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DNA-Binding-Defective Mutants of the Epstein-Barr Virus Lytic Switch Activator Zta Transactivate with Altered Specificities

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The Epstein-Barr virus BRLF1 and BZLF1 genes are the first viral genes transcribed upon induction of the viral lytic cycle. The protein products of both genes (referred to here as Rta and Zta, respectively) activate expression of other viral genes, thereby initiating the lytic cascade. Among the viral antigens expressed upon induction of the lytic cycle, however, Zta is unique in its ability to disrupt viral latency; expression of the BZLF1 gene is both necessary and sufficient for triggering the viral lytic cascade. We have previously shown that Zta can activate its own promoter (Zp), through binding to two Zta recognition sequences (ZIIIA and ZIIIB). Here we describe mutant Zta proteins that do not bind DNA (referred to as Zta DNA-binding mutants [Zdbm]) but retain the ability to transactivate Zp. Consistent with the inability of these mutants to bind DNA, transactivation of Zp by Zdbm is not dependent on the Zta recognition sequences. Instead, transactivation by Zdbm is dependent upon promoter elements that bind cellular factors. An examination of other viral and cellular promoters identified promoters that are weakly responsive or unresponsive to Zdbm. An analysis of a panel of artificial promoters containing one copy of various promoter elements demonstrated a specificity for Zdbm activation that is distinct from that of Zta. These results suggest that non-DNA-binding forms of some transactivators retain the ability to transactivate specific target promoters without direct binding to DNA.

Epstein-Barr virus (EBV) is a lymphotropic human herpesvirus that latently infects B lymphocytes, resulting in a concomitant growth transformation of the infected cell. Infection is closely associated with several human cancers, including nasopharyngeal carcinoma and African Burkitt's lymphoma, and also plays a role in several lymphoproliferative diseases in immunocompromised individuals (for a review, see reference 29). In vitro the transforming potential of EBV is evidenced by its ability to immortalize B lymphocytes to grow indefinitely in culture. Immortalization is achieved through the expression of a relatively small subset of EBV-encoded genes that serve to establish and maintain cellular transformation (for a review, see reference 45).

Propagation of EBV from host to host is dependent upon the activation of an estimated 100 or more viral genes, culminating in the production of infectious virions (2, 4, 9, 27). While these genes remain quiescent during latency, a switch in the genetic program leading to the expression of viral replication associated genes can be accomplished in vitro by treatment of latently infected B lymphocytes with various reagents, including phorbol esters, butyrate, ionophore, and anti-immunoglobulin (2, 28, 37, 46, 48, 51). Activation of the lytic cascade by treatment with anti-immunoglobulin results initially in the expression of two viral genes, BZLF1 and BRLF1, which exhibit similar induction kinetics (maximal mRNA levels are reached between 2 and 4 h postinduction) (14, 46). The protein products of both the BZLF1 (referred to here as Zta, but also called ZEBRA and EB1) and BRLF1 (Rta) genes have been shown to be transcriptional activators (8, 10, 12, 25, 26). Expression of Zta and Rta leads to the activation of early genes and ultimately viral replication. Of all the viral transactivators examined, Zta is unique in that its expression alone can initiate the entire lytic cascade (10, 11, 40), and regulation of Zta expression appears to be central to regulating entry into the lytic cycle.

We have previously identified multiple elements within the BZLF1 promoter (Zp) that play a role in regulating BZLF1 expression (15, 16). Several of these elements bind cellular transcription factors that are responsive to phorbol ester stimulation (15). Zp also contains two Zta binding sites (ZIIIA and ZIIIB) that are important for autoactivation (16, 33, 49).

Zta shares several structural similarities with other transcription factors. As described by Farrell et al. (12), Zta contains a basic region with homology to the DNA-binding domains of the AP-1 family of transcription factors (Fig. 1). In addition, Zta binds DNA as a dimer and contains a region carboxyl terminal to the basic region that mediates dimerization (7, 33). Although Zta's dimerization domain does not contain a heptad repeat of leucines, a hallmark of the bZIP family of transcription factors, mutagenesis studies indicate that dimerization does indeed occur through a coiled-coil interaction (18, 31).

