

The DNA-dependent ATPase Activity of Vaccinia Virus Early Gene Transcription Factor Is Essential for Its Transcription Activation Function*

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Vaccinia virus early transcription factor (VETF) activates the transcription of early gene templates by the viral RNA polymerase. VETF is a heterodimeric protein that binds to transcription promoters and has an associated DNA-dependent ATPase activity. The small subunit of VETF has sequences resembling two motifs commonly found in ATPases: an A-type ATP binding motif and a DEAH box. To investigate the functional role of the ATPase activity, we have analyzed the effect of mutations in each of the putative ATPase motifs. Recombinant VETF was expressed in HeLa cells using a vaccinia virus/T7 RNA polymerase system. Simultaneous expression of both subunits of VETF was required to obtain soluble protein with promoter binding, DNA-dependent ATPase, and transcription activation functions. The mutants with altered ATPase motifs retained promoter binding activity but had no detectable ATPase activity and no ability to activate transcription. The DEAH box mutant was shown to dominantly repress transcription activation by wild-type VETF. These results indicate that the DNA-dependent ATPase activity of VETF is essential for its transcription activation function.

Vaccinia virus is a member of the poxvirus family. These viruses characteristically have a large DNA genome and replicate in the cytoplasm of host cells. Approximately 200 separate proteins are encoded by the vaccinia genome. Viral gene expression is tightly regulated at the level of transcription initiation to ensure that the timing of synthesis of individual gene products occurs at specific points in the virus' life cycle (1, 2). Known genes can be grouped into the early, intermediate, and late classes that are defined according to the relative time of transcription initiation after the virus enters the host cell. Transcription of all three classes of genes appears to be carried out by a multisubunit RNA polymerase that is virus-encoded. Transcription promoters for each of the three gene classes have characteristic nucleotide sequence elements around the start site for transcription that appear to interact with class specific transcription factors (1-5).

Transcription of vaccinia virus early gene templates *in vitro* by the viral RNA polymerase is stimulated by the protein

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vaccinia early transcription factor (VETF¹; Ref. 6). Purified VETF binds early transcription promoters with high affinity (6, 7). Promoter binding by VETF appears to activate transcription by recruiting the RNA polymerase to the promoter (8, 9). Purified VETF also has an associated ATPase activity that is activated by DNA (10); however, the role of the ATPase is uncertain. Because ATP hydrolysis is essential for transcription *in vitro* (11, 12), it has been tempting to speculate that the ATPase associated with VETF is essential for transcription. In addition, ATP has been shown to destabilize the VETF-promoter complex (13), suggesting that ATP hydrolysis may function in recycling of VETF in multiple rounds of transcription or may destabilize the transcription initiation complex for progression into the elongation phase of RNA synthesis.

VETF appears to be a heterodimer of 70- and 83-kDa polypeptides (6, 10). The two polypeptides are encoded by the vaccinia D6 and A8 open reading frames, respectively (14, 15). The sequence of the D6 gene predicts a protein product with sequences resembling two motifs commonly found in ATP-binding proteins. The sequence HIMGSGKT beginning at amino acid 45 is similar to the consensus sequence GXXGKGK (where X is any amino acid) of the A-type ATP binding site (16), sometimes called the P-loop (17). In addition, at amino acids 135-138 there is the sequence DEAH that has come to be associated with a subfamily of ATP-dependent nucleic acid helicases (18). The presence of these two motifs in the small subunit of VETF has suggested that the small subunit may be responsible for the ATPase activity associated with VETF and has fueled the speculation that VETF may be the factor responsible for the ATP requirement in transcription. To test these hypotheses, we have expressed the vaccinia D6 and A8 gene products in human cells using the hybrid vaccinia-T7 expression system. We have determined that both the A8 and D6 subunits are required for all the biochemical activities associated with VETF. Point mutations in the putative A-type ATP binding site and the DEAH motif resulted in complete loss of ATPase and transcription stimulatory activities. These results demonstrate that the ATPase activity of VETF is essential for its transcription activation function.

