JOURNAL OF VIROLOGY, June 1994, p. 3803–3808
0022-538X/94/$04.00+0

Vol. 68, No. 6

# Genetic Analysis Indicates that the Human Foamy Virus Bel-1 Protein Contains a Transcription Activation Domain of the Acidic Class

WADE S. BLAIR,[1] HAL BOGERD,[1] AND BRYAN R. CULLEN[1,2]*

*Howard Hughes Medical Institute[1] and Section of Genetics,[2] Duke University Medical Center, Durham, North Carolina 27710*

Received 21 January 1994/Accepted 18 March 1994

**Human foamy virus encodes a nuclear regulatory protein, termed Bel-1, that serves as a potent activator of viral transcription. Mutational analysis has identified a small, discrete activation domain within Bel-1 that is highly active in both higher and lower eukaryotic cells. Here, we demonstrate that the activation domain of Bel-1 is highly dependent on the *ADA2* transcriptional adaptor for biological activity in yeast cells, a property previously shown to be a hallmark of the VP16 class of acidic transcriptional activators (S. L. Berger, B. Pina, N. Silverman, G. A. Marcus, J. Agapite, J. L. Regier, S. J. Triezenberg, and L. Guarente, Cell 70:251–265, 1992). Using genetic selection in yeast cells, we have derived a set of point mutants within the Bel-1 activation domain that display a qualitatively similar loss in activation potential when examined in either yeast or human cells. These data indicate that the Bel-1 activation domain functions similarly in both lower and higher eukaryotes and strongly suggest that Bel-1 belongs to the VP16 class of acidic transcription factors.**

The primate foamy viruses encode a potent transcriptional *trans* activator of their homologous long terminal repeat promoter element (17, 24, 28, 34). In the case of human foamy virus (HFV), the prototype of this class of complex retroviruses, this is a 300-amino-acid (300-aa) nuclear regulatory protein, termed Bel-1, that is essential for HFV replication in culture (17, 22). Because the target sequence for Bel-1 is located entirely 5′ to the start site for viral transcription, it has been proposed that Bel-1 acts via a DNA target element (17, 19, 28, 34). However, both the precise DNA sequence requirements for Bel-1 function and whether Bel-1 interacts directly or indirectly with its target sites remain to be determined.

Recent mutational analyses of both Bel-1 and the analogous Taf *trans* activator of simian foamy virus have demonstrated that these proteins contain a small, highly conserved transcription activation domain located proximally to their carboxy termini (8, 15, 23, 33). It has, in particular, been demonstrated that fusion of either of these closely similar activation domains to the DNA-binding domain of GAL4 results in the efficient induction of transcription from minimal promoter elements that contain GAL4 DNA-binding sites. Of interest, the Bel-1 activation domain was found to be fully functional not only in mammalian cells but also in cells of lower eukaryotic origin (8).

The apparently ubiquitous activity of the activation domain of Bel-1 suggested that it belongs to the acidic class, the only class of activation domains known to demonstrate this property (14). However, inspection of the Bel-1 activation domain does not reveal any particular preponderance of acidic amino acids or any evident homology to well-characterized acidic activation domains such as that in the herpes simplex virus VP16 protein (6, 8, 11, 33).

Recently, Berger et al. (3) reported that overexpression of a GAL4 fusion protein containing a potent acidic activation domain, such as that in VP16, is toxic in yeast cells. This toxicity, as well as the ability of these fusion proteins to function as effective transcriptional activators at lower levels of expression, was found to be dependent on the functional expression of the yeast *ada2* gene. *ada2* was therefore proposed to function as a specific transcriptional adaptor for at least some *trans* activators of the acidic class (3). Here, we demonstrate that overexpression of a GAL4/Bel-1 fusion protein is also toxic in yeast cells. Both the toxicity and the activation potential of the GAL4/Bel-1 hybrid are shown to be highly dependent on the functional expression of the ADA2 protein. The hypothesis that the function of this domain is conserved in higher and lower eukaryotic cells was strongly supported by the observation that a series of point mutants introduced into the Bel-1 activation domain exhibit closely similar phenotypes in both in vivo contexts. On the basis of these findings, we therefore propose that the Bel-1 and VP16 transcription activation domains belong to the same functional class.

## MATERIALS AND METHODS

**Expression plasmids.** Plasmid pGAL4/BEL(260-290), which directs the expression of the GAL4 DNA-binding domain (aa 1 to 147) fused to the minimal Bel-1 activation domain (aa 260 to 290) in mammalian cells, was derived from the GAL4 DNA-binding domain expression plasmid pSG424 as previously described (8, 30). Mutated Bel-1 activation domains were substituted into pGAL4/BEL(260-290) in place of the wild-type sequence to generate plasmids pB1 to pB11. The derivation of the chloramphenicol acetyltransferase (*cat*) gene-based indicator plasmid pG6(−83)HIVLTRΔTAR has been described elsewhere (31).

