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Pattern of aromatic and hydrophobic amino acids critical for one of two subdomains of the VP16 transcriptional activator

(transcriptional activation/herpes simplex virus/site-directed mutagenesis/virion protein Vmw65/α-trans-inducing factor)

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ABSTRACT **Structural features of the transcriptional activation domain of the herpes simplex virion protein VP16 were examined by oligonucleotide-directed mutagenesis. Extensive mutagenesis at position 442 of the truncated VP16 activation domain (Δ456), normally occupied by a phenylalanine residue, demonstrated the importance of an aromatic amino acid at that position. On the basis of an alignment of the VP16 sequence surrounding Phe-442 and the sequences of other transcriptional activation domains, we subjected leucine residues at positions 439 and 444 of VP16 to mutagenesis. Results from these experiments suggest that bulky hydrophobic residues flanking Phe-442 also contribute significantly to the function of the truncated VP16 activation domain. Restoration of amino acids 457–490 to various Phe-442 mutants partially restored activity. Although the pattern of amino acids surrounding Phe-473 resembles that surrounding Phe-442, mutations of Phe-473 did not dramatically affect activity; in fact, Phe-475 appears more sensitive to mutations than does Phe-473. We infer that the two regions of VP16 (amino acids 413–456 and 457–490) possess unique structural features, although neither is likely to be an amphipathic α-helix or an "acidic blob." These results, considered with previous *in vitro* activation and inhibition studies, suggest that the two subdomains of VP16 affect transcription by different mechanisms.**

The mechanisms that regulate the rate of transcriptional initiation depend in part on factors termed transcriptional activators (1, 2). Several models have been proposed to explain how activators work. They may function by directly or indirectly contacting a component of the basal transcription complex, thereby speeding up the ordered assembly of that complex at the promoter (1–6). Activators could also increase the rate of transcriptional initiation by relieving the inhibition caused by histones, thus allowing the transcription complex to form (7–11). A third model posits that activators transform a preinitiation or initiation complex into an elongation complex, allowing transcription to proceed (12–14). These models need not be mutually exclusive; in fact, evidence exists for the participation of the transcriptional activation domain of VP16 (a prototypical activator) in several of these mechanisms. Because an understanding of how activators function is crucial to our understanding of transcriptional regulation, we have undertaken a detailed and systematic mutagenesis study of VP16.

VP16 [also known as α-trans-inducing factor (α-TIF) and Vmw65] is the component of the herpes simplex virus 1 virion that specifically activates transcription of the viral immediate early (IE) genes (15, 16). Many transcriptional activator proteins have two domains: one conferring specific association with promoter sequences, usually a DNA-binding domain, and a second domain for regulatory function (1, 2, 17).

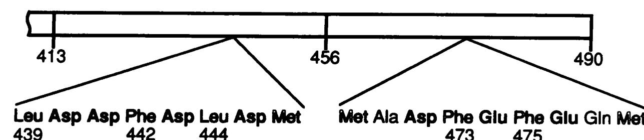

FIG. 1. Schematic representation of the VP16 activation domain (amino acids 413–490). The truncated VP16 activation domain (Δ456) lacks residues 457–490 (24, 31). Portions of the amino acid sequence are shown, using hollow type for hydrophobic amino acids and bold type for acidic amino acids.

In the case of VP16, the amino-terminal region of the protein interacts with host cell factors that bind to IE gene promoter elements (18–23), while the transcription activation function resides in the carboxyl-terminal 80 amino acids (24–28).

Various transcriptional activation domains, including the domain of VP16, are rich in acidic amino acids (29, 30). Previous work from our laboratory (31) demonstrated that net negative charge was necessary but not sufficient to account for the strong activation ability of VP16; other elements of protein structure also contributed to the activity. Giniger and Ptashne (32) proposed that acidic activation domains form amphipathic α-helices, and indeed insertion of a potentially helix-breaking proline residue at position 442 of the truncated VP16 activation domain (codons 413–456) abolished activity. However, replacing this proline residue with helix-compatible but nonaromatic amino acid residues (Ala, Ser) did not restore activity to the VP16 activation domain. Furthermore, substitution of two prolines simultaneously at positions 432 and 436 within the predicted α-helix also had no effect on activity. Here we report further mutations of VP16 that demonstrate the importance of an aromatic moiety at position 442 and strengthen the suggestion that the activity of this domain does not depend upon an amphipathic α-helix.