MATERIALS AND METHODS

Recombinant VETF Expression—Vaccinia virus open reading frames (ORFs) D6 and A8 were placed in the phagemid vector pUC119 (19) and were subjected to site directed mutagenesis by the method of Kunkel *et al.* (20) to place a *Nde*I restriction site at the initiation codons. The two internal *Nde*I cleavage sites in each ORF were

¹ The abbreviations used are: VETF, vaccinia early transcription factor; ORF, open reading frame; VGF, vaccinia growth factor; Ni-NTA, nickel-nitrilo.

inactivated by mutations that did not alter protein coding potential. The genes were then transferred independently to the bacteriophage T7 expression vector pET-BS that was obtained from S. Shuman, Sloan-Kettering Memorial Cancer Center. The vector has T7 transcription promoter and terminator elements (21) in the plasmid Bluescript KSII+ (Stratagene, Inc.). Site-directed mutagenesis was used to introduce six tandem histidine codons prior to the termination codon for each ORF to produce polypeptides with carboxyl-terminal extensions of His₆. All mutations were verified by DNA sequencing using Sequenase (U. S. Biochemical Corp.).

For protein expression, 5–7 × 10⁷ HeLa cells in monolayer were infected with 10–12 plaque-forming units per cell of vaccinia virus TF7-3 that expresses the T7 RNA polymerase (22). After 1.5 h, the cells were transfected with 100 µg of the vaccinia D6 and/or A8 expression plasmid by the calcium phosphate precipitation method (23). After 48 h, cells were harvested and lysed by dounce homogenization in buffer B (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 1 mM dithiothreitol, 0.01% Nonidet P-40, 0.2 mM phenylmethylsulfonyl fluoride, and 5 mM benzamidine) at 4 °C. Particulate matter was removed by centrifugation at 15,000 × g for 45 min. Recombinant proteins were purified by virtue of the affinity of the (His)₆ tails for nickel support resins (24). The soluble fraction was incubated with 0.4 ml of nickel-nitrilo-agarose (Ni-NTA-agarose; Qiagen, Inc.) for 1 h. The resin was washed twice with buffer B and twice with buffer B containing 10 mM imidazole, pH 8.0, each for 10 min. The bound protein was eluted by two washes with buffer B containing 60 mM imidazole, pH 8.0. Native VETF was purified from virion extracts as described previously (6). Protein was quantitated by Coomassie Blue binding (25).

Biochemical Assays—DNA binding was determined by electrophoretic gel shift analysis of the vaccinia growth factor (VGF) promoter as described previously (6). Electrophoresis buffer consisted of 6.7 mM Tris-HCl, pH 7.5, 3.3 mM sodium acetate, and 0.1 mM EDTA. Binding reactions contained 100 ng of poly(dI-dC)·poly(dI-dC) as a nonspecific competitor DNA except when binding was quantitated, no competitor was used.

ATPase activity was determined by release of PO₄ from [γ -³²P]ATP (26). Where indicated, double-stranded plasmid DNA was present as a cofactor at 20 µg/ml. The effectiveness of DNA to serve as a cofactor for the ATPase is the same regardless of whether the DNA contains VETF binding sites.²

Transcription activity was determined with vaccinia virus RNA polymerase purified as described previously (9). The template consisted of a synthetic idealized promoter in front of a G-less cassette 400 nucleotides in length (6). Reactions were conducted with 1 mM ATP and CTP, and 0.07 mM [α -³²P]UTP. RNA products were extracted and analyzed by electrophoresis on 4% polyacrylamide gels containing 6 M urea (6).

Proteins were analyzed by SDS-polyacrylamide gel electrophoresis (27) and immunoblotting (28) on 12.5% polyacrylamide gels. Antibody directed against the VETF subunits were generated by immunization of rabbits with recombinant D6 and A8 gene products made in bacteria (14).