Both the multicopy, 2μm-based yeast expression plasmid pGAL4 and the single-copy, ARS-CEN-based yeast plasmid pYCplacIII have been described previously (4, 9). In pGAL4, the *ADH1* promoter is located 5′ to residues 1 to 117 of the GAL4 DNA-binding domain. Sequences corresponding to the activation domains of VP16 (aa 413 to 490), TFE3 (aa 2 to 89), p65 (aa 416 to 550), and HAP4 (aa 330 to 553) (1–3, 6) were amplified by PCR with primers that introduced unique *Xba*I

* Corresponding author. Mailing address: Howard Hughes Medical Institute, Duke University Medical Center, Box 3025, Durham, NC 27710. Phone: (919) 684-3369. Fax: (919) 681-8979.

and *Eco*RI restriction endonuclease sites and were then inserted 3′ to, and in frame with, the GAL4 DNA-binding domain by using these sites. Identical activation domain sequences were also amplified with primers that introduced flanking *Bam*HI sites prior to insertion 3′ to the *ADH1* promoter and GAL4 DNA-binding domain in pYCplacIII. The yeast expression plasmids pY-GAL4/BEL(1-300), pY-GAL4/BEL(260-290), pY-GAL4/TAF(1-308), pY-GAL4/TAF (264-308), and pY-GAL4/TAT(2-86) have been described previously (4, 8).

pADA2 was constructed by PCR amplification of the full-length *ada2* gene from yeast strain PSY316 (3) genomic DNA using primers that introduced unique flanking *Nhe*I sites. The amplified *ada2* gene was then introduced into plasmid pVT101UR (35), at a unique *Xba*I site; the resulting pADA2 plasmid expresses the *ada2* gene under control of the *ADH1* promoter.

**Yeast growth and transformation.** All pGAL4-derived yeast plasmids contain the yeast HIS3 marker and were transformed into the yeast strain PSY316, PSY316ada2$^-$ (3), or GGY1::171 (10) as previously described (4). After 3 days on supplemented synthetic dextrose plates lacking histidine (His$^-$), yeast colony growth was scored. pGAL4-derived yeast plasmids were also cotransformed with pADA2, which contains the URA3 marker, into yeast strain PSY316ada2$^-$. After 3 days of selection on His$^-$, uridine-deficient dextrose plates, colony growth was scored.

**Analysis of transcriptional activity in yeast cells.** Yeast ARS-CEN plasmids encoding GAL4 hybrids were cotransformed into yeast strain PSY316 or PSY316ada2$^-$ with the reporter plasmid pLGSD5 (13), which contains the β-galactosidase (β-Gal) gene under the control of 5′-promoter-proximal GAL4 DNA-binding sites. pY-GAL4/BEL(260-290) yeast plasmid derivatives were transformed into yeast strain GGY1::171, which contains an integrated β-Gal gene flanked 5′ by GAL4 DNA-binding sites (10). Three days after growth selection, colonies were transferred into synthetic sucrose liquid media and incubated until cultures were at equivalent optical density units. Yeast cell extracts were then assayed for β-Gal activity as described elsewhere (4).

**Mutation of the Bel-1 activation domain.** Sequences corresponding to the Bel-1 activation domain (aa 260 to 290) were amplified by PCR under conditions that favor single nucleotide substitution mutations (20) with primers that introduce unique flanking *Xba*I and *Eco*RI restriction endonuclease sites. Briefly, the PCR was carried out under conditions limiting for dATP (final concentration, 10 mM) in the presence of 100 mM dTTP, 100 mM dCTP, and 100 mM dGTP. The PCR buffer used was purchased from Perkin-Elmer Cetus, with $MnCl_2$ and $MgCl_2$ added to final concentrations of 50 and 2 mM, respectively. Amplified products were then introduced into the pGAL4 yeast expression plasmid at the unique *Xba*I and *Eco*RI sites. The resulting plasmids were then pooled and transformed into the yeast strain GGY1::171. After 3 days of selection on His$^-$ plates, large colonies were harvested and plasmid DNA was recovered by yeast cell lysis, phenol-chloroform extraction, and ethanol precipitation. Recovered plasmid DNA was sequenced to identify mutations in the encoded Bel-1 activation domain. Plasmids which contained mutations in the Bel-1 activation domain were then analyzed for transcriptional activation potential as described above.

