponding to the 3′ ends of ORFs *A8L* and *D6R* were overexpressed in *Escherichia coli* and used to prepare antisera that bound to the larger and smaller subunits, respectively, of affinity-purified VETF. Immunoblot analysis of proteins from infected cells indicated that both subunits are expressed exclusively in the late phase of infection, just prior to their packaging in virus particles. The two subunits of VETF have no significant local or overall amino acid sequence homology to one another, to other entries in biological sequence data bases including bacterial σ factors, or to recently determined sequences of some eukaryotic transcription factors. The 70-kDa subunit, however, has motifs in common with a superfamily of established and putative DNA and RNA helicases.**

Vaccinia virus, a member of the poxvirus family of large cytoplasmic DNA viruses, contains ≈200 genes that can be divided into at least three regulatory classes—early, intermediate, and late (for a review, see ref. 1). All of the enzymes and protein factors required for early transcription are present within the virus particle and are brought into the cytoplasm of the cell at the time of infection. Extracts of infectious virus particles are able to correctly initiate and terminate transcription of an appropriate template *in vitro* and also to cap and polyadenylate newly synthesized or added mRNA (2). Many of the components of the early transcriptional apparatus have been isolated and purified. These include a multisubunit DNA-dependent RNA polymerase and a multifunctional heterodimeric capping enzyme that modifies the 5′ end of the nascent RNA (3, 4) and also is required for transcriptional termination (5, 6). Two additional enzymes, a poly(A) polymerase that selectively adds adenylate residues to the free 3′ ends of mRNAs without apparent primer specificity (7, 8) and an RNA (nucleoside-2′)methyltransferase that modifies the penultimate nucleotide of the cap (9, 10), have been isolated and characterized.

Vaccinia virus RNA polymerase alone is incapable of initiating transcription *in vitro* from double-stranded DNA templates (11, 12), but when supplemented with the vaccinia early transcription factor (VETF) (13), it can initiate transcription from vaccinia virus early gene promoters. VETF activity copurifies with two polypeptides of M_r 82,000 and M_r 77,000 and sediments in density gradients at a rate that is consistent with a dimeric structure (14). The binding site for VETF within early promoters coincides with the "critical region" element, in which sequence changes drastically modulate transcription (15, 16). The consensus AAAAAAT-GAAAAAA(A or T)A has been derived for this element, whose 3′ end occurs ≈13 base pairs (bp) upstream of the early mRNA start site. A DNA-dependent ATPase activity is associated with VETF (14), consistent with an ATP requirement for vaccinia early transcription (17, 18). Thus far, no factors other than RNA polymerase, VETF, and capping enzyme, which may be associated with one another in a transcription complex (19), have been shown to be necessary for transcription of early genes *in vitro*, and regulatory regions other than the early promoter element and the early transcription termination signal (20) have not been identified.

It remains unclear how many of the enzymes and factors used for vaccinia virus transcription are virus-encoded and whether any are derived from the host cell. Evidence has been obtained that most, if not all, of the tightly bound RNA polymerase subunits are encoded within the virus genome (21), although a role for the host-cell RNA polymerase II has been suggested (22, 23). The capping enzyme subunits are also virally encoded (24–26), as are some other virion-derived enzymes including a DNA-dependent ATPase (14, 27) and a topoisomerase (28, 29). In this communication, we report that both subunits of VETF are encoded by vaccinia virus late genes, consistent with a cascade mechanism of gene regulation.

MATERIALS AND METHODS

VETF Assays. DNA electrophoretic mobility-shift assays were performed essentially as described by Yuen *et al.* (15) with the 99-bp *Bam*HI–*Eco*RI fragment of plasmid pPG107-6, 3′-end-labeled with [α-^{32}P]dATP, as probe. One strand of this fragment contains the sequence 5′-ACAATATATTATT-AGTTTAAAAAATTGAAAAACTAGGAAATCCTT-TCATAACTTGTCATAAACACACACTG-3′ flanked by some additional sequences, including those derived from the polylinker of pBluescript KS(+) (Stratagene). *In vitro* transcription reactions were performed essentially as described by Broyles *et al.* (13).