After the discovery of the importance of Phe-442, the amino acid sequence of the VP16 activation domain was compared to the sequences of other transcriptional activation domains. The sequences of several transcriptional activation domains were aligned, using as a guide six bulky hydrophobic residues of the VP16 activation domain (31). In a number of different types of activation domains, bulky hydrophobic residues are observed at positions that can be aligned with Leu-439 and Leu-444 of VP16, on either side of the critical Phe-442 (see Fig. 1). The only direct test of this pattern in another activation domain has recently been reported by Hardwick *et al.* (33), who found that this pattern is important for activation by the Rta protein of Epstein–Barr virus. In the work reported herein, these two leucine residues of VP16 were also targeted for mutagenesis; substitution of small hydrophobic or hydrophilic amino acids for either leucine greatly decreased activity.

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Abbreviations: IE, immediate early; tk, thymidine kinase.
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We also show that the addition of amino acids 457–490 to the truncated VP16 activation domain partially restores the activities of truncated VP16 mutants inactivated by changes at Phe-442. Within this added region, a phenylalanine at position 473 is found in a context similar to that surrounding Phe-442. Changing this residue to nonaromatic amino acids only modestly affected the activity of this region. Instead, a neighboring phenylalanine (at position 475) was somewhat more sensitive to mutations. Our work suggests that two regions of the VP16 activation domain have different structural elements and may have different functions for transcriptional activation.

MATERIALS AND METHODS

Mutagenesis and Cloning. *Sal* I/*Bam*HI fragments corresponding to the truncated (codons 411–456) or full-length (codons 411–490 plus 7 bp of 3′ nontranslated sequence) activation domain of VP16 (24) were inserted into M13mp19 (34). Oligonucleotide-directed mutagenesis was performed as described (31, 35, 36). Mutations were identified by dideoxy sequencing (37), and double-stranded phage DNAs containing the desired mutant VP16 activation domains were harvested. The *Sal* I/*Bam*HI fragments encoding the altered activation domains were cloned downstream of codons 1–410 by using the expression vector pMSVP16 (24).

Transient Transfection Assay. Mouse L cells were grown in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% fetal calf serum (HyClone). Cells (8×10^5 per 60-mm culture plate) were transfected by DEAE-dextran (38) with an expression plasmid pMSVP16 (50 ng), a reporter plasmid (2 μg of pICP4-tk) containing IE regulatory sequences fused to the body of the herpes simplex virus 1 thymidine kinase (tk) gene (24), and an internal control plasmid (2 μg of pMSV-tk) (39). Total RNA was harvested 48 hr after transfection as described (24).

Primer Extension Assay. A ^{32}P-labeled primer complementary to tk RNA was incubated with each RNA sample. The annealed primer was extended with avian myeloblastoma virus reverse transcriptase (Life Sciences, St. Petersburg, FL). Samples were treated with RNase A at 20 μg/ml, extracted with phenol/chloroform, and precipitated. The RNAs expressed from PICP4-tk and PMSV-tk yielded primer extension products of 81 and 55 bases, respectively; these products were separated by electrophoresis on a 9% acrylamide denaturing gel in 0.5× TBE buffer (1× TBE buffer = 90 mM Tris borate, pH 8.3/2 mM EDTA) and were detected by autoradiography.

Quantitation of Primer Extension Products. The developed film and the dried gel were aligned and portions of the gel corresponding to bands on the film were cut out. Cerenkov activity of each gel slice was detected by a 5-min scan on the tritium setting of a Packard 300 liquid scintillation counter. Alternatively, the dried gel was used to expose a phosphorus screen, and the image was detected by a Molecular Dynamics (Sunnyvale, CA) PhosphorImager. Radioactivity of each band was determined by using the IMAGEQUANT program and volume integration with correction for background.