**Mammalian cell culture and analysis.** HeLa cells and COS cells were maintained as previously described (15, 17) and were transfected by the calcium phosphate procedure and the DEAE-dextran procedure, respectively (7). Activity of GAL4/Bel-1 fusion proteins was assayed in HeLa cells by cotransfection of the pGAL4/BEL(260-290) plasmid, or mutant derivatives, together with the indicator plasmid pG6(83)HIV LTRΔTAR (31). Induced chloramphenicol acetyltransferase activity was assayed at ~48 h posttransfection as previously described (26).

Appropriate expression of mutant derivatives of pGAL4/BEL(260-290) was confirmed by Western blot (immunoblot) analysis. Briefly, COS cells were transfected with either the parental GAL4 DNA-binding domain expression plasmid pSG424 (30), pGAL4/BEL(260-290), or mutant derivatives of pGAL4/BEL(260-290). At 48 h after transfection, transfected COS cell cultures were harvested in Laemmli sample buffer (18), boiled, and subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Resolved proteins were then transferred to nitrocellulose and analyzed by Western blot using a 1:1,000 dilution of a rabbit polyclonal antiserum directed against the DNA-binding domain of GAL4 (aa 1 to 147) (8, 10) followed by $^{125}$I-protein A. Reactive proteins were visualized by autoradiography.

## RESULTS

It has been demonstrated that overexpression of hybrid proteins consisting of the GAL4 DNA-binding motif fused to a potent acidic activation domain is toxic in yeast cells and that this toxicity is mediated by the functional expression of the yeast *ada2* gene product (3). To examine whether the Bel-1 and Taf activation domains also displayed this property, we constructed a series of yeast expression plasmids containing the GAL4 DNA-binding domain (aa 1 to 117) fused to the full-length Bel-1 and Taf proteins or to the minimal Bel-1 and Taf C-terminal activation domains (aa 260 to 290 and 264 to 308, respectively). These hybrid proteins were then expressed under the control of the constitutive alcohol dehydrogenase (*ADH1*) promoter element present in the multicopy pGAL4 yeast expression plasmid (4). Control constructs either lacking a functional activation domain or containing the GAL4 DNA-binding domain (aa 1 to 117) fused to potent acidic activation domains derived from the mammalian transcription factors VP16 (aa 413 to 490), TFE3 (aa 2 to 89), and NF-KB/p65 (aa 416 to 550) were also tested, as was a plasmid containing the GAL4 DNA-binding domain fused to the human immunodeficiency virus type 1 Tat protein (aa 2 to 86). These plasmids were introduced into either the PSY316(wt) or PSY316ada2$^-$ yeast strains that differed only in whether they possessed a functional copy of the *ada2* gene (3). The transformed cells were then selected for functional expression of the *HIS3* gene product encoded within these pGAL4-based yeast expression plasmids.

As shown in Table 1, introduction into the parental or the *ada2* yeast cells of vectors that expressed the GAL4 DNA-binding domain alone or a fusion of GAL4 to human immunodeficiency virus type 1 Tat resulted in large numbers of healthy yeast colonies upon selection for growth on His$^-$ plates. In contrast, all three vectors expressing fusions of GAL4 to known acidic activation domains gave visible colonies only in the context of the *ada2* yeast strain during the same selection period. Similarly, plasmids expressing fusions of GAL4 to either the Bel-1 or the Taf activation domain also gave rise to large numbers of transformants in the *ada2* yeast strain but either no detectable transformants (Taf) or only minute colonies (Bel-1) when introduced into the parental strain. The specificity of the requirement for ADA2 was further demonstrated by the finding that the growth of the *ada2* cells containing these hybrid transcription factors was dramatically inhibited upon introduction of a plasmid express-

TABLE 1. Effects of overexpression of GAL4 fusion proteins on yeast colony growth

| Activation domain (aa)[a] | Colony growth[b] | | |
|---|---|---|---|
| | WT | *ada2* | *ada2* + ADA2[c] |
| None | ++ | ++ | ++ |
| Tat (2–86) | ++ | ++ | ND |
| VP16 (413–490) | − | ++ | − |
| TFE3 (2–89) | − | ++ | − |
| p65 (416–550) | − | ++ | − |
| Bel-1 (1–300) | ± | ++ | − |
| Bel-1 (260–290) | ± | ++ | − |
| Taf (1–308) | − | ++ | ND |
| Taf (264–308) | − | ++ | ND |

[a] Fused to the C terminus of the GAL4 DNA-binding domain. None, GAL4 DNA-binding domain only.