Purification of VETF. VETF was purified from vaccinia virions by a method similar to that of Broyles *et al.* (13). Soluble proteins were extracted from purified virions with sodium deoxycholate and fractionated over two DEAE-cellulose columns and a single-stranded DNA-agarose column. For some preparations, DNA-Sepharose affinity column purification was performed as described by Broyles *et al.* (13).

A DNA–magnetic bead affinity procedure was developed for use as a final purification step prior to protein sequencing.

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Abbreviations: ORF, open reading frame; VETF, vaccinia virus early transcription factor.

A ligand for this procedure was made as follows: two direct repeats of the insert of plasmid pPG107-6 were cloned by cleaving separate samples of the plasmid with *Bam*HI and *Eco*RI and filling the 3′ recessed ends, then cutting with either *Kpn* I or *Dra* III (which cleave the plasmid once within the vector sequences), and ligating the smaller *Kpn* I–*Bam*HI (or *Dra* III–*Eco*RI) fragment to the larger *Kpn* I–*Eco*RI (or *Dra* III–*Bam*HI) fragment. The process was iterated four times to create plasmid pPG114-19, which contains 32 direct repeats of the insert of pPG107-6. Approximately 400 μg of purified pPG114-19 was linearized with *Eco*RI, then extracted with phenol, and precipitated with ethanol. The linearized DNA was incubated briefly with [α-^{32}P]dATP and Sequenase v2.0 (United States Biochemical), after which cold dATP and Bio-21-dUTP (Clontech) were added and the incubation was continued. At the end of the incubation, the DNA was desalted over Sephadex G-50, precipitated with ethanol, and dissolved in 350 μl of TE buffer (10 mM Tris·HCl/1 mM EDTA, pH 8.0). A suspension of M-280 magnetic beads in 3.6 ml of storage buffer (Dynal) was magnetically fractionated, and the beads were washed twice with 6 ml of phosphate-buffered saline containing 1 mg of bovine serum albumin per ml, twice with 6 ml of 1 M NaCl, and once with 6 ml of TE. After removal of the TE buffer, the beads were incubated overnight with the biotinylated DNA solution before washing twice with 3 ml of TE and monitoring the proportion of radioactivity in the solid phase.

The DNA-affinity beads were separated from the TE buffer and suspended in a preparation of partially pure VETF (from either the single-stranded DNA-agarose or DNA-Sepharose affinity step) in buffer A [50 mM Tris·HCl, pH 8.0/0.1 mM EDTA/0.01% Nonidet P-40/1 mM dithiothreitol/10% (vol/vol) glycerol] containing 70 mM NaCl. After a 30-min incubation followed by magnetic fractionation and removal of the supernatant, the beads were washed twice with 50 ml of 100 mM KCl in buffer A (10 min each wash) and then incubated three times with 1 ml of 400 mM KCl in buffer A and once with 2 M KCl in buffer A. Buffer A (1 ml) was then added to each of the four eluates before concentrating them to ≈0.2 ml by using a Centricon 10 (Amicon) and restoring the volumes to 1 ml with fresh buffer A. Proteins present at various stages of purification were analyzed by sodium dodecyl sulfate (SDS)/8–18% gradient polyacrylamide gel electrophoresis (PAGE) (30). These analytical gels were stained with silver (Bio-Rad).

N-Terminal Sequencing. Proteins were resolved by MZE 3328.IV PAGE (31), transferred to a polyvinylidene difluoride membrane (Millipore), and lightly stained (32). Membrane-bound proteins were N-terminally sequenced by using an Applied Biosystems 477A running program O3RPTH.