Residues 1 through 153 of Zta do not appear to influence either dimerization or DNA binding but play a role in activation of transcription. Sequences that are crucial for activation of transcription lie between residues 27 and 78, since deletion of these sequences results in a severe impairment of transactivation (6, 13, 22, 34). The Zta activation domain has been shown to bind the TATA box-binding factor TBP and to stabilize the interaction of TBP with DNA (34). Amino acids between 25 and 86 were shown to be crucial for this interaction, suggesting that binding to TBP could play an important role in Zta transcriptional activation. Indirect evidence sug-

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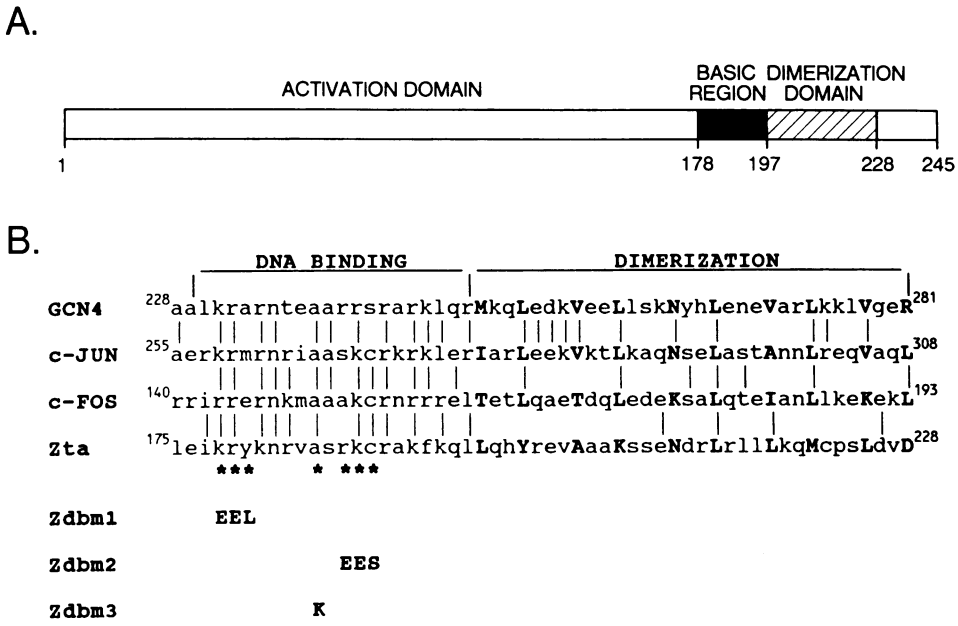

FIG. 1. (A) Schematic illustration of the functional domains in Zta. (B) Alignment of the DNA-binding and dimerization domains of Zta with other members of the bZIP family of transcription factors (12). The specific mutations introduced into the basic region of Zta to generate Zdbm1, Zdbm2, and Zdbm3 are indicated.

gests that the basic/DNA-binding region and the dimerization domain also play a role in activation of transcription (13, 22).

In the past few years it has become clear that one mode of regulating the activity of transcription factors is through post-translational modifications that affect DNA binding (for example, see references 1, 3, 5, 36, and 38). In this paper we show that site-directed mutations introduced into the DNA-binding domain of Zta, which abrogate DNA binding, also ablate Zta recognition site-dependent transactivation. However, all of these mutants retain the ability to activate Zp, and we show that transactivation by Zta DNA-binding mutants (Zdbm) is dependent upon promoter elements that bind cellular transcription factors. These observations indicate that modifications that inhibit the ability of transcription factors to bind DNA may not only inhibit activation through specific recognition sequences but may also impart additional activities that are dependent upon distinct sets of *cis* elements.

MATERIALS AND METHODS

Cell culture, transfections, and CAT assays. The EBV-negative Burkitt's lymphoma cell line DG75 was propagated at 37°C in RPMI 1640 medium (GIBCO Laboratories) supplemented with 10% fetal bovine serum. Transfections were performed with DEAE-dextran as described previously (13). Cells were harvested ca. 40 h posttransfection, and chloramphenicol acetyltransferase (CAT) activity was determined as described by Gorman et al. (24). The extent of acetylation was assessed by counting the acetylated species of chloramphenicol with a Betascope (Betagen).