Determination of Mutant Protein Stability. Mouse L cells were transfected with 10 μg of wild-type or mutant pMSVP16 as described above. Forty-eight hours after transfection, cells were lysed in SDS buffer (50 mM Tris·HCl, pH 7.5/0.2 M NaCl/20 mM EDTA/0.5% SDS) and sonicated, and total protein was precipitated with acetone. Protein pellets were resuspended in sample buffer and electrophoresed on a 4% polyacrylamide stacking gel followed by an SDS/10% polyacrylamide resolving gel. Proteins were electrophoretically transferred to nitrocellulose by using a Western Mini Transphor TE22 apparatus (Hoefer) and VP16 protein was detected by anti-VP16 antisera (24). Primary antibody was visualized by using a biotinylated secondary antibody and avidin/biotinylated enzyme complex (Vector Laboratories), the substrate for which was 4-chloro-1-naphthol.

RESULTS

Importance of an Aromatic Amino Acid at Position 442. Previous results from this laboratory (31) suggested that Phe-442 is critical for function of the truncated VP16 activation domain (Δ456). To more thoroughly test this hypothesis, additional amino acid substitutions were made at position 442. Activities of VP16 mutants were determined by transient transfection assays in which a plasmid expressing the VP16 gene was cotransfected with a reporter plasmid bearing the herpes simplex virus 1 tk gene under the control of a VP16-responsive promoter and an internal control plasmid consisting of the tk gene regulated by the Moloney murine sarcoma virus long terminal repeat promoter. Total RNA was harvested and the amount of tk RNA was quantitated by primer extension assay and scintillation counting. In our assays, the full-length VP16 protein activates expression of the reporter by 20-fold, and the truncated (Δ456) protein activates approximately 12-fold.

Of the 19 possible changes at position 442, 17 have been generated and their activities have been tested. Substitution of the other two aromatic amino acids, tyrosine and tryptophan, for Phe-442 decreased but did not abolish function (Fig. 2). No other substitution mutants had an activity greater than 15% relative to wild-type Δ456 (Table 1). The substitution of bulky hydrophobic residues (Leu, Ile, and Met) and the smaller hydrophobic residue Ala reduced activity to 10–15% of Δ456. All other substitution mutants were no more than 10% active (i.e., equivalent to controls lacking VP16 protein), with the exception of the asparagine substitution (11%). Interestingly, increasing the net negative charge by substitution of acidic residues (Glu, Asp) at position 442 had no positive effect. Immunoblots probed with polyclonal antisera directed against VP16 showed no significant differences in size or stability of the various mutant proteins (data not shown). These results conclusively support the previous suggestion (31) that the aromatic character of amino acid 442 is particularly important and that other hydrophobic residues at this position are less effective but retain some function.

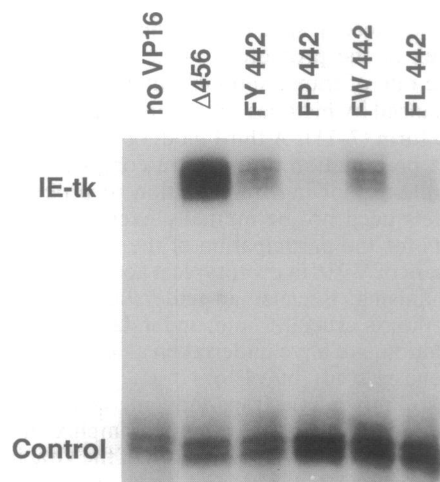

FIG. 2. Autoradiogram of primer extension assay reflecting the activities of truncated VP16 proteins altered at Phe-442. FY442 indicates Tyr substitution, etc., in the standard one-letter code. Positions of the reporter (IE-tk) and internal control primer extension products are indicated. The size and stability of every mutant protein reported in this paper were confirmed by Western blotting and immunodetection by anti-VP16 antisera (data not shown).