Tryptic Peptide Analysis. For internal sequence analysis, proteins were separated by SDS/PAGE (30), transferred to nitrocellulose, and digested with trypsin essentially as described by Aebersold *et al.* (33). The resulting peptides were eluted from the nitrocellulose, separated by microbore reverse-phase high pressure liquid chromatography, and sequenced. The peptide sequences were compared with a vaccinia virus protein sequence library by using the computer program of Pearson and Lipman (34).

***Escherichia coli* Overexpression.** Regions of the vaccinia virus genome containing open reading frames (ORFs) *D6R* and *A8L* were amplified *in vitro*, ligated with *Nde* I– and *Bam*HI-cut pET3c (35), and cloned in *E. coli* DH5α to give pPG118 and pPG119, respectively. The smaller *Bam*HI subfragment of pPG118 was recloned in the *Bam*HI site of pET3b (pPG139) for expression of a fusion protein consisting of the C-terminal portion (amino acids 420–710) of the 82-kDa VETF protein with 12 extra amino acids added to the N terminus. pPG119 was treated with *Nde* I and phage T4 DNA ligase to make a truncated plasmid, pPG128, encoding the C-terminal portion (amino acids 378–637) of the 70-kDa VETF protein. For expression by bacteriophage T7 RNA polymerase, pPG139 and pPG128 were transferred to *E. coli* BL21(DE3) and induced with 0.4 mM isopropyl β-D-thiogalactopyranoside (36). The overexpressed proteins were extracted from polyacrylamide by diffusion and were injected into New Zealand White rabbits in Freund's complete adjuvant. Boosters with antigen in incomplete Freund's adjuvant were given 2 weeks after the initial injection.

Immunodetection of VETF. BSC-1 cells infected with vaccinia virus (WR) at a multiplicity of 10 plaque-forming units per cell were harvested at various times after commencement of infection, lysed in 50 mM Tris·HCl, pH 8.0/1 mM EDTA/100 mM NaCl/1 μg of aprotinin per ml/100 μg of phenylmethanesulfonyl fluoride per ml/0.5% Nonidet P-40 and centrifuged briefly. The resulting supernatants, as well as purified VETF, were subjected to SDS/PAGE before transferring to nitrocellulose (BA85, Schleicher & Schuell), probing with antisera, and developing with ^{125}I-labeled protein A by standard techniques (37).

RESULTS

Affinity Purification of VETF. VETF was isolated from purified virus particles by a procedure based on that of Broyles *et al.* (13). An additional final purification step of DNA–magnetic bead affinity purification was used with a DNA ligand that consisted of 32 direct repeats of an early promoter containing several "up" mutations (16). VETF activity, as measured by a DNA electrophoretic mobility shift assay, was eluted from the affinity beads with two proteins whose apparent molecular masses were estimated by SDS/PAGE to be 82 and 70 kDa. The larger polypeptide was identical in size to that previously reported for VETF (13), but the other one was smaller by ≈7 kDa. The affinity-purified VETF was both necessary and sufficient to direct specific initiation by highly purified preparations of vaccinia virus RNA polymerase *in vitro* (data not shown).

VETF obtained by using the DNA–magnetic affinity beads was superior in both yield and purity to that obtained by using the DNA-Sepharose procedure described previously (13). The new affinity step separated VETF from numerous contaminating species in the size range 11–95 kDa, as visualized by gradient SDS/PAGE. Fig. 1 shows the separation of VETF from two particularly abundant polypeptides with molecular masses that are similar to those of the transcription factor.

Identification of the Genes Encoding VETF Subunits. The proteins in both the partially purified and affinity-purified VETF preparations were blotted and lightly stained with Coomassie blue (Fig. 1). The bands corresponded to the 82- and 70-kDa proteins were subjected to N-terminal sequencing. A 15-amino acid sequence (Met-Arg-Tyr-Ile-Val-Ser-Pro-Gln-Leu-Val-Leu-Gln-Val-Gly-Lys) was obtained for the 82-kDa protein from both preparations and was found to be a perfect match to the N-terminal sequence of the predicted 82,274-Da product of ORF *A8L*. This ORF is situated close to the left end of the *Hin*dIII A fragment of the vaccinia virus genome (Fig. 2). A portion of the sequence of *A8L* has been reported (39); the sequence of the remainder was determined in this laboratory (N. B. Cole and B.M., unpublished data). The amino acid sequence predicted from *A8L* is given in Fig. 2.