Electrophoretic mobility shift assay (EMSA). Zta in vitro expression plasmids were prepared and linearized by standard procedures (39). In vitro transcripts were generated with T3 RNA polymerase (Ambion) and then by in vitro translation of a fraction of the transcription reaction, employing wheat germ extract (Promega) with [^{35}S]methionine according to the manufacturer's specifications.

Equivalent amounts of each Zta protein were mixed with either a ^{32}P-labeled BZLF1 promoter fragment containing bp −221 to +14 or ^{32}P-labeled double-stranded oligonucleotides containing specific *cis* elements present in the BZLF1 promoter in 25 μl of 10 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES; pH 7.9), 10% (vol/vol) glycerol, 60 mM KCl, 0.1 mM EDTA, 0.25 mM dithiothreitol, and 0.5 μg of poly(dI-dC). Binding reactions were carried out for 20 min at 22°C and loaded onto a 6% polyacrylamide gel (20:1, acrylamide-bisacrylamide) in 0.5× TBE (1× TBE is 90 mM Tris, 64.6 mM boric acid, 2.5 mM EDTA [pH 8.3]).

Nuclear extraction. Cells (DG75; 10^7) were transfected with 2 μg of effector plasmid, as described above, and harvested 40 h later. The cells were suspended in 200 μl of buffer A (10 mM HEPES [pH 7.9], 10 mM KCl, 1 mM EDTA) with 0.5% Nonidet P-40, subjected to one freeze-thaw cycle, and incubated at 4°C for 15 min. Leaky nuclei were recovered by brief centrifugation, and the supernatant was designated the low-salt fraction. The crude nuclear pellet was resuspended in 200 μl of buffer C (20 mM HEPES [pH 7.9], 0.42 M KCl, 1 mM EDTA) and incubated 20 min at 4°C. After centrifugation, the supernatant (high-salt fraction) was collected for immunoblot analysis. An equivalent percentage of the low- and high-salt fractions was fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted to nitrocellulose. Immunoblot analysis was performed with an anti-Zta polyclonal antiserum by standard procedures (39).

Plasmid construction. All mutant Zta expression vectors were derived from the previously described Zta expression vector (13) containing the simian virus 40 (SV40) early promoter and enhancer upstream from the genomic BZLF1 gene. This expression vector drives the expression of full-length Zta which has been engineered to contain the SV40 large T antigen nuclear localization signal (PKKKRKV) at the carboxyl terminus of the protein. Mutations in the DNA-binding domain were introduced by site-directed mutagenesis (20). Zdbm1 deletion mutants were generated by a two-step cloning proce-

dure used previously (13). First, a *Sca*I restriction site (AGT ACT) was introduced at various positions in the coding sequence by site-directed mutagenesis. Introduction of the *Sca*I sites resulted in the mutation of two adjacent residues to serine (AGT) and threonine (ACT). The internal deletion mutants were generated by mixing and matching amino-terminal regions from various mutants with carboxyl-terminal regions from other mutants through their respective *Sca*I sites.

The Zp reporter deletion series used for these studies was derived from a previously described deletion series (18). To decrease background transcription, promoter-CAT sequences were transferred to the pGL2 vector (Promega) in place of the luciferase gene. This vector contains a poly(A) signal upstream from the promoter fragment which provided a significant reduction in background transcription. Luciferase constructs containing fragments of the BZLF1 promoter were generated by cloning the relevant promoter fragments into the pGL2 vector upstream of the luciferase reporter gene.