Table 1. Relative activities of truncated (Δ456) VP16 mutants bearing amino acid substitutions at position 442

Residue at position 442	Relative activity, % of WT Δ456
Tyr*	30 ± 10
Trp	36 ± 6
Leu	14 ± 2
Pro*	≤10
Ala*	14 ± 3
Ser*	≤10
Gly	≤10
Val	11 ± 2
Ile	14 ± 2
Met	12 ± 1
Lys	≤10
Arg	≤10
His	≤10
Asp	≤10
Glu	≤10
Asn	11 ± 2
Gln	≤10

Relative activities are calculated as the ratio of the reporter signal (IE-tk) to the internal control signal, normalized to the activity of wild-type Δ456. Means and standard deviations were calculated from at least four independent transfections.
*Activities previously reported (31).

Hydrophobic Residues Flanking Phe-442 Also Contribute to Activity. Alignment of the amino acid sequences of several transcriptional activation domains revealed an intriguing pattern of bulky hydrophobic residues (31). In the VP16 activation domain such residues include leucines at positions 439 and 444 flanking Phe-442 (Fig. 1). To determine if these leucines contribute to the activity of the truncated VP16 activation domain, single amino acid substitutions were made and tested in transient transfection assays. Substitution at either position of a small hydrophobic (Ala) or a hydrophilic residue (Ser) for leucine diminished activity significantly (Fig. 3). Substitution of another bulky hydrophobic residue (Val) or an aromatic hydrophobic residue (Phe) decreased activity to a lesser extent. A bulky hydrophobic amino acid was perhaps a slightly better substitute than an aromatic hydrophobic amino acid at either position. We conclude that the pattern of hydrophobic residues observed among different transcriptional activation domains is an indicator of amino acids important to the function of the truncated VP16 activation domain.

Addition of Amino Acids 457–490 Partially Restores Activity to Phe-442 Mutants. All of the mutations described above were tested in a VP16 activation domain truncated at amino acid 456. Previous deletion analyses demonstrated that this truncated activation domain possesses approximately 50% of wild-type activity (24). We wished to know if the presence of the last 34 amino acids of the activation domain could restore activity to those truncated proteins inactivated by mutations of Phe-442. Therefore we placed Phe-442 substitutions in the context of the full-length activation domain. Addition of amino acids 457–490 partially restored activity to VP16 mutants such that relative activities of the full-length proteins reflected the activities of the corresponding truncated mutants (Fig. 4). For example, truncated FA442 activated expression approximately 2-fold (15% of the activity of its truncated parent), whereas full-length FA442 activated expression approximately 9-fold (45% of wild-type full-length VP16). Similarly, the 4-fold activation by the truncated FW442 protein (approximately 35% of Δ456 activity) was increased to 14-fold activation (70% of wild-type VP16) upon addition of the remainder of the activation domain. Thus, replacing the distal domain restores partial activity to formerly defective Δ456 mutants.

Proximal and Distal Subdomains of VP16 Have Unique Structural Features. Examination of the sequence of residues 457–490 revealed a pattern of hydrophobic and aromatic residues similar to that surrounding Phe-442 (Fig. 1). This sequence similarity and the results of adding back amino acids 457–490 suggested that the activities of the proximal (amino acids 410–456) and distal (amino acids 457–490) subdomains of VP16 might rely on similar structural features. Two phenylalanines (Phe-473 and Phe-475) were identified within a pattern of acidic and hydrophobic residues similar to that surrounding Phe-442. Amino acid substitutions at these two positions were made, both in the wild-type Phe-442 background and in combination with the FA442 mutation. When the activities of the single mutants were tested in transient transfection assays, we saw little or no effect of substitutions at positions 473 or 475 in the context of wild-type Phe-442 (data not shown). Furthermore, when substitutions at positions 473 or 475 were tested in combination with the FA442 mutation, we observed that the types of