RESULTS

Expression of Recombinant VETF—We have used the hybrid vaccinia virus/T7 expression system (22) to produce recombinant VETF. The vaccinia virus ORFs D6 and A8 were placed behind the bacteriophage T7 phi10 promoter in separate plasmid constructs. Six histidine codons were inserted at the 3' end of each ORF to permit affinity purification of histidine-tailed target proteins on Ni-NTA-agarose (24). The plasmids were transfected individually or in combination into HeLa cells that had been infected with a vaccinia virus expressing the bacteriophage T7 RNA polymerase. Soluble fractions of cell cytoplasms were adsorbed onto Ni-NTA-agarose, and unbound proteins were removed by repeated washes. Histidine-tailed proteins were eluted with 60 mM imidazole. Affinity-purified proteins were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting with anti-VETF antibodies. The protein from cells transfected with both the D6 and A8 ORF expression vectors contained

additional polypeptides of 70 and 83 kDa relative to protein purified from untransfected control cells (Fig. 1). These polypeptides co-migrated with the VETF subunits and reacted with antibodies specific for the two VETF subunits. These results demonstrated that soluble A8 and D6 polypeptides were produced by this expression procedure. Typical preparations of recombinant VETF had yields of 30–50 µg of protein that were estimated to be about 50% pure. Affinity purified protein from cells transfected with the D6 expression vector alone contained soluble D6 polypeptide with undetectable amounts of the A8 polypeptide. VETF polypeptides were not detected in protein purified from cells transfected with the A8 expression plasmid alone. When expressed alone, all of the A8 polypeptide was found in the insoluble particulate fraction (data not shown).

Affinity-purified protein from cells transfected with both the A8 and D6 expression plasmids was tested for DNA-dependent ATPase, promoter binding, and transcription activator functions associated with VETF purified from virions. The recombinant VETF preparation had an abundant ATPase activity (Fig. 1C). The ATP hydrolytic activity was stimulated 10–20-fold by DNA, consistent with the properties of the VETF-associated ATPase (10). In the presence of DNA, the recombinant VETF had a *K_M* for ATP of 0.2 mM, similar to the value reported for VETF purified from virus core particles (10). Purified D6 polypeptide also exhibited a low level of ATPase activity that was about 3-fold over background. No significant ATP hydrolysis was detected in protein from untransfected control cells or in protein from cells transfected with the A8 expression vector alone.

Recombinant VETF was tested for promoter binding by electrophoretic gel shift analysis. Recombinant VETF bound the VGF gene promoter, producing a protein-DNA complex identical in mobility to that seen with VETF purified from vaccinia virions (Fig. 1D). Protein from cells transfected with the A8 expression vector alone did not exhibit appreciable promoter binding activity. The recombinant D6 polypeptide produced a very weak promoter complex, yielding about 8% of the protein-promoter complex relative to that obtained with both subunits.

The transcription activation activity of recombinant VETF was determined using purified vaccinia RNA polymerase and a DNA template with a synthetic vaccinia early promoter (Fig. 1E). The transcription activity of this preparation of RNA polymerase was stimulated by the recombinant VETF to a maximum of about 15 times greater than that obtained with the RNA polymerase alone. Protein purified from cells expressing only D6 or A8 gene products or untransfected cells did not enhance the transcription activity of the RNA polymerase significantly. The results described here demonstrate that soluble VETF was expressed in this system and that both the D6 and A8 gene products were required for the promoter binding, DNA-dependent ATPase, and transcription activation functions of VETF.

Mutational Analysis of the VETF ATPase Motifs—The functional role of ATP hydrolysis in transcription activation by VETF was addressed by mutation of the two ATPase motifs in the D6 polypeptide. This protein has sequences resembling the A-type ATP binding motif (16) and the DEAH signature (18). In ATP-hydrolyzing proteins of known structure, the A-type ATP binding motif forms part of the nucleotide binding fold (29, 30). A conserved lysine residue in this motif has been shown to play a critical role in ATP binding and hydrolysis for numerous ATP binding proteins (31–34). The corresponding lysine (K51) of the VETF small subunit was changed to an isoleucine to test its role in VETF function.

² J. Li and S. S. Broyles, unpublished data.