The band corresponding to the 70-kDa VETF protein in both the partially purified and affinity-purified preparations (Fig. 1) was refractory to Edman degradation in repeated attempts at N-terminal sequencing, probably through being N-terminally blocked. Therefore, the 70-kDa VETF protein band from the partially purified preparation was digested with trypsin, and the resulting peptides were resolved by mi-

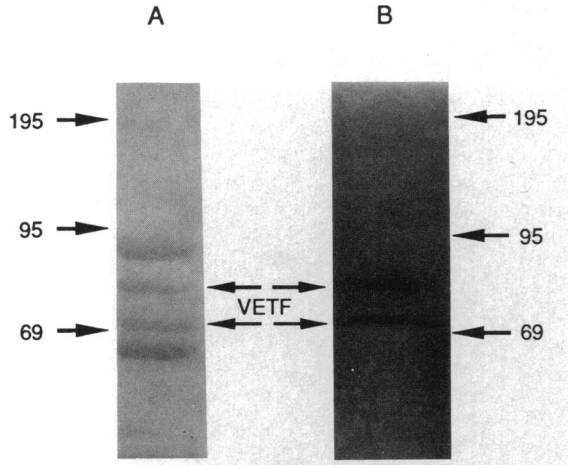
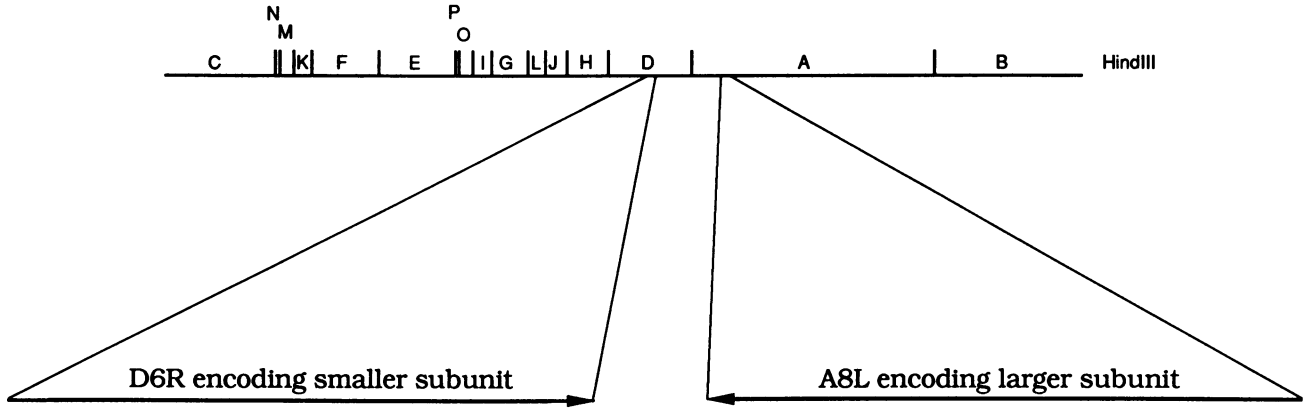


FIG. 1. Coomassie blue-stained nitrocellulose membranes showing proteins with molecular masses >50 kDa present before (*A*) and after (*B*) DNA–magnetic bead affinity purification. VETF is separated from two particularly abundant species with molecular masses similar to those of the two VETF proteins. Contaminating proteins in the size range 11–50 kDa were also removed by affinity purification. Bands labeled VETF were used for protein sequencing.