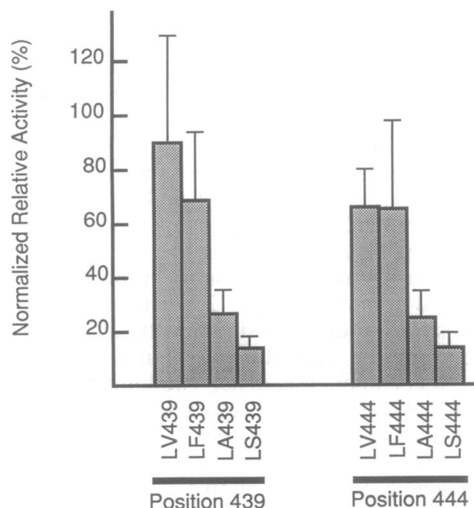

FIG. 3. Effects of amino acid substitutions for Leu-439 or Leu-444 of truncated VP16. Rectangles indicate mean relative activities calculated (as for Table 1) from at least five independent transfections; error bars represent 1 SD.

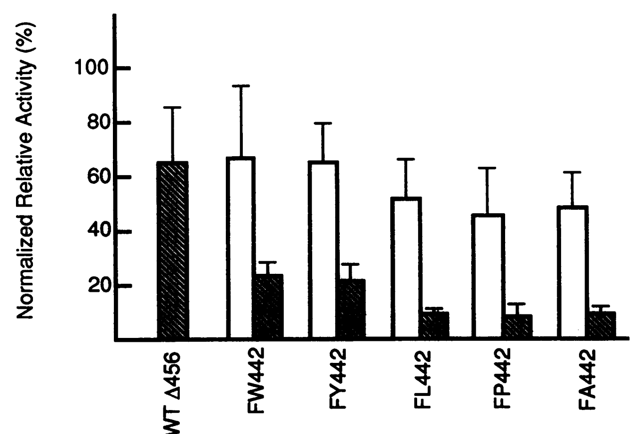

FIG. 4. Relative activities of full-length and truncated VP16 mutants bearing substitutions for Phe-442. Rectangles indicate mean relative activities calculated from at least five independent transfections; error bars represent 1 SD. Activities for this figure have been normalized to the activity of full-length wild-type VP16. Open rectangles represent full-length VP16 mutants; hatched rectangles represent truncated mutants.

substitutions that inactivated the proximal subdomain had much less effect on the activity of the distal subdomain (Fig. 5). Of the substitutions at position 473, the proline and alanine substitutions were not significantly less active than leucine or tyrosine substitutions.

Substitutions at position 475 had more pronounced effects. Proline or alanine substitutions decreased activity to approximately 50% of that of full-length FA442, while substitution of bulky hydrophobic residues leucine or valine decreased activity to approximately 80% and 65%, respectively. The substitution of a tyrosine residue had no significant effect on activity. Thus, similar kinds of mutations at corresponding positions in the 442 region and the 473 region differ quantitatively in their effect on VP16 activity. We conclude from these results that the proximal and distal subdomains of VP16 have distinct structural features dependent on different patterns of amino acids.

DISCUSSION

Using oligonucleotide-directed mutagenesis and transient transfection assays, we have analyzed the effects of amino acid substitutions on the activities of both truncated and full-length VP16 activation domains. In the truncated (Δ456) domain, we have now mutated residue Phe-442 to nearly all other residues. Aromatic amino acids were the best substitutes for Phe-442, retaining approximately 30–35% of the activity of wild-type Δ456. Other hydrophobic substitutions reduced activity to between 10% and 15% of wild type, while all other substitutions reduced activity to no more than 10% of wild type, essentially indistinguishable from controls in which no VP16 is present. Mutational analysis of leucine codons 439 and 444 revealed that, although these positions are somewhat less sensitive to mutation, hydrophobic residues at these positions also contribute to the activity of the truncated VP16 activation domain. Thus, using the pattern of bulky hydrophobic residues previously observed (31) among a variety of transcriptional activation domains, we have accurately predicted the importance of such residues in the truncated VP16 domain. The importance of this pattern has also been revealed in mutational analyses of the Epstein–Barr virus Rta protein (33), suggesting that this pattern fits at least a subset of activator proteins. Whether this pattern has predictive value for other classes of activation domains remains to be tested.