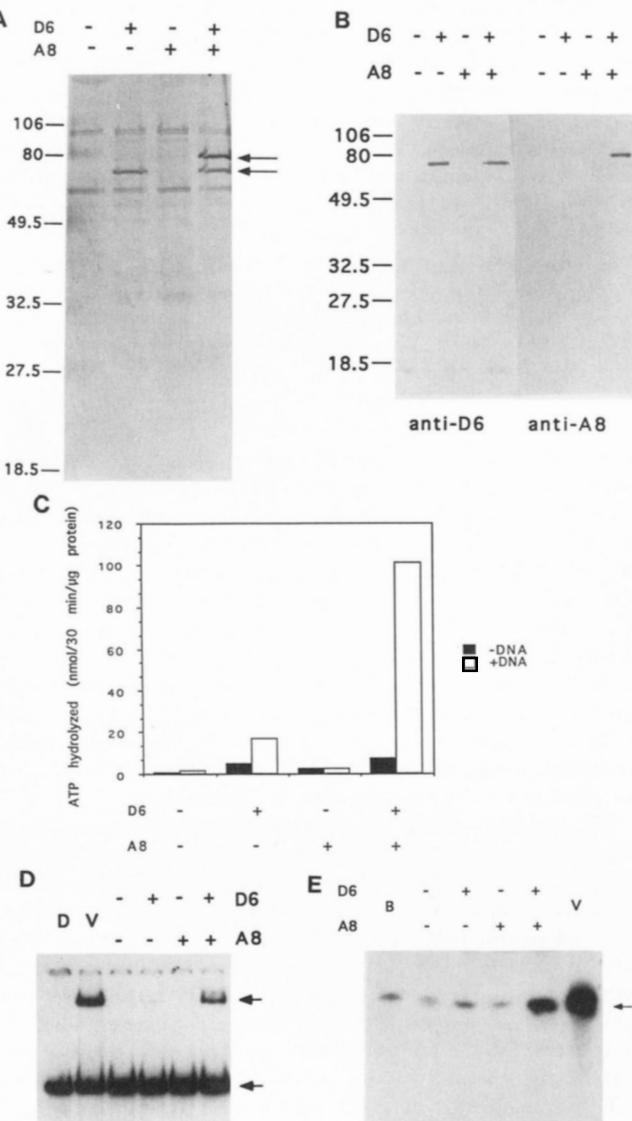


FIG. 1. Characterization of recombinant VETF. Protein from cells transfected with plasmid vectors expressing the vaccinia D6 and/or A8 gene products with His₆ tags were purified by affinity for Ni-NTA-agarose. Proteins were analyzed for polypeptide composition by SDS-polyacrylamide gel electrophoresis and silver staining (A). The polypeptides with the mobilities of the A8 and D6 gene products are indicated by the upper and lower arrows, respectively. Protein size standards (in kilodaltons) are given on the left. VETF-associated polypeptides were assayed by immunoblotting with anti-D6 or anti-A8 antibodies (B). ATPase activity was determined in the absence (closed bars) or presence (open bars) of DNA (C). VETF promoter binding activity was assayed by electrophoretic gel shift (D). The free DNA probe and that shifted by VETF binding are the lower and upper bands, respectively. Lane (D) is DNA probe alone, and lane (V) is the complex formed with VETF purified from virions. The assay for transcription factor activity was performed with purified vaccinia virus RNA polymerase and a synthetic early promoter template (E). Lane (B) is addition of buffer only. The mobility of the RNA product is denoted by an arrow. The D6 and A8 expression vectors used in transfections are indicated as (+), and (-) indicates that vector was absent.

The DEAH motif is believed to participate in magnesium ion interaction via the acidic amino acid side chains (35). The glutamate residue within the DEAH motif of the mammalian translation initiation factor eIF-4A has been shown to be important for ATP hydrolysis (33). The appropriate glutamate residue in the VETF small subunit (E136) was changed to glutamine to test its function.

The mutant VETF proteins were expressed by a slightly different strategy than that described above. In this case, VETF was expressed with the His₆ tag on the D6 polypeptide only. We have found that the His₆ tag need be present on only one of the VETF subunits for purification of the heterodimer on the Ni-NTA-agarose resin. Omission of the histidine tag from the A8 polypeptide ensured that all the purified recombinant VETF was the D6 mutant version and not wild-type virus D6 polypeptide that had assembled with recombinant A8 polypeptide.