[b] Plasmids expressing GAL4 fusion proteins were transformed into yeast strain PSY316(wt) (WT) or PSY316ada2$^-$ (*ada2*) (3). Transformants were selected for 3 days on His$^-$ dextrose plates and scored for growth as follows: ++, 200 to 500 large colonies; ±, 200 to 500 small colonies; −, no visible colonies.

[c] Fusion protein expression plasmids were cotransformed with pADA2 into yeast strain PSY316ada$^-$, selected on His$^-$ Ura$^-$ dextrose plates for 3 days, and scored for growth. ND, not done.

ing the ADA2 protein. As shown in Table 1, when plasmids expressing the hybrid transcription factors and the full-length ADA2 protein were cotransformed into the *ada2* yeast strain, no detectable transformants were recovered.

A further characteristic of acidic activation domains of the VP16 class in yeast cells is that their activation function is also highly dependent on ADA2 expression (3). In order to examine this property, it was necessary to circumvent the toxicity associated with high-level expression of these GAL4 fusion proteins. We therefore introduced sequences corresponding to the *ADH1* promoter and a subset of the above-mentioned GAL4 hybrids into the single-copy yeast expression plasmid YCplacIII (9). The resulting plasmids were transformed into the wild-type or *ada2* yeast strains together with the indicator plasmid pLGSD5, which contains the β-Gal gene under the control of a minimal CYC1 promoter and 5′-promoter-proximal GAL4 DNA-binding sites (13). After 3 days, transformants were grown in liquid media under appropriate selection conditions (see Materials and Methods) until cultures were at equivalent optical densities. Yeast cell extracts were then prepared and assayed for β-Gal activity. As shown in Table 2, both the GAL4/VP16 and the GAL4/TFE3 fusion proteins retain <3% of their activation potential in the *ada2* yeast strain compared with the wild-type yeast. Similarly, the GAL4/Bel-1 fusion protein, while clearly less active than the GAL4/VP16 hybrid, also lost ~99% of its activation potential when examined in the absence of a functional *ada2* gene product. In contrast, a hybrid protein consisting of the GAL4 DNA-binding domain fused to the activation domain of the yeast transcription factor HAP4 was, as previously reported by Berger et al. (3), only modestly affected by the loss of *ada2*. The level of expression of each of these fusion proteins was similar in both the parental and *ada2* yeast cells, as determined by Western blot analysis (data not shown), thus eliminating trivial reasons for this phenotypic difference. Overall, it therefore appears that the Bel-1 activation domain is similar to those of VP16 and TFE3, and dissimilar to that of HAP4, in that it is highly dependent on the ADA2 protein for transcriptional activation function in yeast cells.

TABLE 2. Transcriptional activation in yeast cells by GAL4 fusion proteins expressed from single-copy plasmids

| Activation domain (aa) | β-Gal activity[a] | |
|---|---|---|
| | WT | *ada2* |
| None | 1 | 20 |
| VP16 (413–490) | 15,320 | 320 |
| TFE3 (2–89) | 758 | 18 |
| HAP4 (330–553) | 4,100 | 980 |
| Bel-1 (260–290) | 271 | 3 |

[a] Determined as described in Materials and Methods after cotransformation of yeast strain PSY316(wt) (WT) or PSY316ada2$^-$ with single-copy yeast plasmids encoding the indicated GAL4 hybrids and the reporter plasmid pLGSD5. The data are milli-optical density units per milliliter of extract, corrected for any dilution of extract required to maintain samples in the measurable range.

**Genetic selection of Bel-1 activation domain mutants.** The data presented in Tables 1 and 2 reveal that the biological activity of the Bel-1 activation domain in yeast cells, in terms of both toxicity and transcriptional activation, is highly dependent on the functional expression of ADA2. This strongly suggests, as indeed previously proposed for the VP16 activation domain (3), that toxicity in yeast cells results from trapping of essential transcription factors at inappropriate genomic sites. Therefore, mutations that reduce the activation potential of this Bel-1 sequence should also attenuate toxicity in yeast cells. To test this hypothesis, we therefore devised a genetic screen to identify missense mutations in the Bel-1 activation domain that relieve its toxicity in yeast cells. The DNA sequence encoding the Bel-1 activation domain (aa 260 to 290) was amplified by PCR under conditions that favor the incorporation of single nucleotide missense mutations (20). The amplified DNA fragment was then cloned into the multicopy pGAL4 fusion protein yeast expression plasmid described above and transformed into the ADA2$^+$ yeast strain GGY1::171. Large yeast colonies were then isolated, and the plasmid DNA was recovered and subjected to sequence analysis. Plasmids containing potentially interesting mutations in the Bel-1 activation domain, i.e., missense rather than nonsense or frameshift mutations, were then analyzed for transcriptional activity by reintroduction into the yeast strain GGY1::171 (10), which contains an integrated β-Gal gene under the control of a $GAL_{UAS}$. In addition, these Bel-1 activation domain mutants were also cloned into the analogous mammalian GAL4 fusion protein expression plasmid pSG424 (30) and analyzed for activity in HeLa cells by using the reporter plasmid pG6(−83)HIVLTRΔTAR, which contains the *cat* gene under the control of a minimal human immunodeficiency virus type 1 long terminal repeat promoter element flanked by 5′-promoter-proximal GAL4 DNA-binding sites (31).