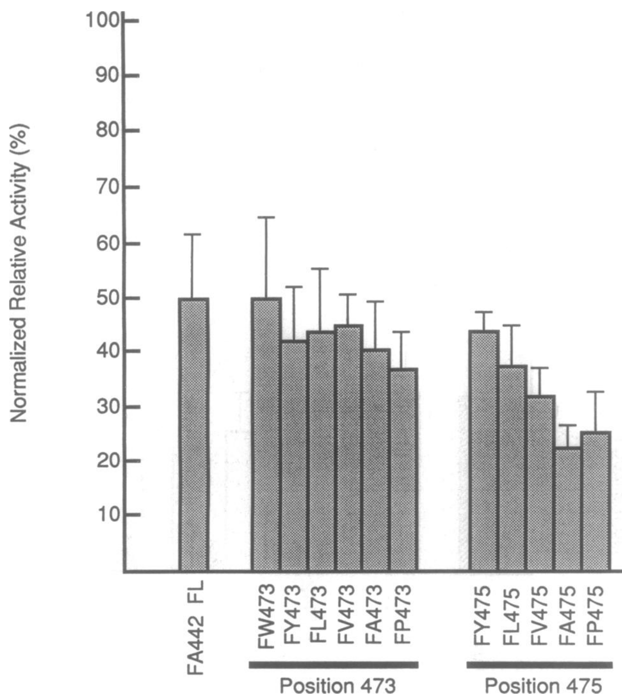

FIG. 5. Effects of amino acid substitutions at Phe-473 or Phe-475 of VP16, tested in the context of the FA442 mutation. Rectangles indicate mean relative activities (normalized to full-length wild-type VP16) calculated from at least five independent transfections; error bars represent 1 SD. FA442 FL represents the activity of the FA442 mutation in full-length VP16 (taken from Fig. 4).

These new results strengthen our previous arguments against prior hypotheses about structural features of acidic activation domains. On one hand, VP16 seems not to fit the "acid blob or negative noodle" hypothesis (40), which (stated simplistically) suggests that the activity of such domains is primarily a function of net negative charge (30, 41). Many of our mutations of VP16 have no effect on net charge, and yet they have dramatic effects on transcriptional activation. Furthermore, increasing the net negative charge by replacing Phe-442 with Asp or Glu had detrimental rather than beneficial effects on VP16 activity. Apparently, having an aromatic or hydrophobic residue at position 442 is of greater importance than is the net charge of the domain. On the other hand, our results are also inconsistent with a model of VP16 as an amphipathic α-helix (32, 42). We have previously shown that introducing two potentially helix-breaking proline residues at positions 432 and 436 in the predicted helix of truncated VP16 has no effect on transcriptional activation (31), suggesting that the predicted helix (if it does exist) is not necessary for VP16 function. Here, we show the deleterious effect of replacing Phe-442 with any of a number of residues predicted to maintain an amphipathic α-helix, suggesting further that such a structure is not sufficient for activity. These conclusions from our mutational analysis are reinforced by recent spectroscopic studies of the VP16, GCN4, and GAL4 activation domains (refs. 43–45; S. A. Johnston, personal communication) that found little evidence of helical structure.

Although our mutations in the truncated VP16 activation domain indicate that an aromatic amino acid is strongly preferred at position 442, we do not yet understand the reason for this preference. The two simplest ideas for the role of Phe-442 are either that it is necessary for maintaining the structure of the domain or that it is directly involved in interactions with target proteins in the activation mechanism. This question can be addressed by probing the structure of VP16, using spectroscopic or crystallographic methods, and by exploring the association of VP16 with putative target proteins.