BGCAT was generated by transferring the β-globin TATA box from pG4CAT (13) into the −159ZpCAT reporter, with the concomitant removal of Zp sequences. Double-stranded oligonucleotides corresponding to relevant promoter elements were then cloned upstream from the minimal β-globin promoter: 1xZII, 5′-CTAGACGTCCCAAACCATGACATCAC AGAGGAG-3′ and 5′-GATCCTCCTCTGTGATGTCATG GTTTGGGACGT-3′; 1xZIIIB, 5′-CTAGACAGGCATTGC TAATGTACC-3′ and 5′-GATCGGTACATTAGCAATGC CTGT-3′; 1xZIB, 5′-CCGGGCACCAGCTTATTTTAGACA CTTCT-3′ and 5′-CTAGAGAAGTGTCTAAAATAAGCTG GCC-3′; 3xZIB, 5′-CCGGGACCAGCTTATTTTAGACAC TTACCAGCTTATTTTAGACACTTACCAGCTTATTTTA GACACTTT-3′ and 5′-CTAGAAAGTGTCTAAAATAAG CTGGTAAGTGTCTAAAATAAGCTGGTAAGTGTCTAA AATAAGbpCTGGTC-3′; 1xZIC, 5′-CCGGGCCTCCTCCT CTTTTAGAAACTATT-3′ and 5′-CTAGAATAGTTTCTA AAAGAGGAGGAGGC-3′; 3xZIC, 5′-CCGGGCTCCTCC TCTTTTAGAAACTACTCCTCCTCTTTTAGAAACTACT CCTCCTCTTTTAGAAACTAT-3′ and 5′-CTAGATAGTT TCTAAAAGAGGAGGAGTAGTTTCTAAAAGAGGAGG AGTAGTTTCTAAAAGAGGAGGAGC-3′; 1xCRE, 5′-CT AGATCAAATTGACGTCATGGTAAA-3′ and 5′-GATCT TTACCATGACGTCAATTTGAT-3′; 1xAP-1, 5′-CTAGAT AAAGCATGAGTCAGACACCTCT-3′ and 5′-GATCAGA GGTGTCTGACTCATGCTTTAT-3′; 1xAP-2, 5′-CTAGAT CCCCAGGCTCCCCAG-3′ and 5′-GATCCTGGGGAGC CTGGGGAT-3′; 1xNF-κB, 5′-CTAGAGGGACTTTCCG-3′ and 5′-GATCCGGAAAGTCCCT-3′; and 1xSP-1, 5′-CTA GAGTCGGGGCGGCGCGG-3′ and 5′-GATCCCGCGCCG CCCCGACT-3′.

RESULTS

Zdbm do not bind DNA in vivo but retain the ability to transactivate Zp. For the studies described in this paper we have focused on two different mutations introduced into the basic region of Zta, which we have previously shown abrogate Zta DNA binding in vitro (see Zdbm1 and Zdbm2 [Fig. 1]) (18, 47). In the course of characterizing these mutants, we unexpectedly observed that they retained the ability to transactivate Zp. Cotransfection of the EBV-negative Burkitt's lymphoma cell line DG75 with a CAT reporter plasmid containing the BZLF1 promoter (−221ZpCAT) and the appropriate control or Zta expression vector resulted in a significant increase in activity with either the Zdbm1 or the Zdbm2 expression vectors (Fig. 2A). In the experimental results shown, Zta transactivated −221ZpCAT 600-fold while Zdbm1