As shown in Fig. 1, this genetic selection identified 11 distinct mutants of the GAL4 activation domain that resulted in attenuated toxicity in yeast cells. Remarkably, all but two of the observed missense mutations were located within, or immediately adjacent to, the highly conserved 15-aa core activation domain of Bel-1 (15, 35), while approximately one-third of these missense mutations resulted in the insertion of basic amino acids. Analysis of the transcriptional activation potential of these mutant Bel-1 activation domains in yeast cells (Table 3) revealed that all displayed reduced activity, as predicted. Of particular interest was the tryptophan 279-to-arginine mutation, which, either alone or in combination with other point mutations, totally blocked the activity of this chimeric transcription factor. Also totally inactive was mutant B9, which contains mutations at methionine 273 and tyrosine 283.

Analysis of the biological activity of these mutant Bel-1

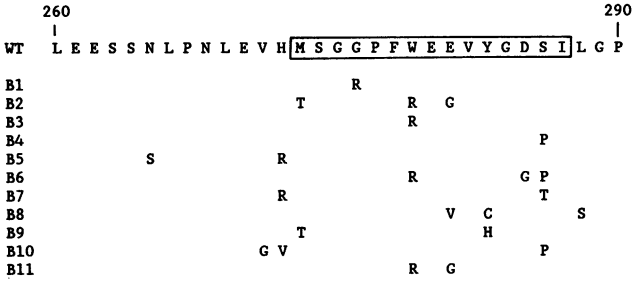


FIG. 1. Mutations in the Bel-1 activation domain isolated by genetic selection. Eleven distinct mutants designated B1 to B11 were isolated after PCR mutagenesis and genetic selection in yeast strain GGY1::171. Wild-type (WT) Bel-1 residues 260 to 290 are depicted by the one-letter amino acid code at the top. Residues corresponding to the conserved region of the HFV Bel-1 and SFV Taf activation domains are boxed (15). The substituted residues present in mutant Bel-1 activation domains are indicated directly under their corresponding positions in the wild-type Bel-1 sequence.

activation domains as GAL4 fusion proteins in HeLa cells revealed a very close correlation with the activity in yeast cells. In particular, mutants lacking all activity in yeast cells, e.g., B2 and B9, were also totally inactive in HeLa cells, while mutants displaying partial activity in yeast cells, e.g., B5 and B10, were also partially active in HeLa cells. To eliminate the trivial possibility that these mutant fusion proteins are simply highly unstable or otherwise grossly aberrant when expressed in primate cells, we examined their expression in mammalian cells by Western blot analysis. As shown in Fig. 2, all of these fusion proteins are, in fact, expressed at comparable levels and migrate at the same relative molecular mass.

TABLE 3. Transcriptional activity in yeast and HeLa cells of mutant GAL4/Bel-1 hybrids

| Activation domain (aa)[a] | Relative activity[b] | |
|---|---|---|
| | Yeast | HeLa |
| None | <1 | <1 |
| Bel-1[c] | 100 | 100 |
| B1 | 73 | 7 |
| B2 | <1 | <1 |
| B3 | <1 | <1 |
| B4 | 77 | 10 |
| B5 | 52 | 5 |
| B6 | <1 | <1 |
| B7 | 15 | 40 |
| B8 | 7 | 4 |
| B9 | <1 | <1 |
| B10 | 22 | 20 |
| B11 | <1 | <1 |

[a] The precise sequence of each of the Bel-1 activation domain mutants (B1 to B11) is given in Fig. 2. None, transformation with the parental pGAL4 (yeast) or pSG424 (HeLa) GAL4 DNA-binding domain expression plasmid.