In addition to the analysis of the truncated activation domain, we have begun to examine the role of the extreme carboxyl-terminal or distal subdomain of VP16 (amino acids 457–490). Adding this subdomain onto defective Phe-442 mutants partially restored transcriptional activity (Fig. 4). Intriguingly, the pattern of acidic and bulky hydrophobic amino acids surrounding Phe-473 in this subdomain strikingly resembles the pattern surrounding Phe-442. Furthermore, a linker insertion mutation at codon 471 reportedly abolished transcriptional activity of VP16 (26). However, the types of amino acid substitutions that significantly affected the activity of the truncated activation domain had quantitatively different effects upon the distal subdomain. Specifically, Phe-473 (best aligned with Phe-442) was relatively insensitive to mutations, whereas Phe-475 (best aligned with Leu-444) was somewhat more sensitive. These results imply that the pattern of acidic and hydrophobic amino acids, although useful in predicting important residues surrounding Phe-442, does not correctly describe key residues of the distal subdomain. On the other hand, although secondary structure prediction algorithms suggest that residues 468–478 might fold into an amphipathic α-helix, our mutational analysis

argues against this notion. First, a proline substitution for Phe-473 had little effect on the apparent activity of the distal subdomain. Second, the replacement of Phe-475 with Ala should be compatible with an amphipathic helix, and yet it had the greatest effect on the activity of this subdomain. We conclude that the proximal and distal subdomains both contribute to the overall activity of VP16, but the structural features necessary for the function of the two subdomains must differ considerably.

A number of potential mechanisms for transcriptional activation are now being investigated. The mechanism may involve a direct interaction of activators with basal transcription factors. For example, both TFIID (the TATA-binding protein; refs. 46 and 47) and TFIIB (48) reportedly can bind to the activation domain of VP16 and, less efficiently, to the proximal subdomain itself. Alternatively or perhaps additionally, activators such as VP16 might operate through adaptor proteins (also termed coactivators or mediators), as suggested by both biochemical and genetic experiments (49–52).

Although its activity is less than that of the full-length activation domain, the proximal subdomain of VP16 is clearly sufficient to activate transcription independent of the distal subdomain, perhaps by virtue of interactions with basal factors. The role of the distal subdomain, however, remains ambiguous. Its presence may support or stabilize the proximal subdomain and therefore compensate for mutations of Phe-442 that would otherwise disable the proximal subdomain. Alternatively, the distal subdomain may have an independent ability to activate transcription; the ability of the full-length but not the truncated protein to interact with a putative adaptor protein (49) supports this contention. In contrast, however, deletion of the proximal subdomain (codons 413–443) abolished the activity of the one mutant tested (24). Additional deletion mutants should permit testing this hypothesis more thoroughly.