SDS-polyacrylamide gel electrophoresis and immunoblotting analyses showed that the affinity purified wild-type and mutant versions of VETF contained both the D6 and A8 polypeptides (Fig. 2). Thus, the A8 polypeptide must have associated with the tagged D6 proteins as a heterodimer. The fact that the mutant proteins retained the ability to dimerize suggested that the amino acid substitutions did not grossly affect the structure of the protein.

The two VETF mutant proteins were analyzed for the biochemical activities described above. Gel shift analysis with the VGF promoter showed that both mutants had the ability to bind the VGF promoter. The promoter DNA binding

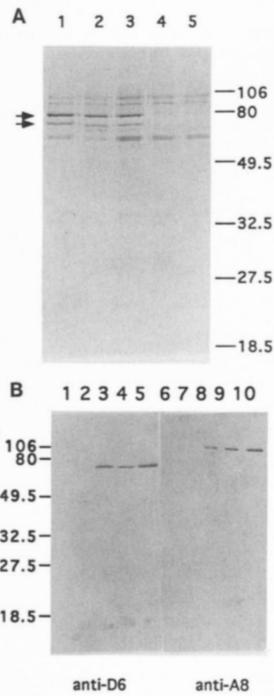


FIG. 2. Polypeptide composition of VETF mutants. Wild-type and ATPase motif mutant versions of VETF were expressed with the His₆ tag on the D6 polypeptide only, and proteins were purified on Ni-NTA-agarose. Proteins were analyzed by SDS-polyacrylamide gel electrophoresis and silver staining (A). Expression vectors for protein expression were: A8 plus D6 E136Q (lane 1), A8 plus D6 K51I (lane 2), A8 plus wild-type D6 (lane 3), A8 alone (lane 4), and none (lane 5). Proteins also were analyzed by immunoblotting using anti-D6 or anti-A8 antibodies (B). Expression vectors for protein expression were: none (lanes 1 and 6), A8 alone (lanes 2 and 7), A8 plus wild-type D6 (lanes 3 and 8), A8 plus D6 K51I (lanes 4 and 9), and A8 plus D6 E136Q (lanes 5 and 10). The mobilities of protein size standards (in kilodaltons) are shown.

activity was resistant to competition with non-specific DNA indicating that the binding was specific (Fig. 3). The promoter binding activity of the K51I mutant appeared to be somewhat lower than that of the wild-type and the E136Q mutant. This was analyzed in greater detail by titration of the protein with its promoter ligand. Scatchard analysis showed that wild-type recombinant VETF bound the VGF promoter with a K_D of about 0.39 nM (Table I). This is comparable to the K_D of 0.27 nM seen with VETF purified from virions. The E136Q mutant bound the promoter similarly with a K_D of about 0.18 nM. The K51I mutant exhibited lower affinity for the promoter DNA with an association constant of about 2.5 nM. Therefore, the E136Q mutation did not appreciably alter the affinity of VETF for its promoter binding site, however the K51I mutation resulted in about a 10-fold reduction in affinity for the promoter.

The two VETF mutants were analyzed for their ability to hydrolyze ATP. Time course experiments showed that both had no detectable activity above the background (Fig. 4). Thus, mutation of the putative A-type and DEAH motifs in the D6 polypeptide completely inactivated the ATPase activity associated with VETF supporting the contention that these motifs constitute sites required for interaction with ATP.

The two VETF mutants were also tested for their effect on transcription by the vaccinia RNA polymerase. These experiments were performed in two different ways. First, purified RNA polymerase, which has a low level of basal transcription activity, was titrated with wild-type and mutant VETF proteins to assess their ability to stimulate transcription. The wild-type recombinant VETF was capable of stimulating the transcription activity of the RNA polymerase by approximately 10-fold under these conditions (Fig. 5). The two VETF

mutants did not produce appreciably stimulate transcription under the same conditions, indicating that the mutant forms of VETF lacked transcription activator function. In another experiment, the RNA polymerase was mixed with a fixed amount of wild-type VETF and titrated with either more wild-type or the mutant E136Q VETF in a transcription reaction. The E136Q mutant VETF was chosen for this experiment because its affinity for the promoter is comparable to that of wild-type VETF. The additional wild-type VETF in the transcription reaction produced increased amounts of RNA product by about 2-fold (Fig. 6). Titration with the same amount of the E136Q mutant caused a reduction in transcription activity by approximately 80%. Therefore, the E136Q mutant interfered with the transcription activation by wild-type VETF.