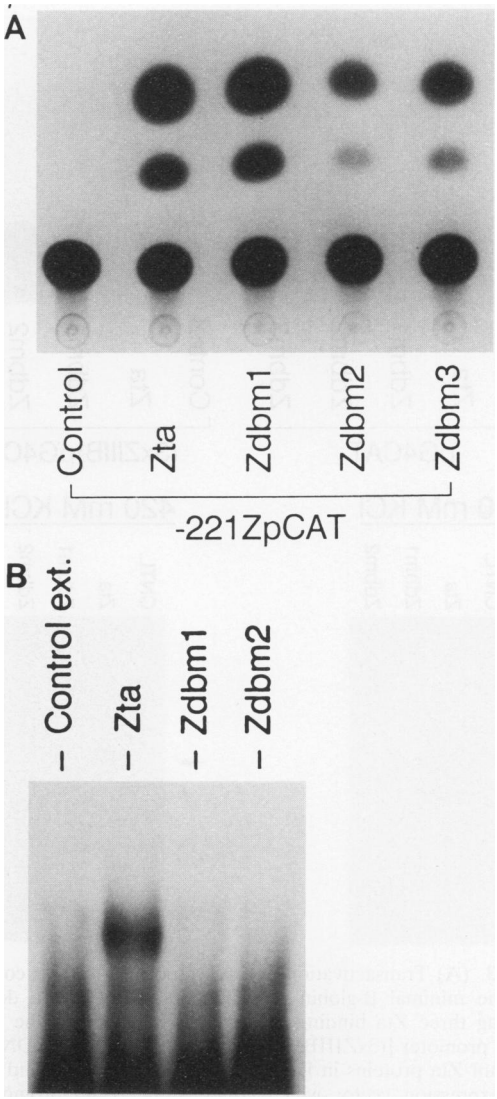

FIG. 2. (A) Transactivation of a CAT reporter containing the BZLF1 promoter (−221 to +14 bp) by Zta and Zdbm1, Zdbm2, and Zdbm3 in DG75 cells. (B) EMSA employing a ^{32}P-labeled fragment of Zp (−221 to +14 bp) and equivalent amounts of in vitro-translated Zta, Zdbm1, and Zdbm2.

and Zdbm2 increased CAT activity 800- and 80-fold, respectively. To extend this observation, we generated a third Zta mutant (Zdbm3 [Fig. 1]) which introduced a lysine in place of an alanine at residue 185. This mutation had previously been shown by others to abrogate DNA binding (22). Zdbm3 was also able to transactivate −221ZpCAT (120-fold) (Fig. 2A). Taken together, these results indicated that Zta mutants which lack the ability to specifically bind DNA retain the ability to transactivate the BZLF1 promoter. It should be noted that in addition to DG75 cells, we have also observed Zdbm transactivation of the −221ZpCAT reporter plasmid in several other cell lines, including the Burkitt's lymphoma cell lines Ramos and clone-13, as well as in the cervical carcinoma cell line HeLa (data not shown).

It had previously been demonstrated that Zdbm do not bind in vitro to Zta response elements (ZREs) (18, 22, 47); to address the possibility that the observed transactivation of Zp by Zdbm occurred through direct DNA binding to another

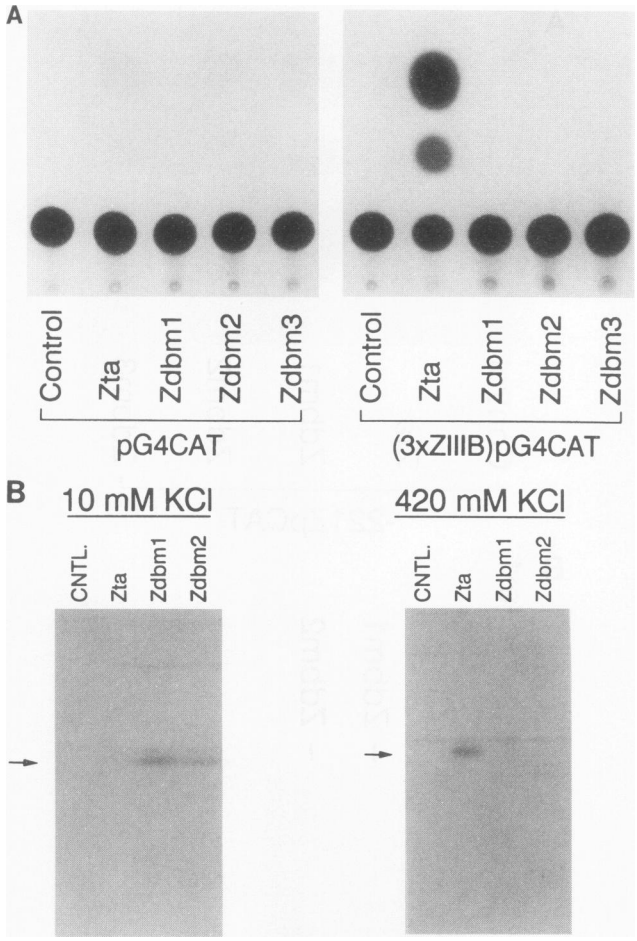

FIG. 3. (A) Transactivation of CAT reporter plasmids containing either the minimal β-globin promoter (pG4CAT) or a derivative containing three Zta binding sites cloned upstream of the minimal β-globin promoter [(3xZIIIB)pG4CAT] by wild-type and DNA-binding mutant Zta proteins in DG75 cells. The control plasmid was the parent expression vector which contains the SV40 promoter and enhancer cloned into the Bluescript vector (Stratagene). (B) Low-salt extraction of Zdbm1 and Zdbm2 from permeabilized nuclei of transiently transfected DG75 cells. Zta and Zdbm proteins were detected by immunoblot analysis with a rabbit polyclonal anti-Zta serum. CNTL., control.