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1. Ptashne, M. (1988) *Nature (London)* **335**, 683–689.
2. Mitchell, P. J. & Tjian, R. (1989) *Science* **245**, 371–378.
3. Buratowski, S., Hahn, S., Guarente, L. & Sharp, P. A. (1989) *Cell* **56**, 549–561.
4. Lewin, B. (1990) *Cell* **61**, 1161–1164.
5. Ptashne, M. & Gann, A. A. F. (1990) *Nature (London)* **346**, 329–331.
6. Wang, W., Carey, M. & Gralla, J. D. (1992) *Science* **255**, 450–453.
7. Workman, J. L., Taylor, I. C. A. & Kingston, R. E. (1991) *Cell* **64**, 533–544.
8. Croston, G. E., Kerrigan, L. A., Lira, L. M., Marshak, D. R. & Kadonaga, J. T. (1991) *Science* **251**, 643–649.
9. Laybourn, P. J. & Kadonaga, J. T. (1991) *Science* **254**, 238–245.
10. Kornberg, R. D. & Lorch, Y. (1991) *Cell* **67**, 833–836.
11. Felsenfeld, G. (1992) *Nature (London)* **355**, 219–224.
12. Spencer, C. A. & Groudine, M. (1990) *Oncogene* **5**, 777–786.
13. Rougvie, A. E. & Lis, J. T. (1990) *Mol. Cell. Biol.* **10**, 6041–6045.
14. Kerppola, T. K. & Kane, C. M. (1991) *FASEB J.* **5**, 2833–2842.
15. Post, L. E., Mackem, S. & Roizman, B. (1981) *Cell* **24**, 555–565.
16. Campbell, M. E. M., Palfreyman, J. W. & Preston, C. M. (1984) *J. Mol. Biol.* **180**, 1–19.
17. Brent, R. & Ptashne, M. (1985) *Cell* **43**, 729–736.
18. McKnight, J. L. C., Kristie, T. M. & Roizman, B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7061–7065.
19. Gerster, T. & Roeder, R. G. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6347–6351.
20. Preston, C. M., Frame, M. C. & Campbell, M. E. M. (1988) *Cell* **52**, 425–434.
21. O'Hare, P. & Goding, C. R. (1988) *Cell* **52**, 435–445.
22. O'Hare, P., Goding, C. R. & Haigh, A. (1988) *EMBO J.* **7**, 4231–4238.
23. Triezenberg, S. J., LaMarco, K. L. & McKnight, S. L. (1988) *Genes Dev.* **2**, 730–742.
24. Triezenberg, S. J., Kingsbury, R. C. & McKnight, S. L. (1988) *Genes Dev.* **2**, 718–729.
25. Sadowski, I., Ma, J., Triezenberg, S. & Ptashne, M. (1988) *Nature (London)* **335**, 563–564.
26. Werstuck, G. & Capone, J. P. (1989) *Gene* **75**, 213–224.
27. Cousens, D. J., Greaves, R., Goding, C. R. & O'Hare, P. (1989) *EMBO J.* **8**, 2337–2342.
28. Greaves, R. & O'Hare, P. (1989) *J. Virol.* **63**, 1641–1650.
29. Hope, I. A. & Struhl, K. (1986) *Cell* **46**, 885–894.
30. Ma, J. & Ptashne, M. (1987) *Cell* **48**, 847–853.
31. Cress, W. D. & Triezenberg, S. J. (1991) *Science* **251**, 87–90.
32. Giniger, E. & Ptashne, M. (1987) *Nature (London)* **330**, 670–672.
33. Hardwick, J. M., Tse, L., Applegren, N., Nicholas, J. & Veliuona, M. A. (1992) *J. Virol.* **66**, 5500–5508.
34. Norrander, J., Kempe, T. & Messing, J. (1983) *Gene* **26**, 101–106.
35. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 488–492.
36. Zoller, M. J. & Smith, M. (1982) *Nucleic Acids Res.* **10**, 6487–6500.
37. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
38. Lopata, M. A., Cleveland, D. W. & Sollner-Webb, B. (1984) *Nucleic Acids Res.* **12**, 5707–5717.
39. Graves, B. J., Eisenman, R. N. & McKnight, S. L. (1985) *Mol. Cell. Biol.* **5**, 1948–1958.
40. Sigler, P. B. (1988) *Nature (London)* **333**, 210–212.
41. Gill, G. & Ptashne, M. (1987) *Cell* **51**, 121–126.
42. Zhu, Q. L., Smith, T. F., Lathrop, R. H. & Figge, J. (1990) *Proteins Struct. Funct. Genet.* **8**, 156–163.
43. Donaldson, L. & Capone, J. P. (1992) *J. Biol. Chem.* **267**, 1411–1414.
44. Van Hoy, M., Hansen, A. & Kodadek, T. (1992) *J. Am. Chem. Soc.* **114**, 362–363.
45. O'Hare, P. & Williams, G. (1992) *Biochemistry* **31**, 4150–4156.
46. Stringer, K. F., Ingles, C. J. & Greenblatt, J. (1990) *Nature (London)* **345**, 783–786.
47. Ingles, C. J., Shales, M., Cress, W. D., Triezenberg, S. J. & Greenblatt, J. (1991) *Nature (London)* **351**, 588–590.
48. Lin, Y.-S. & Green, M. R. (1991) *Cell* **64**, 971–981.
49. Berger, S. L., Cress, W. D., Cress, A., Triezenberg, S. J. & Guarente, L. (1990) *Cell* **61**, 1199–1208.
50. Kelleher, R. J., III, Flanagan, P. M. & Kornberg, R. D. (1990) *Cell* **61**, 1209–1215.
51. Pugh, B. F. & Tjian, R. (1990) *Cell* **61**, 1187–1197.
52. Berger, S. L., Piña, B., Silverman, N., Marcus, G. A., Agapite, J., Regier, J. L., Triezenberg, S. J. & Guarente, L. (1992) *Cell* **70**, 251–265.