The DNA binding domain of the vaccinia virus early transcription factor small subunit is an extended helicase-like motif

Jing Li and Steven S. Broyles*

Department of Biochemistry, Purdue University, West Lafayette, IN 47907-1153, USA

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ABSTRACT

The vaccinia virus early transcription factor (VETF) is an ATP-dependent activator of the early class of viral genes. VETF is a heterodimeric protein that binds an initiator-like element surrounding the start site of transcription. Previous studies indicated that the small subunit of VETF contacts the promoter DNA. We have taken a mutational approach to determine sequences in the VETF small subunit that are important for DNA binding. Two types of sequences were targeted for mutation: ones resembling motifs that are conserved in the nucleic acid helicase family and positively charged residues in predicted α-helices. Mutations affecting transcription activation were clustered in two regions. One mutation that impaired DNA binding is located near the N-terminus within the putative ATP-binding pocket that comprises helicase domain I. DNA binding was also severely reduced by mutations in a sequence resembling helicase domain VI and two putative α-helices that flank this domain in the C-terminal third of the polypeptide. These results indicate that the DNA binding domain in the small subunit of VETF is not isolated within a separable domain as is the case with most transcription factors, but rather, spans most of the length of the 637 residue polypeptide. A model for VETF structure is suggested in which the active site for ATP hydrolysis is integrated within an extended DNA-binding domain such that the structure and function of each domain influences that of the other.

INTRODUCTION

The initiation of transcription is the most frequently utilized control point in the regulation of gene expression. In order to initiate RNA synthesis, the RNA polymerase must be directed to appropriate sites on the genome where polymerization of the RNA chain will ensue. Genomic site selection is modulated by sequence-specific DNA binding proteins that may positively or negatively affect the ability of the RNA polymerase to assemble into a pre-initiation complex or may influence the progression of the pre-initiation complex into RNA synthesis.

Much of our current understanding of the mechanistic aspects of transcription factors derives from knowledge of the functional domains of these proteins and how these domains physically interact with DNA, RNA polymerases, and other transcription factors.

Most DNA-binding transcription factors can be described as having a modular structure in which the different functional domains can be physically separated from one another while maintaining domain function. For example, the protein domain conferring DNA recognition is usually confined to a relatively short linear sequence of amino acids that can bind cognate DNA sequence elements with specificity and affinity similar to that of the intact protein. Also transcription 'activation' domains responsible for contacting other proteins can maintain function when tethered to an unrelated DNA binding protein.

The vaccinia virus early transcription factor (VETF) is an activator of transcription of the early class of genes by the viral RNA polymerase. VETF is a heterodimeric protein that binds to the promoter DNA spanning 40 nt surrounding the start site of transcription, and thus resembles nuclear initiator element binding proteins. DNA binding activates an ATP hydrolytic activity intrinsic to VETF that is essential for its transcription activation function. The role for ATP hydrolysis in transcription is not clear, but it has been shown that ATP hydrolysis accelerates the rate of dissociation of VETF from DNA. Promoter binding by VETF also is believed to have a role in assembly of it and other components of the viral transcription machinery into virion particles.

The sequences of the two VETF subunits has not provided any substantial clues as to the functional domains of the protein. There are no obvious similarities to DNA binding or activation domains of other transcription activators. Previous studies implicated the small subunit of VETF in DNA binding. Photoaffinity labeling studies with brominated promoter DNA identified the small subunit as interacting with DNA on the upstream side of the transcription start site. In addition, mutations in the gene encoding the VETF small subunit from temperature-sensitive viruses were shown to impair the factor's affinity for promoter DNA.

* To whom correspondence should be addressed

† Present address: Laboratory of Immunobiology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA
While the sequence of the small subunit of VETF does not resemble that of previously described DNA binding domains, it does bear reasonable similarity to several subdomains described for the DEAH subfamily of nucleic acid helicases. Good candidates for helicase domains I, II, III and VI have been identified (9). We previously demonstrated that domains I and II were essential for VETF's ATPase and transcription activation activities (4). Domain I with the consensus sequence GXXGKKT is believed to lie in the adenosine nucleotide binding pocket, and domain II with the sequence DEAH is considered to be the site of interaction with the magnesium ion cofactor. Several eukaryotic transcription factors including yeast SWI2/SNF2 (10,11), Drosophila brahma (12) and potential mammalian counterparts (13,14), as well as the 89 kDa subunit of the basal transcription factor BTF2/TFIIH (15,16) possess amino acid sequences conserved among the helicase family. While BTF2/TFIIH has been demonstrated to have DNA helicase activity (16), the role for the helicase domain in the regulation of transcription by these proteins is unknown.