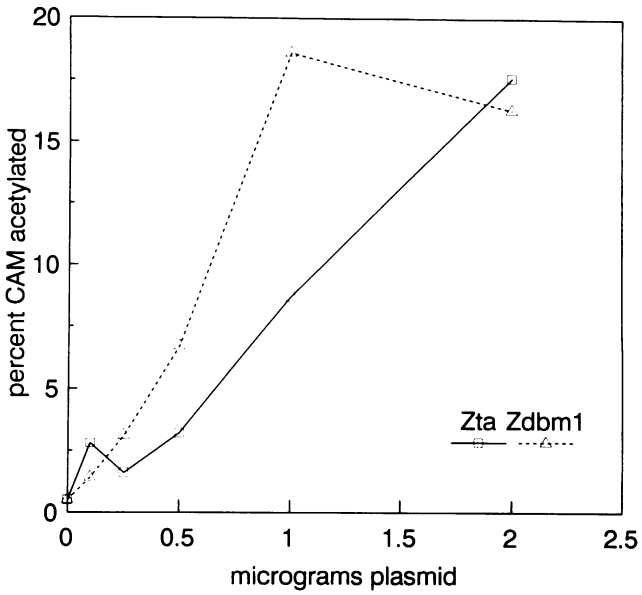

FIG. 4. Transactivation of −221ZpCAT in DG75 cells as a function of amount of transfected Zta and Zdbm1 expression vector. −221ZpCAT DNA (2 μg) was cotransfected with the indicated amount of effector plasmid. The appropriate amount of a control expression plasmid, containing the SV40 promoter and enhancer, was added to each transfection mixture such that the total amount of effector plasmid plus control plasmid was 2 μg. CAM, chloramphenicol.

site(s) in Zp, an EMSA was performed with in vitro-synthesized Zta, Zdbm1, and Zdbm2 and a labeled DNA fragment containing the entire region of Zp present in the −221ZpCAT reporter construct (Fig. 2B). No binding of either mutant to Zp could be detected in this assay, although significant binding of wild-type Zta was observed. To further confirm that Zdbm do not retain any ability to specifically bind to and activate through Zta binding sites in vivo, these mutants were assayed for their ability to transactivate a minimal reporter plasmid containing three copies of a ZRE derived from the BZLF1 promoter [(3XZIIIB)pG4CAT] (13). DG75 cells were cotransfected with the (3XZIIIB)pG4CAT reporter and either a control plasmid containing only the SV40 promoter/enhancer or expression plasmids encoding Zta, Zdbm1, Zdbm2, or Zdbm3. As shown in Fig. 3A, although a greater than 800-fold increase in CAT activity from (3XZIIIB)pG4CAT was observed with Zta, no detectable increase in CAT activity was observed with Zdbm1, Zdbm2, or Zdbm3. Thus, each of the mutations introduced into the DNA-binding domain of Zta appears to be sufficient to abrogate specific DNA binding to ZREs in vivo.

To further examine the DNA-binding properties of Zdbm in vivo, a nuclear extraction experiment employing different salt concentrations was carried out to determine the affinity of these mutants for genomic DNA (Fig. 3B). Expression plasmids encoding Zta, Zdbm1, or Zdbm2 were transfected into DG75 cells, and 40 h posttransfection the cells were harvested, freeze-thawed, and subjected to low-salt extraction and then to high-salt extraction as described in Materials and Methods. As shown in Fig. 3B, the mutants Zdbm1 and Zdbm2 were easily extracted with low salt (10 mM KCl), while Zta remained associated with genomic DNA and was recovered in the high-salt fraction (0.42 M KCl). This further indicated that Zdbm1 and Zdbm2 are not tightly associated with genomic DNA in vivo.