crobore reverse-phase high-pressure liquid chromatography. N-terminal sequences of three of the peptides were determined to be Ile-Ser-Tyr-Tyr-Glu-Met-Pro-Asp-Lys-Asp-Leu-Pro-Thr-Ile-Arg, Phe-Ser-Tyr-Ala-Asp-Ile-Ser-Glu-Pro-Val-Asn-Val-Tyr-Leu-Leu, and Asn-Tyr-Ile-Asp-Asp-Ile-Val-Asn-Gly-His-Phe-Phe-Val-Ser-Asn-Lys and were found to be perfect matches to sequences within the predicted 73,832-Da product of vaccinia virus ORF *D6R* (Fig. 2). *D6R* is situated towards the center of the vaccinia virus genome within the *Hin*dIII D fragment (Fig. 2), whose complete sequence has been determined (38). The amino acid sequence predicted from *D6R* is shown in Fig. 2. Since the VETF preparation from which the peptide sequences were determined had not been affinity-purified, the identity of ORF *D6R* was confirmed by using a specific antiserum (below).

Protein Sequence Analysis. There are no significant homologs of either subunit of VETF in a library of translated GenBank version 63.0 sequences, which includes the amino acid sequences of a number of bacterial σ factors. In addition, no significant homology was found between VETF and the sequenced eukaryotic general transcription initiation factors, human RAP30 (40) and yeast TFIID (41–44).

Despite the current absence of homologous sequences, VETF contains some motifs in common with cellular proteins. The 70-kDa subunit contains, starting at residue 48, a match to the motif Gly-Xaa-Gly-Lys-(Ser or Thr), which can form the "A" site of an NTP binding motif and is highly conserved in ATPases (ref. 45 and references therein) and a superfamily of established and putative DNA and RNA helicases (46). The 70-kDa protein contains matches to two additional motifs that are highly conserved within the helicases (46)—namely, Asp-Glu-Xaa-His (at residue 135) and Gln-Xaa-Xaa-Gly-Arg-Xaa-Xaa-Arg (at residue 450) (Fig. 2). The correlation between the latter two motifs, in that helicases containing Asp-Glu-Ala-Asp in place of Asp-Glu-Xaa-His also contain His-Xaa-Xaa-Gly-Arg-Xaa-Xaa-Arg in place of Gln-Xaa-Xaa-Gly-Arg-Xaa-Xaa-Arg, is found in the 70-kDa protein. The predicted sequences of the two VETF proteins show an absence of strongly basic, acidic, or glutamine- or proline-rich regions characteristic of various classes of nuclear initiation factors (41, 47–51). A leucine "zipper" motif (52) starts at residue 45 in the 82-kDa protein and is the only one greater than two heptads in length. Although the zipper is atypical, being of the form Leu-Xaa$_6$-Ile-Xaa$_6$-Leu-Xaa$_6$-Leu-Xaa$_6$-Leu-Xaa$_6$-Ile, characteristics favoring its being a dimerization domain would include its occurrence within a region predicted to be strongly

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MNTGIIDLFDNHVDSIPTILPHQLATLDYLVRTIIDENRSVLLFHIMGSGKTIIALLFAL 60
VASRFKKVYILVPNINILKIFNYNMGVAMNLFNDEFIAENIFIHSTTSFYSLNYNDNVIN 120
YNGLSRYNNSIFIVDEAHNIFGNNTGELMTVIKNKNKIPFLLLSGSPITNTPNTLGHIID 180
LMSEETIDFGEIISRGKKVIQTLLNERGVNVLKDLLKGRISYYEMPDKDLPTIRYHGRKF 240
LDTRVVYCHMSKLQERDYMITRRQLCYHEMFDKNMYNVSMAVLGQLNLMNNLDTLFQEQD 300
KELYPNLKINNGVLYGEELVTLNISSKFKYFINRIQTLNGKHFIYFSNSTYGGLVIKYIM 360
LSNGYSEYNGSQGTNPHMINGKPKTFAIVTSKMKSSLEDLLDVYNSPENDDGSQLMFLFS 420
SNIMSESYTLKEVRHIWFMTIPDTFSQYNQILGRSIRKFSYADISEPVNVYLLAAVYSDF 480
NDEVTSLNDYTQDELINVLPFDIKKLLYLKFKTKETNRIYSILQEMSETYSLPPHPSIVK 540
VLLGELVRQFFYNNSRIKYNDSKLLKMVTSVIKNKEDARNYIDDIVNGHFFVSNKVFDKS 600
LLYKYENDIITVPFRLSYEPFVWGVNFRKEYNVVSSP                         637