In the current study, we have constructed 24 point mutants in the VETF small subunit in order to determine sequences important for DNA binding. Mutations in two regions widely separated in the linear sequence of the protein were found to impair the ability of the protein to interact with DNA: the N-terminus at helicase domain I and a region surrounding the sequence that resembles helicase domain VI near the C-terminus.

### MATERIALS AND METHODS

#### Production of wild-type and mutant VETF

A hybrid vaccinia virus/T7 expression system was used to express proteins in HeLa cells (4). Site-directed mutagenesis was used to introduce nucleotide changes in the VETF small subunit gene (vaccinia open reading frame D6R) by the method of Kunkel et al. (17). The VETF small subunit was modified to possess a (His)6 tag at its C-terminus. The procedure for protein production and purification was as described previously (4) with the following modifications. Approximately 3 x 10^8 HeLa cells growing in monolayer culture were infected with vaccinia virus strain vTF7-3 that expresses the bacteriophage T7 RNA polymerase (18) at a multiplicity of 20 p.f.u./cell. The cells were transfected with 300 μg each of the plasmids directing the expression of the VETF small subunit and large subunit devoid of a (His)6 tag. Cells were harvested after 72 h, and the proteins were purified from the cytoplasmic fraction on nickel-NTA-agarose (4). After adsorption of protein, the nickel--NTA-agarose was washed with buffer A (150 mM NaCl, 50 mM Tris, pH 8.0, 1 mM dithiothreitol, 0.01% NP-40) containing 10 mM imidazole. (His)6-tagged proteins were eluted with buffer A containing 80 mM imidazole. The proteins were purified further by chromatography on DEAE and phosphocellulose. The affinity purified protein was first loaded onto a DEAE-cellulose column equilibrated with 0.1 M NaCl in buffer B (50 mM Tris pH 8.0, 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% NP-40, 10% glycerol). The flow through fraction was diluted by half with buffer B and loaded onto a phosphocellulose column equilibrated with 50 mM NaCl in buffer B. VETF was recovered by step elution with 0.25 M NaCl in buffer B.

### Biochemical assays

Transcription reactions were performed with purified vaccinia virus RNA polymerase (0.2–0.5 U) and the indicated form of VETF (~5 ng) as described previously (3). The DNA template consisted of a synthetic early promoter directing transcription of a 400 nt G-less cassette. RNA products were resolved by electrophoresis on a 6 M urea-4% polyacrylamide gel and visualized by autoradiography. Transcription reactions were titrated with each form of VETF to determine optimal activation since these reactions respond to VETF in a bimodal fashion (3).

Promoter DNA binding activity was determined by electrophoretic gel shift analysis using a DNA fragment containing the vaccinia growth factor gene promoter (2). Protein–promoter DNA complexes were quantitated by densitometry of autoradiograms with a LKB Ultrascan II densitometer. ATPase activity was determined by release of phosphate from [γ-32P]ATP as described (2). DNA cofactor was included in ATPase reactions where specified. Limited trypsin digestion of VETF mutants was performed as described previously (7). Digestion products were analyzed by immunoblotting using antibodies specific for the large and small subunits of VETF.

### RESULTS

#### Production of VETF mutants

Previous results demonstrated that both subunits of the heterodimeric VETF were required to preserve the activity and solubility of the protein (4). The small subunit remained soluble when expressed alone, but had no measurable DNA binding activity. The large subunit was insoluble when expressed alone. Despite not having high affinity for DNA, there are two lines of evidence that the small subunit participates in contacting promoter DNA. Temperature-sensitive mutations mapping in the gene encoding the small subunit were shown to impair VETF's DNA binding activity (7), and photoaffinity labeling experiments identified the small subunit specifically as contacting DNA (8). In an attempt to locate amino acids in this polypeptide participating in DNA-binding, we have adopted a mutational approach. Initial deletion experiments showed that removal of as little as 50 amino acids from the C-terminus or 22 amino acids from the N-terminus of the 637 polypeptide resulted in loss of solubility (data not shown), therefore a point mutation strategy was employed. Only amino acids that are conserved among all poxvirus ETFs of known sequence which includes vaccinia (strains WR and Copenhagen), variola, shope fibroma and fowlpox virus were examined in this study. Replacements were designed to minimize perturbation of the local secondary structure as predicted by a structure algorithm (24). The N-terminus adjacent to the putative ATP binding pocket (domain I) was targeted for mutation because a temperature-sensitive mutation at residue 25 was previously shown to impair DNA binding (7).