[b] Activity in yeast cells was measured as β-Gal activity observed after transformation of the wild-type or mutant pY-GAL4/BEL(260-290) plasmid into yeast strain GGY1::171 (10) and is given relative to the activity observed with the wild-type Bel-1 activation domain, which is arbitrarily set at 100. Similarly, activity in HeLa cells was measured as chloramphenicol acetyltransferase activity resulting from cotransfection of mutant pGAL4/BEL(260-290) plasmids with the indicator plasmid pG6(−83)HIVLTRΔTAR (31) and is, again, given as a percentage of the activity observed upon cotransfection of the wild-type pGAL4/BEL(260-290) plasmid with indicator.

[c] aa 260 to 290, wild type.

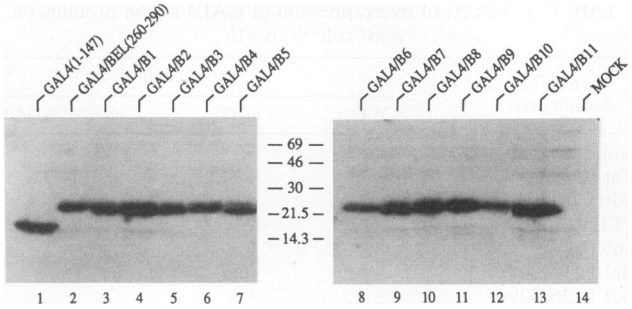


FIG. 2. Western blot analysis of GAL4/BEL(260-290) transcription factor hybrids in mammalian cells. COS cell cultures were transfected with the indicated wild-type (lane 2) or mutant (lanes 3 to 13) pGAL4/BEL(260-290) plasmids or the parental GAL4 DNA-binding domain expression plasmid pSG424 (lane 1) or were mock transfected (lane 14). At ~48 h following transfection, total cell protein was harvested in Laemmli sample buffer, resolved by SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose. Fusion proteins were then detected by Western blot analysis using a GAL4-specific rabbit antiserum followed by $^{125}$I-protein A. Protein molecular mass markers were run in parallel and are indicated (in kilodaltons) between the panels.

## DISCUSSION

Eukaryotic transcription factors can generally be divided into two functional domains (16, 25). A binding or specificity domain directs the factor to its appropriate target sequence by direct binding to the DNA or, as in the case of VP16, by mediating a specific interaction with another sequence-specific DNA-binding protein (11, 16, 25). The activation or effector domain then induces transcriptional activation by facilitating the recruitment of factors involved in initiation complex assembly to the promoter (5, 25, 29).

Mutational analyses of the HFV Bel-1 protein and the related simian foamy virus Taf protein have led to the identification and rough delineation of both binding and activation domains. In the case of the 300-aa Bel-1 protein, the sequences required for targeting to the HFV long terminal repeat reside between approximately residue 80 and residue 210, while the Bel-1 effector domain extends from approximately residue 250 to residue 290 (15, 33). The latter domain can be further subdivided into a relatively weak activation domain that extends from approximately residue 250 to 266 and a somewhat more potent activation domain that extends from residue 266 to 290 (15, 33). Only the more C terminal of these two Bel-1 activation domains is functional in the context of GAL4 DNA-binding domain chimeras.

Although data from several laboratories are in general agreement with the Bel-1/Taf domain organization delineated above, very little is known about the mechanism of action of these domains. It remains, for example, entirely unclear whether Bel-1 directly interacts with the DNA or is instead recruited to the HFV long terminal repeat by a cellular cofactor. Similarly, it has remained uncertain whether the Bel-1 activation domain, and particularly the more potent and highly conserved C-terminal activation motif of Bel-1/Taf, is a novel activation domain or is instead a member of one of the established classes of transcriptional activators (8, 25, 33). Because the mechanism of action of such domains remains controversial (5, 12, 29, 32), it is not currently possible to classify activation domains on the basis of biochemical criteria. However, the demonstration by Guarente and coworkers (3) that the VP16 class of acidic activators is defined by a

requirement for specific coactivators in yeast cells does provide a genetic approach to this question.

In this article, we demonstrate that the conserved Bel-1/Taf C-terminal activation domain is, like those of VP16 and NF-κB(p65), toxic when expressed at high levels in yeast cells as a GAL4 DNA-binding motif fusion (Table 1). This toxicity is specifically dependent on the yeast ADA2 coactivator. Similarly, this Bel-1 domain is also similar to acidic activation domains of the VP16 class, but distinct from the HAP4 activation domain, in also being highly dependent on ADA2 for transcriptional activation (Table 2). These data therefore strongly suggest that the Bel-1 activation domain functions analogously to acidic activation domains of the VP16 and NF-κB(p65) class in yeast cells.