MRYIVSPQLVLQVGKGQEVERALYLTPYDYIDEKSPIYYFLRSHLNIQQPEIVKRHILLT 60
LRMTQLKGYLGNLLDIKDDIIIYSHKNNLEYSYVDNTIFNPFVYTQKKTLLKNDSFLYNV 120
YPGACDFLVIWVARACDTSIPEFGSYEDVDNNIIKFETMLMEVFPQLDLDITVESKFNNI 180
FRTNLKLTGLKKIIQRVQDLDINYKSLLSRYDEHFINMTGNHFILNDEQLNLSIWDLDGT 240
LALSSDGDTVMINNVKLFTDLVSDIDTQMERIKGDITYKVHLATPINSRIKLDIETSFIF 300
IETATNNILLSSDKKISIILAKNHISIKVKNHIPNIEKYFTFLVIAINAMFNSVQKSADF 360
TKVETVYWSRICQNTKNKNRKPIIINYLDPGMKKISNNFYKSDEKEVFINDNGIMFTCMD 420
PLGKYNKVGFLNIFHDMRKYCIPCCFLHDQSHRSTFSSCVHQIDVEKKIVSPYILNFGKV 480
VTESKMSFLPIIFDAFLNDGMTANMEQDNKRLKETSGYHIVRCCAGDDIVRLRTTSDIIQ 540
FVNEDKNILIVNDMVYFPMNASDIGKKIHILIQEIVHEVMIVKKKESSDKIDFFPPNYKL 600
LKDLFPKQTIQTPIQSDAGMVLTTDGFYIDGKLFNEDLSSKYVTFTKNVIASDAVAKYFS 660
PLFKYVISEAKDRFIKTWMINIMIHMNVDPNNIIPTLEKYYPNSGRAQIN           710
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FIG. 2. Predicted amino acid sequences of the 82- and 70-kDa VETF proteins and the positions of the corresponding genes on the vaccinia virus genome. The predicted sequence of the 70-kDa protein is from Niles *et al.* (38). The predicted sequence of the 82-kDa protein from amino acids 1 through 533 was from Van Meir *et al.* (39) and from amino acids 534 through 710 was from this laboratory (N. B. Cole and B.M., unpublished data). Thick underlines denote sequenced peptides as well as the arginine or lysine residue N-terminal to each trypsin cleavage site, thin underlines show motifs discussed in the text, and boldface residues are the N-terminal-most amino acids of the portions of the two genes overexpressed in *E. coli*.