Several properties of VETF indicate that DNA binding and the ATPase are interrelated. The ATPase activity is DNA-dependent, and ATP hydrolysis results in dissociation of VETF from promoter DNA (5). Because ATP hydrolysis and DNA binding influence each other, we hypothesize that one protein domain may participate in both functions. To test this notion, we have targeted sequences believed to be important for ATP hydrolysis for mutation. We previously demonstrated that mutation of...
residues conserved in helicase domains I and II, which are also conserved in other non-helicase ATPases, resulted in inactivation of ATPase activity and transcription activation activity by VETF (4). As other targets for mutation, the sequences resembling domains III and VI of nucleic acid helicases were chosen. The best candidate for the SAT sequence of domain III is SGS at residues 164–166. The serine and threonine residues in this domain have been shown to be essential for function for the herpes simplex I DNA helicase UL9 (19), the RNA helicase translation initiation factor eIF4A (20), and the pre-mRNA splicing factor PRP2 that is a putative RNA helicase (21). Therefore we altered the sequence at amino acids 164–166 of the VETF small subunit to AGG. A good match to the consensus QXXGRXXR of domain VI in the VETF small subunit is QILGRSIR at residues 450–457. The arginine residues in this sequence were shown previously to be essential for the helicases UL9 (19) and eIF4A (22). Accordingly, we constructed the double mutant R454SIR457 to GSIG to test the importance of this sequence for VETF function. In addition, the importance of potential y-helical regions was tested because DNA-binding contacts are frequently mediated by protein helices (23). The basic residues in putative helices were chosen because basic residues frequently mediate DNA contacts.

The central region of the polypeptide was not chosen for mutation because the mutant V278M from a temperature-sensitive virus was shown previously to have a severely distorted tertiary structure as determined by sensitivity to proteolysis by trypsin (7). The global change in structure of this mutant suggested that residue 278 may be important for protein folding and may reside in the core of the protein. Therefore this region of the protein was avoided in this study.

Mutant proteins were expressed in HeLa cells using a vaccinia virus/T7 RNA polymerase system (4). The C-terminus of the small subunit was tagged with a (His)$_6$ sequence to facilitate affinity purification of the mutant protein, and the protein was co-expressed with native large subunit lacking the tag. All 24 mutants were recovered in soluble form. Purified protein preparations consisted primarily of the 77 and 83 kDa polypeptides characteristic of VETF (data not shown). These polypeptides were shown to react with antibodies specific for the two VETF subunits by immunoblotting (data not shown). Because only the small subunit possessed the (His)$_6$ tag, the retention of the large subunit in the affinity purified preparations demonstrated that all mutant small subunits maintained the ability to associate with the large subunit in a heterodimer. In addition, limited proteolysis by trypsin failed to detect structural alterations expected of misfolded proteins (7).

**Transcription activation by VETF mutants**

The mutant VETFs were tested initially for their ability to activate transcription from an early viral promoter by the vaccinia RNA polymerase. The purified RNA polymerase had almost no detectable transcription activity alone (Fig. 1). Protein recovered from cells transfected with the vector directing expression of the VETF large subunit alone weakly stimulated transcription activity. We attribute this low level of activity to a minor contamination with native VETF expressed from the virus vector. Wild-type VETF activated transcription ~30-fold relative to the RNA polymerase alone. As reported previously (4), mutation of lysine 51 at the putative ATP binding pocket completely inactivated transcription factor activity. Mutations H22O, Q23M and H45A adjacent to the nucleotide binding pocket at the N-terminus also impaired activity. The mutations K431A in one putative $\alpha$-helix, and mutations K504Q and K512A in a second putative helix, completely abrogated activity. Mutation K510A in the latter helix partially abrogated activity. Mutations K505A and K514A in the same helix did not affect activity appreciably. The loss of activity by the double mutant K504QK505Q can be ascribed to K504 since altering K505 alone had no effect. Mutations R32Q and R39Q near the N-terminus had no effect on activity, nor did K301A, K391A, K357Q, K394A, R434A, K566A, K573Q, K595A and K599A that lie in putative $\alpha$-helices.