The correlation between the transcriptional activation potential and the toxicity of acidic activation domains in yeast cells suggested a strategy to genetically select missense mutations of the Bel-1 activation domain that led to a lower level of toxicity and, hence, a lower ability to activate transcription. A genetic screen for such mutants in fact identified 11 distinct Bel-1 mutants that were both less toxic and less active as transcription factors when analyzed in yeast cells (Fig. 1; Table 3). The finding that the Bel-1 mutants generated by this screen were of widely varying biological activity provided a means to test whether the Bel-1 activation domain functioned analogously in both yeast and mammalian cells. In particular, our finding that the level of biological activity in yeast cells was a highly accurate predictor of transcriptional activation in HeLa cells (Table 3) strongly supports the hypothesis that the Bel-1 activation domain functions by similar mechanisms in both eukaryotic settings.

Although acidic activation domains, and particularly that in VP16, have been extensively studied, neither their biological target(s) in vivo nor the primary amino acid sequence that defines this class of activators has been fully defined (5, 6, 12, 21, 29, 32). However, recent data suggest that a critical feature may be a specific pattern of aromatic and hydrophobic amino acids in an otherwise somewhat acidic sequence environment (27). From this perspective, it is of interest that the down mutations in the Bel-1 activation domain (Fig. 1) frequently involved the insertion of basic amino acids and that the mutations that entirely eliminated activation function modified either a hydrophobic tryptophan residue or an aromatic tyrosine to a charged amino acid (Fig. 1).

The clear link between toxicity and transcriptional activation in yeast cells, together with the evidence suggesting that acidic activation domains function by similar mechanisms in both higher and lower eukaryotes, implies that genetic selection in yeast cells may provide a generally useful technique to identify residues important in transcriptional activation. In this article, we have used this approach to identify several interesting mutations of the Bel-1 activation domain. However, it appears likely that this technique could be used successfully on any activator belonging to the VP16-like acidic class.

## ACKNOWLEDGMENTS

We thank S. Goodwin for secretarial assistance and L. Guarente, M. Ptashne, T. Kadesch, M. Green, and P. Luciw for reagents used in this research.

This work was supported by the Howard Hughes Medical Institute.

## REFERENCES

1. **Ballard, D., E. Dixon, N. Peffer, H. Bogerd, S. Doerre, B. Stein, and W. Greene.** 1992. The 65-kDa subunit of human Nf-κB functions as a potent transcriptional activator and a target for U-Rel-mediated repression. Proc. Natl. Acad. Sci. USA **89:**1875–1879.
2. **Beckman, H., L. K. Su, and T. Kadesch.** 1990. TFE3: a helix-loop-helix protein that activates transcription through the immunoglobulin enhancer μE3 motif. Genes Dev. **4:**167–179.
3. **Berger, S. L., B. Pina, N. Silverman, G. A. Marcus, J. Agapite, J. L. Regier, S. J. Triezenberg, and L. Guarente.** 1992. Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. Cell **70:**251–265.
4. **Bogerd, H. P., R. A. Fridell, W. S. Blair, and B. R. Cullen.** 1993. Genetic evidence that the Tat proteins of human immunodeficiency virus types 1 and 2 can multimerize in the eukaryotic cell nucleus. J. Virol. **67:**5030–5034.
5. **Choy, B., and M. R. Green.** 1993. Eukaryotic activators function during multiple steps of preinitiation complex assembly. Nature (London) **366:**531–536.
6. **Cress, W. D., and S. J. Triezenberg.** 1991. Critical structural elements of the VP16 transcriptional activation domain. Science **251:**87–90.
7. **Cullen, B. R.** 1987. Use of eukaryotic expression technology in the functional analysis of cloned genes. Methods Enzymol. **152:**684–704.
8. **Garrett, E. D., F. He, H. P. Bogerd, and B. R. Cullen.** 1993. Transcriptional *trans* activators of human and simian foamy viruses contain a small, highly conserved activation domain. J. Virol. **67:**6824–6827.
9. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:**527–534.
10. **Gill, G., and M. Ptashne.** 1987. Mutants of GAL4 protein altered in an activation function. Cell **51:**121–126.
11. **Goding, C. R., and P. O'Hare.** 1989. Herpes simplex virus Vmv65-octamer binding protein interaction: a paradigm for combinatorial control of transcription. Virology **173:**363–367.
12. **Goodrich, J. A., T. Hoey, C. J. Thut, A. Admon, and R. Tjian.** 1993. Drosophila $TAF_{II}40$ interacts with both a VP16 activation domain and the basal transcription factor TFIIB. Cell **75:**519–530.
13. **Guarente, L., R. Yocum, and P. Gifford.** 1982. A GAL10-CYC1 hybrid yeast promoter identifies the GAL4 regulatory region as an upstream site. Proc. Natl. Acad. Sci. USA **79:**7410–7414.
14. **Hahn, S.** 1993. Structure(?) and function of acidic transcription activators. Cell **72:**481–483.
15. **He, F., J. D. Sun, E. D. Garrett, and B. R. Cullen.** 1993. Functional organization of the Bel-1 *trans* activator of human foamy virus. J. Virol. **67:**1896–1904.
16. **Keegan, L., G. Gill, and M. Ptashne.** 1986. Separation of DNA binding from the transcription-activating function of a eukaryotic regulatory protein. Science **231:**699–704.
17. **Keller, A., K. M. Partin, M. Löchelt, H. Bannert, R. M. Flügel, and B. R. Cullen.** 1991. Characterization of the transcriptional *trans* activator of human foamy retrovirus. J. Virol. **65:**2589–2594.
18. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
19. **Lee, K. J., A. H. Lee, and Y. C. Sung.** 1993. Multiple positive and negative *cis*-acting elements that mediate transactivation by *bel1* in the long terminal repeat of human foamy virus. J. Virol. **67:**2317–2326.
20. **Leung, D. W., E. Chen, and D. V. Goeddel.** 1989. A method for random mutagenesis of a defined RNA segment using a modified polymerase chain reaction. Technique **1:**11–15.
21. **Leuther, K. K., J. M. Salmeron, and S. A. Johnston.** 1993. Genetic evidence that an activation domain of GAL4 does not require acidity and may form a β sheet. Cell **72:**575–585.
22. **Löchelt, M., H. Zentgraf, and R. M. Flügel.** 1991. Construction of an infectious DNA clone of the full-length human spumaretrovirus genome and mutagenesis of the *bel1* gene. Virology **184:**43–54.
23. **Mergia, A., L. W. Renshaw-Gegg, M. W. Stout, R. Renne, and O. Herchenröeder.** 1993. Functional domains of the simian foamy virus type 1 transcriptional transactivator (Taf). J. Virol. **67:**4598–4604.
24. **Mergia, A., K. E. S. Shaw, E. Pratt-Lowe, P. A. Barry, and P. A. Luciw.** 1991. Identification of the simian foamy virus transcriptional transactivator gene (*taf*). J. Virol. **65:**2903–2909.
25. **Mitchell, P. J., and R. Tjian.** 1989. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins.