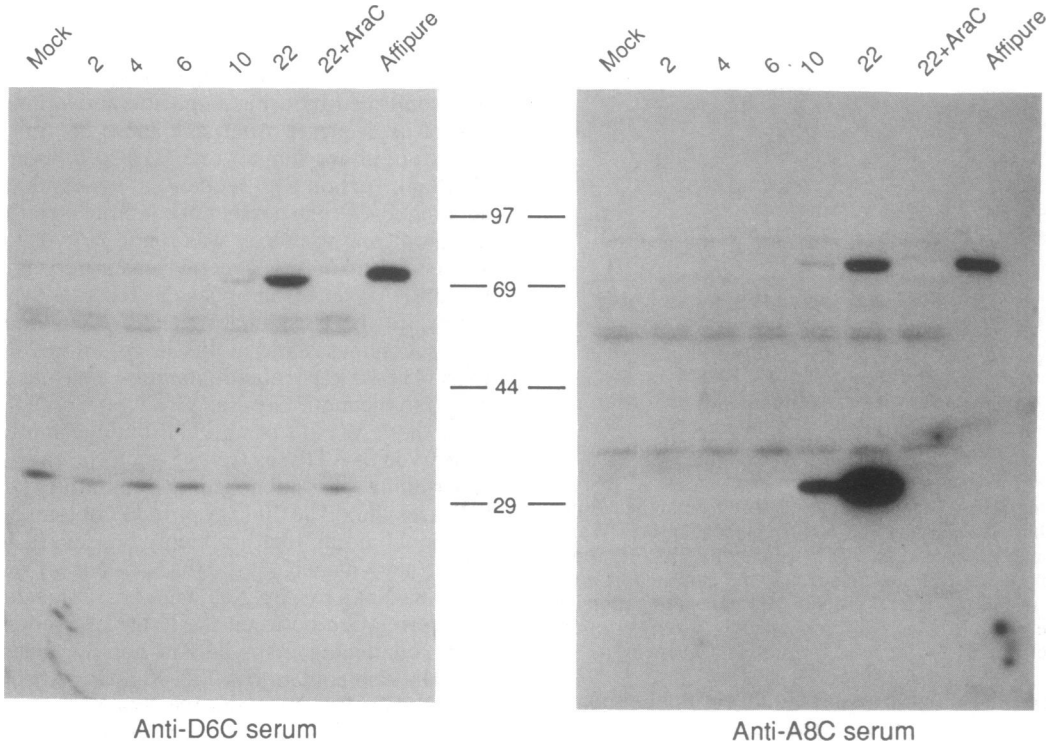

FIG. 3. Time course of VETF synthesis during a vaccinia virus infection. At the indicated times after infection, the cells were lysed, and the proteins were subjected to SDS/PAGE. The resolved proteins were transferred to a nitrocellulose membrane and incubated with anti-D6C and anti-A8C sera and ^{125}I-labeled protein A. Autoradiographs are shown. The positions of electrophoretic size markers (kDa) are indicated.

α-helical and the presence of small hydrophobic residues placed centrally within the heptads. In addition, there is a sequence Cys-Xaa-Xaa-Cys-Xaa$_{14}$-Cys-Xaa-His starting at residue 441 of the 82-kDa protein, which is an atypical variant of known zinc-finger motifs (53, 54).

Reactivity of VETF with Antisera to A8L and D6R Fusion Proteins. The 3′ portions of *A8L* and *D6R*, encoding amino acids 420–710 of the 82-kDa protein and 378–637 of the 70-kDa protein (Fig. 2), were expressed in *E. coli* in order to produce rabbit antisera. The anti-A8C and anti-D6C sera reacted specifically with the 82- and 70-kDa polypeptides, respectively, of affinity-purified VETF (Fig. 3), as verified by staining of the blots (data not shown). Since the *A8L* gene had been identified solely from the N-terminal sequence of the affinity-purified 82-kDa polypeptide, the reactivity of that polypeptide with antisera against the predicted C-terminal sequence of the *A8L* gene confirmed the correctness of our identification. The reactivity of the affinity-purified 70-kDa protein with antibody made to the sequence encoded by the D6R product was also significant, since the polypeptide used for sequencing was purified from material that had not been subjected to an affinity step.