The double mutant R454SIR457 to GSIG in helicase-like domain VI also lost all activity. The mutation of helicase-like motif III S164G5166 to AGA but had no effect on transcription activity.

**Promoter binding activity of VETF mutants**

Mutants defective for transcription activation were characterized further to determine the basis for the defect. The capacity of mutant proteins to bind promoter DNA was determined by electrophoretic mobility shift. Of these mutants, four were found to be significantly impaired in their ability to bind promoter DNA: K504Q, K512A, the double mutant K504QK505Q and R454SIR457G (Fig. 2). DNA binding was assessed in a more...
quantitative fashion by assay of binding as a function of promoter DNA concentration and Scatchard analysis of the data. The results revealed that none of the mutants suffered complete loss of promoter binding activity, however the mutants described above had a 4-6-fold higher dissociation constants relative to wild-type VETF (Table 1). Quantitative analysis revealed that mutants H45A and K514A were also impaired, having a ~3-fold higher dissociation constant. All other mutants had dissociation constants for DNA binding close to that of wild-type. For example, mutants K391A and K599A that are normal transcription activators exhibited a KD for promoter binding that was also normal (Table 1).

Table 1. Dissociation constants for promoter DNA binding by mutant VETFs

<table>
<thead>
<tr>
<th>VETF</th>
<th>$K_D$ (nM)</th>
<th>Ratio $K_D/K_D$ wild-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td>K51Q</td>
<td>1.6</td>
<td>0.89</td>
</tr>
<tr>
<td>H22Q</td>
<td>0.6</td>
<td>0.33</td>
</tr>
<tr>
<td>Q23M</td>
<td>1.6</td>
<td>0.89</td>
</tr>
<tr>
<td>H45A</td>
<td>6.2</td>
<td>3.4</td>
</tr>
<tr>
<td>K431A</td>
<td>4.9</td>
<td>2.7</td>
</tr>
<tr>
<td>K504Q</td>
<td>8.2</td>
<td>4.6</td>
</tr>
<tr>
<td>K510A</td>
<td>5.8</td>
<td>3.2</td>
</tr>
<tr>
<td>K512A</td>
<td>7.7</td>
<td>4.3</td>
</tr>
<tr>
<td>R454SIR457GSIG</td>
<td>10.9</td>
<td>6.1</td>
</tr>
<tr>
<td>K391A</td>
<td>1.3</td>
<td>0.72</td>
</tr>
<tr>
<td>K599A</td>
<td>1.3</td>
<td>0.72</td>
</tr>
</tbody>
</table>

DNA-dependent ATPase activity of mutant VETFs

The ATPase activity of VETF is stimulated 20-30 fold in the presence of high concentrations of DNA (2). Because the ATPase is DNA-dependent, this activity can provide information on the DNA binding capability of the factor. At DNA concentrations that confer optimal activity on wild-type VETF, several of the mutants exhibited reduced ATPase activity (Fig. 3). As reported previously (4), mutation of K51 at the putative active site for ATP hydrolysis reduced activity to negligible levels. The mutations H22Q, Q23M and H45A clustered on the amino terminal side of the active site also severely impaired activity. The mutations R32Q and R39Q in this same region had no effect (data not shown). The mutant R454SIR457 to GGSIG also had negligible ATPase activity. Several mutants including K431A, K504Q, K510A, K512A and K514A exhibited ATPase activity that was in the range of 50-80% that of wild-type VETF. All other mutants had activities that were approximately normal as is shown for mutants K391A and K599A (Fig. 3).