DISCUSSION

To investigate the structure and function of VETF, we have sought the expression of recombinant transcription factor. Initially, a variety of strategies for expression in bacteria was attempted, however very little, if any, soluble protein was obtained.² Using the vaccinia virus/T7 RNA polymerase system, we have expressed VETF in mammalian cells in a soluble form. The expression constructs were designed to direct the synthesis of proteins with a six histidine repeat at the carboxyl termini to permit facile purification on nickel resins. The purified recombinant VETF exhibited promoter binding, DNA-dependent ATPase, and transcription activator functions previously described for VETF purified from virus particles.

The characteristics of the recombinant VETF support several previous hypotheses regarding its structure and function. First, both the D6 and A8 vaccinia virus gene products were required to observe any of the biochemical activities of VETF, supporting the contention that the factor is a heterodimer (6, 10). In addition, the fact that both the D6 and A8 gene products were retained on Ni-NTA-agarose when only the D6 polypeptide had a histidine tag further indicates that VETF is a heterodimer of the two polypeptides. Finally, mutational analysis of the two putative ATP binding motifs in the small subunit of VETF provides evidence that the ATPase associated with VETF is inherent to the transcription factor.

A low level of each of the three biochemical activities ascribed to VETF was observed with the protein purified from cells transfected with the D6 gene alone. We believe this likely is due to assembly of small amounts of the recombinant D6 polypeptide with native A8 subunit encoded by the virus. The evidence described here indicates that, in solution, VETF is a heterodimer of the two polypeptides. While it has not been demonstrated directly, VETF probably associates with the promoter as a heterodimer as well. Glycerol gradient sedimentation analysis of the recombinant D6 polypeptide suggests that it is monomeric (data not shown). Monomeric D6 polypeptide would be expected to shift the electrophoretic mobility of promoter DNA to a lesser extent than that observed when the heterodimer binds the promoter. This, however, was not the case, indicating that the D6 polypeptide does not stably associate with the promoter on its own.

Mutation of the two ATP binding motifs in VETF provides the first evidence that the VETF-associated ATPase is essential for its transcription activator function. This implies that the previously described ATP-dependent destabilization of the VETF-promoter complex (13) is a function necessary for transcription activation. Mutation of both the A-type motif and the DEAH box inactivated both the ATPase and transcription stimulatory activities. Loss of ATPase for one mu-

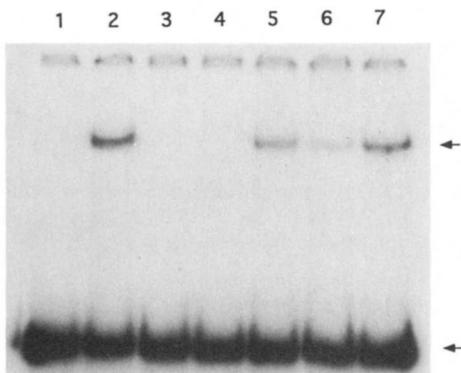


FIG. 3. Promoter binding activity of VETF mutants. Proteins were mixed with 32 P-labeled VGF promoter DNA fragment and analyzed by electrophoresis in a native polyacrylamide gel. The proteins used were: VETF purified from virions (lane 2), proteins from untransfected control cells (lane 3), proteins transfected with the A8 expression vector alone (lane 4), wild-type recombinant D6 (lane 5), A8 plus D6 mutant K51I (lane 6), A8 plus D6 mutant E136Q (lane 7). Lane 1 is the DNA fragment alone. The mobilities of the free DNA and that complexed to VETF are shown by the lower and upper arrows, respectively.