Time of Synthesis of VETF. The specificity of the anti-A8C and anti-D6C sera allowed us to determine the time of synthesis of the individual VETF subunits. Immunoblots were made of proteins extracted from cells at various times after infection with vaccinia virus. Polypeptides corresponding to the VETF subunits were first detected 6 hr after infection, and their abundance increased over the following 16 hr (Fig. 3). No VETF was detected in "late" extracts from cells infected in the presence of cytosine arabinoside, an inhibitor of DNA replication and consequently of "late" gene expression. The slight difference in mobility between the cellular and virion-derived 70-kDa protein, apparent in Fig. 3, was shown by mixing experiments to be due to differences in the sample buffers. In addition to reacting with the larger subunit of VETF, the anti-A8C serum bound to a 31-kDa protein that also appeared late in infection. This small protein was isolated from purified virus particles as well as cytoplasmic extracts but fractionated separately from VETF during purification.

The late synthesis of both subunits of VETF is consistent with the exclusively late transcription of *D6R* (55) and *A8L* (unpublished result).

DISCUSSION

Vaccinia virus provides a unique system for combined biochemical and genetic studies of the regulation of gene expression. In this communication, we demonstrate that VETF is encoded by two nonhomologous vaccinia virus genes, *A8L* and *D6R*. No function had previously been assigned to either gene, though two conditionally lethal temperature-sensitive mutants in *D6R* exhibit normal patterns of DNA and protein synthesis at the restrictive temperature (56). The apparent absence of a defect in early transcription implies to us that when packaging occurs under permissive conditions, these VETF mutations are not subsequently thermolabile. A similar insensitivity has been found with all isolated RNA polymerase mutations, suggesting that the temperature-sensitive step may be the assembly of subunits (57, 58). It has been speculated (59), based solely on sequence considerations, that *D6R* encodes a previously isolated virion phosphohydrolase, NPHII (60). This genetic assignment could now only be true if NPHII were identical to the 70-kDa VETF protein. Although the two proteins are similar in size, they are unlikely to be identical, since they have previously been shown to have different substrate and cofactor preferences for their associated ATPase activities and different sedimentation rates as native enzymes (14, 61).

There are similarities between vaccinia virus early mRNA synthesis and basal transcription mediated by RNA polymerase II. The viral and eukaryotic RNA polymerases have similar subunit structures (11, 62), and sequence similarities exist between eukaryotic RNA polymerase subunits and the two largest RNA polymerase subunits of vaccinia virus or the

closely related cowpox virus (63–65). The vaccinia virus early promoter is about 30 bp in length and contains an essential A+T-rich sequence that specifies the distance to the site of RNA initiation (16). In addition, hydrolysis of ATP or dATP is required for one or more steps in the transition from the initiation to the elongation phase of transcription in both the vaccinia virus early (17, 18) and RNA polymerase II (66, 67) transcription systems. It seems possible, therefore, that a cellular homolog of VETF will be found.

Our finding that both subunits of VETF are expressed late in infection, supports a cascade mechanism of vaccinia virus gene regulation. Evidence that early transcription takes place in partially uncoated virions within the cytoplasm of the infected cell, without the requirement for newly synthesized viral proteins (68, 69), is consistent with the kinetics of VETF synthesis. Apparently, newly synthesized VETF is not intended to be used for early transcription in the cell in which it is synthesized but is destined to be packaged in virus particles for use during a subsequent round of infection. However, the mechanism that prevents the occurrence of transcription from early promoters late during infection is not known. It is possible that additional factors prevent such illicit transcription or that VETF is inactive until modified upon packaging in the virion. Presumably, the virion membrane, which forms within the cytoplasm, prevents early transcription until it is disrupted during virus entry into a new cell. Our identification of the genes encoding VETF and the preparation of antibodies to the polypeptides should help considerably in the elucidation of these questions.

Note Added in Proof. The smaller subunit of VETF shares 25% sequence identity with ORF 4 of *Kluyveromyces lactis* killer plasmid K2 (70). Each of the three helicase motifs is conserved. VETF also is not homologous to the recently published sequences of the eukaryotic basal initiation factors BTF3 (71) and hUBF (72). Additional evidence for ORF *D6R* encoding the 70-kDa subunit of VETF has recently been reported (73).

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