The ATPase activity of VETF mutants was analyzed as a function of ATP concentration, and kinetic parameters for ATP hydrolysis were derived from Lineweaver–Burk plots of the data. The active site mutant K51Q exhibited reduced catalytic efficiency, expressed as the ratio $k_{cat}/K_m$, as expected (Table 2). The largest contribution to the impairment of catalysis by this mutant was from the $k_{cat}$ value. The mutants H22Q, Q23M and H45A were impaired in catalytic efficiency to a lesser degree, having both higher $K_m$ and lower $k_{cat}$ values than wild-type VETF. The mutants K431A, K504Q, K510A and K512A in the putative $\alpha$-helices had slightly reduced catalytic efficiencies for ATP hydrolysis, with small contributions from both the $K_m$ and $k_{cat}$ values. The mutants K391A, K599A and K514A in the putative $\alpha$-helices had slightly reduced catalytic efficiencies for ATP hydrolysis, with small contributions from both the $K_m$ and $k_{cat}$ values. The R454SIR457 mutant in helicase-like domain VI had a severely depressed catalytic efficiency. The $K_m$ and $k_{cat}$ values for hydrolysis by the domain VI mutant were nearly identical to that of the K51Q mutant. $K_m$ and $k_{cat}$ values for the apparently normal transcription activators K391A and K599A were essentially the same as wild-type VETF. The kinetic analyses described here indicate that the residues near the amino terminus adjacent to the putative ATP binding pocket may play a role in ATP hydrolysis, but the residues in the putative $\alpha$-helices in the C-terminal third of the polypeptide are not likely to directly participate in the ATPase activity. The reduced ATPase activity of the latter set of mutants may be the result of reduced DNA binding activity. The kinetic parameters of the domain VI mutant indicate that this sequence may directly participate in ATP hydrolysis. The kinetic parameters of this mutant were essentially indistinguishable from those of the K51Q mutant, strongly suggesting that both sequences are essential for catalysis.
Figure 4. DNA dependence of the ATPase activity of VETF mutants. Protein were incubated with [γ-32P]ATP in the presence of the indicated amount of DNA cofactor for 30 min, and phosphate release was determined. Legends specifying VETF mutants are inset on the upper left of each panel.

Table 2. Kinetic constants for ATP hydrolysis by mutant VETFs

<table>
<thead>
<tr>
<th>VETF</th>
<th>$K_m$ (mM)</th>
<th>$k_{cat}$ (nmol/ng VETF/30 min)</th>
<th>$k_{cat}/K_m \times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>0.077</td>
<td>0.40</td>
<td>5.0</td>
</tr>
<tr>
<td>K51Q</td>
<td>0.27</td>
<td>0.040</td>
<td>0.15</td>
</tr>
<tr>
<td>H22Q</td>
<td>0.40</td>
<td>0.17</td>
<td>0.43</td>
</tr>
<tr>
<td>Q23M</td>
<td>0.43</td>
<td>0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>H45A</td>
<td>0.18</td>
<td>0.12</td>
<td>0.67</td>
</tr>
<tr>
<td>K431A</td>
<td>0.11</td>
<td>0.18</td>
<td>1.6</td>
</tr>
<tr>
<td>K504Q</td>
<td>0.089</td>
<td>0.17</td>
<td>1.9</td>
</tr>
<tr>
<td>K510A</td>
<td>0.093</td>
<td>0.33</td>
<td>3.5</td>
</tr>
<tr>
<td>K512A</td>
<td>0.11</td>
<td>0.11</td>
<td>1.0</td>
</tr>
<tr>
<td>R454SIR457GSIG</td>
<td>0.27</td>
<td>0.036</td>
<td>0.13</td>
</tr>
<tr>
<td>K391A</td>
<td>0.061</td>
<td>0.26</td>
<td>4.3</td>
</tr>
<tr>
<td>K599A</td>
<td>0.054</td>
<td>0.24</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Previous studies demonstrated that the temperature-sensitive mutants A25T and V278M were defective for DNA binding and partially defective for ATP hydrolysis (7). ATPase activity could be restored to these mutants by very high concentrations of DNA suggesting that defective DNA binding was the basis for reduced ATPase activity expected if DNA binding and ATP hydrolysis are coupled. Some of the mutants described in the present study also exhibited this same behavior. Titration of ATPase reactions with increasing DNA concentrations resulted in enhanced ATP hydrolysis for mutants K431A, K504Q, K510A, K512A and K514Q (Fig. 4). This result indicates that the reduced ATPase activity of the mutants is the result of weakened DNA binding. Mutants H22Q, Q23M and H45A showed a modest but detectable response upon titration with high concentrations of DNA. In contrast, the ATPase activity of two mutants showed no detectable response to high DNA concentrations. The K51Q mutant with a presumably impaired active site, exhibited no activity. In addition the mutant R454SIR457 to GGSIG in helicase-like domain VI also had no detectable ATPase activity at any of the DNA concentrations tested.