TABLE I

Association constants for promoter binding

Protein	K_D
	nM
VETF, virion	0.27
VETF, wild-type ^a	0.39
VETF, K51I ^a	2.5
VETF, E136Q ^a	0.18

^a Recombinant proteins.

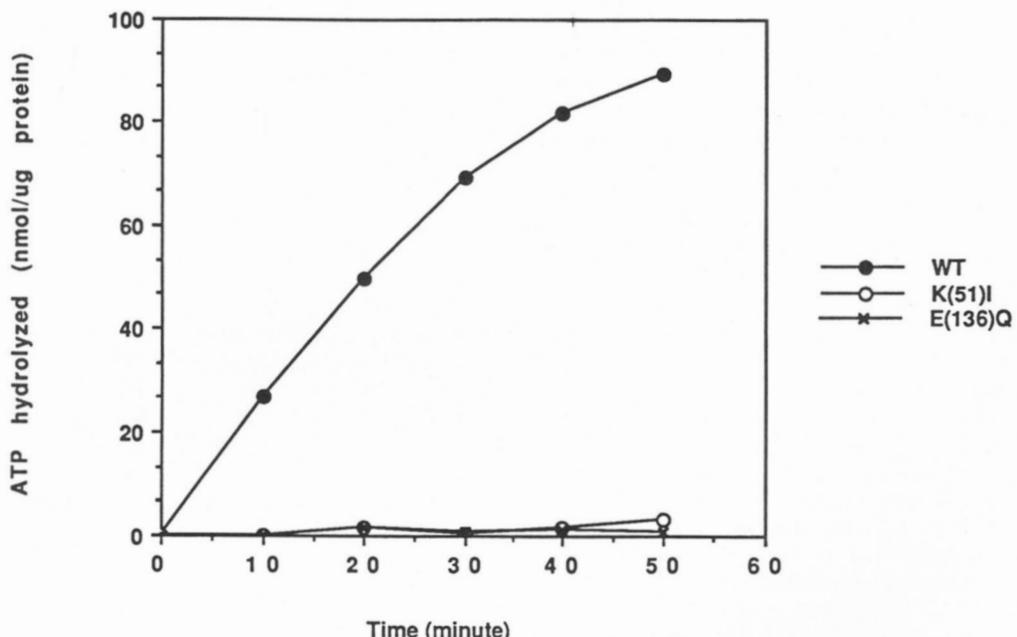


FIG. 4. Time course of ATP hydrolysis by VETF mutants. Approximately 20 ng of protein were incubated with DNA and 0.1 mM ATP. Phosphate released was determined as a function of time for recombinant wild-type VETF (●), the VETF mutant D51I (○), and the VETF mutant E136Q (×).

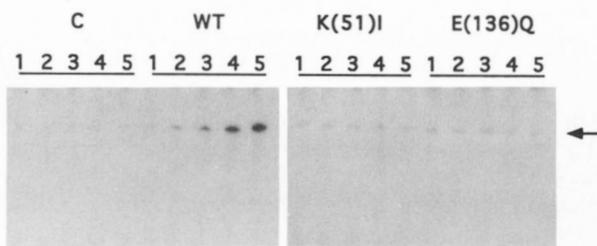


FIG. 5. Transcription activator activity of VETF mutants. Wild-type (WT), mutant K51I, or E136Q mutant recombinant VETF was incubated with vaccinia RNA polymerase, an early promoter DNA template, and radiolabeled nucleotides. RNA products were resolved by electrophoresis on a denaturing polyacrylamide gel. The mobility of the 400-nucleotide RNA product is indicated by an arrow. Reactions contained 0 ng (lane 1), 2.25 ng (lane 2), 4.5 ng (lane 3), 9 ng (lane 4), and 18 ng (lane 5) of the indicated VETF protein. Reactions in panel C are controls titrated with protein purified from cells transfected with the A8 expression plasmid alone.