Science **245**:371–378.
26. **Neumann, J. R., C. A. Morency, and K. O. Russian.** 1987. A novel rapid assay for chloramphenicol acetyltransferase gene expression. BioTechniques **5**:444–447.
27. **Regier, J. L., F. Shen, and S. J. Triezenberg.** 1993. Pattern of aromatic and hydrophobic amino acids critical for one of two subdomains of the VP16 transcriptional activator. Proc. Natl. Acad. Sci. USA **90**:883–887.
28. **Rethwilm, A., O. Erlwein, G. Baunach, B. Maurer, and V. Ter Meulen.** The transcriptional transactivator of human foamy virus maps to the *bel1* genomic region. Proc. Natl. Acad. Sci. USA **88**:941–945.
29. **Roeder, R. G.** 1991. The complexities of eukaryotic transcription initiation: regulation of preinitiation complex assembly. Trends Biochem. Sci. **16**:402–411.
30. **Sadowski, I., and M. Ptashne.** 1989. A vector for expressing GAL4(1-147) fusions in mammalian cells. Nucleic Acids Res. **17**:7539.
31. **Southgate, S. D., and M. R. Green.** 1991. The HIV-1 Tat protein activates transcription from an upstream DNA-binding site: implications for Tat function. Genes Dev. **5**:2496–2507.
32. **Stringer, K. F., C. J. Ingles, and J. Greenblatt.** 1990. Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. Nature (London) **345**:783–786.
33. **Venkatesh, L. K., and G. Chinnadurai.** 1993. The carboxy-terminal transcriptional enhancement region of the human spumaretrovirus transactivator contains discrete determinants of the activator function. J. Virol. **67**:3868–3876.
34. **Venkatesh, L. K., P. A. Theodorakis, and G. Chinnadurai.** 1991. Distinct *cis*-acting regions in U3 regulate *trans*-activation of the human spumaretrovirus long terminal repeat by the viral *bel1* gene product. Nucleic Acids Res. **19**:3661–3666.
35. **Vernet, T., D. Dignard, and D. Y. Thomas.** 1987. A family of yeast expression vectors containing the phage f1 intergenic region. Gene **52**:225–233.