DISCUSSION

We have previously described a mutant VETF small subunit from a temperature-sensitive vaccinia virus with a threonine for alanine substitution at residue 25 (7). This mutant was shown to be defective for DNA binding. Residue 25 is situated amino terminal to the putative active site for ATP hydrolysis. In the present study we have identified several residues in this region of the polypeptide that are important for transcription activation. The H22Q, Q23M, and H45A mutants were all defective as transcription activators. The H22Q and Q23M mutants proved to have defects in ATP hydrolysis but had normal DNA binding activity. The H45A mutant was impaired for DNA binding similar to the A25T mutation described previously. It is interesting to note that this histidine occupies a position in the consensus ATP binding
participate in the coupling of ATP hydrolysis to DNA binding that is essential for the ATPase. This is analogous to the phenotype domain has a direct role in ATP hydrolysis or alternatively, may be located in the same α-helix, all affected transcription activation by impairing DNA binding. Residue K43 predicted to be in a second α-helix also reduced DNA binding when mutated. The functional significance of two helicase-like motifs in the VETF small subunit was tested by replacement of residues that are highly conserved among nucleic acid helicases. The sequence SGS at residues 164–166 resembles the conserved domain III SAT helicase motif both in terms of sequence and proximity to other helicase motifs in the polypeptide. Replacement of both serine residues had no effect on transcription activator activity of VETF. Mutation of the hydroxyl-bearing residues in this motif in the herpes simplex I virus replication factor UL9 that is a DNA helicase (19), the translation initiation factor elf4A that is an RNA helicase (20), and the pre-mRNA splicing factor PRP2 that is a putative RNA helicase (22) all impair the function of the respective proteins. In the case of elf4A, mutation of domain III has been demonstrated to impair the RNA helicase activity without markedly affecting RNA binding or ATPase activity (20), suggesting a direct role for this domain in unwinding duplex RNA. Our results indicate that VETF does not have an equivalent to domain III of helicases. In this vein, VETF has not been demonstrated to have helicase activity despite repeated attempts (unpublished results).

The conserved arginine residues in helicase domain VI have been shown to be essential for the functions of elf4A (22) and herpes UL9 (19). Replacement of both conserved arginines (residues 454 and 457) in the sequence resembling domain VI in the VETF small subunit resulted in a protein with no detectable transcription activator or ATPase activities. Also this mutant had the most severely impaired DNA binding ability of mutants tested, having an equilibrium dissociation constant ($K_D$) of about six times that of wild-type VETF. These results indicate that this domain is essential for a vital function for VETF. The complete loss of ATPase activity under all conditions tested suggests that this domain has a direct role in ATP hydrolysis or alternatively, may participate in the coupling of ATP hydrolysis to DNA binding that is essential for the ATPase. This is analogous to the phenotype observed for elf4A domain VI mutants which were found to have no ATPase or RNA helicase activity and also had impaired RNA binding (22). It would appear that this domain may have similar functions for VETF and elf4A.

The findings of this study indicate that VETF has a domain structure that can be considered atypical of transcription regulator proteins. The assembly of mutants that we have studied is by no means exhaustive, and it is unlikely that all parts of VETF that interact with DNA have been identified. Indeed, not all DNA contacts are likely attributable to the small subunit alone since both subunits are required for stable association with promoter DNA (4). Nevertheless, the results presented here provide a basis for beginning to understand the structure and function of this unusual transcription activator protein. As summarized in Figure 5, the amino acids that were identified as being essential for DNA binding do not appear to be clustered in one region of the polypeptide but span over 400 residues in the sequence. Mutants impairing DNA binding cluster near the N-terminus and in the C-terminal third of the polypeptide. Between these sites are the helicase-like domains I, II and VI that are essential for ATP hydrolysis. The arrangement of the DNA binding domains and domains participating in ATP hydrolysis is indicative of functional domains that are overlapping and interdependent. An ATPase domain flanked by two domains that interact with DNA would suggest that DNA binding influences the folding of the ATPase active site rendering it competent for catalysis. The integration of the DNA binding and functional domains would explain why the ATPase is DNA-dependent and the dissociation of VETF from DNA is coupled to ATP hydrolysis. A plausible model for VETF function is suggested where the protein shifts between different conformations as it is bound to DNA or upon hydrolyzing ATP. Clearly further work will be required to verify this concept.

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