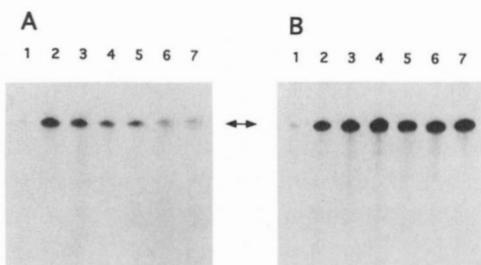


FIG. 6. Repression of transcription by the VETF mutant E136Q. Transcription reactions were conducted with vaccinia RNA polymerase and 14 ng wild-type VETF. The reactions also contained: 0 ng (lane 2), 9 ng (lane 3), 18 ng (lane 4), 27 ng (lane 5), 36 ng (lane 6), and 45 ng (lane 7) VETF mutant E136Q (panel A) or additional wild-type VETF (panel B). Lane 1 is the reaction products from the RNA polymerase with no addition of VETF. The mobility of the 400-nucleotide RNA product is indicated by arrows.

tant (E136Q) converted the activator into a dominant suppressor of wild-type VETF transcription activation. Since the mutant protein bound promoter DNA as tightly as wild-type

VETF, this suggests that the mutant protein competed with wild-type VETF for promoter binding sites but could not activate transcription. Conversion of transcription activators into repressors where the activation domain has been impaired has been described for other activator proteins (36, 37).

Both VETF mutants retained the ability to form a stable complex with the VGF promoter. The DEAH mutant (E136Q) had affinity for the promoter that was comparable to the wild-type factor. Mutation of the A-type ATP binding site resulted in impairment of the affinity of VETF for its promoter binding site. It is possible that replacement of the polar lysine residue with an apolar isoleucine might have impacted the tertiary conformation of the mutant, thereby affecting the integrity of its DNA-binding domain. We have evidence that, in addition to containing the ATPase domain, the small subunit of VETF also contacts the transcription promoter DNA.² It is, therefore, not surprising that a single point mutation could affect both the ATPase and DNA binding properties of the protein. The global structure of this mutant appeared not to be drastically altered since the protein retained its ability to form a heterodimer and remained competent to bind the promoter, albeit with lower affinity.

The reduced affinity for promoter DNA does not explain the loss of DNA-dependent ATPase activity and transcription stimulatory activity of the K51I mutant. These assays were performed with protein and DNA concentrations above the mutant's K_D for promoter DNA. Under these conditions, most of the protein should be bound to DNA and should exhibit any activity that is DNA-dependent. No ATPase or transcription stimulatory activity was detected with this mutant, indicating that lysine 51 likely participates directly in ATP hydrolysis and activation of transcription.

The ATPase motif of VETF is somewhat atypical. The first glycine of the consensus A-type ATP binding site in VETF is replaced with a histidine residue. Another vaccinia ATPase (nucleoside triphosphate phosphohydrolase I) that also is DNA-dependent for its activity, has a histidine residue at the corresponding position of its putative ATP binding site (38). It is tempting to speculate that the histidine residues may

reflect a need for a different structural feature of the ATP binding pocket whose function (and likely tertiary structure) is altered as a result of DNA binding.

There is now substantial evidence that the coupling of transcription activation function to ATP hydrolysis may be a widespread phenomenon (39). In prokaryotes, members of the σ^{54} family of transcription activator proteins utilize ATP hydrolysis to promote open complex formation by the RNA polymerase (40, 41). The yeast snf2 transcription factor has a DNA-stimulated ATPase activity that appears to be required for transcription activation (42). In addition, the eukaryotic TFIIH factor that is required for basal transcription by RNA polymerase II has been reported to possess ATP-dependent DNA helicase activity (43).

The DEAH motif has been observed in a growing group of proteins, a few of which are known ATP-dependent nucleic acid helicases (18). The presence of a DEAH box in the small subunit of VETF has prompted suggestions that VETF is a nucleic acid helicase (44). We have examined this possibility exhaustively and have obtained no evidence that VETF has helicase activity.² We suggest that the DEAH box is not unique to nucleic acid helicases and may be merely reflective of proteins whose conformation may be altered as a consequence of DNA binding, ATP hydrolysis, or both. We, however, cannot rule out the possibility that an accessory protein factor may be required to observe VETF helicase activity.

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