Zdbm1 transactivation of Zp is as efficient as Zta transactivation. Although when taken together the above data strongly indicate that Zdbm transactivation of Zp occurs through a mechanism independent of direct binding of the mutants to DNA, it seemed possible that the observed transactivation was the result of a small percentage of material which was able to bind DNA (e.g., through heterodimerization with a cellular factor or low-affinity binding of the homodimer). If this were the case, then Zdbm1 transactivation would be expected to be inefficient compared with Zta transactivation at subsaturating levels of transactivator. To address this possibility, the efficacy of Zdbm transactivation compared with that of Zta transactivation was determined (Fig. 4). At low concentrations (0.1, 0.25, 0.5, and 1.0 μg) of either effector plasmid, the activity generally increased with the amount of effector DNA added. Notably, at low input plasmid concentra-

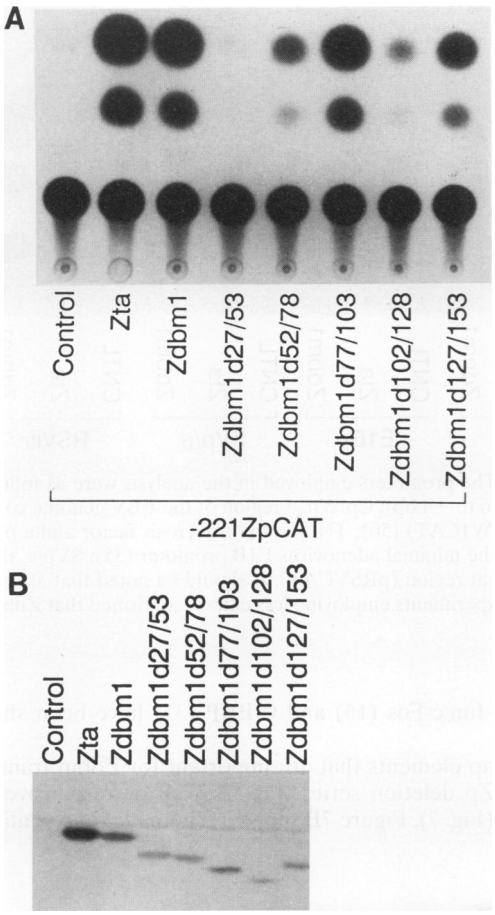

FIG. 5. (A) Determination of the residues in Zdbm1 required for activation of −221ZpCAT. Each mutant is identified by the residues deleted within the amino terminus of Zdbm1. (B) Immunoblot analysis of extracts from DG75 cells transfected with the various Zdbm1 amino-terminal domain deletions. An aliquot of the transiently transfected DG75 cells used to assay CAT activity in panel A was harvested, and the level of expression of each Zdbm1 mutant was determined by immunoblotting with a rabbit polyclonal anti-Zta serum.

tions, transactivation of Zp by Zdbm1 was ca. two- to threefold greater than with Zta. At 2 μg of effector plasmid, however, Zta provided a further increase in activity while Zdbm1 transactivation appeared to have reached saturation. From these results it appeared that Zdbm1 transactivates Zp more efficiently than Zta at low plasmid concentrations. This point was further underscored by the observation that a higher level of Zta than of Zdbm1 was generated from this expression vector, as determined by immunoblot analysis (see below) (Fig. 5B). The difference in the observed levels of expression of Zta and Zdbm1 is most likely due to autoactivation of the SV40 early promoter/enhancer by Zta, but not Zdbm1 (see below) (Fig. 6). The ability of Zdbm1 to activate Zp as well as or better than Zta at subsaturating levels of transactivator, in conjunction with the failure to detect specific binding of Zdbm to Zp and the low-salt extractability of Zdbm from the nucleus of transfected cells, makes it extremely unlikely that Zdbm transactivation is mediated by direct DNA binding.

Sequences within the amino terminus of Zta that are important for transactivation by the wild-type protein are also involved in transactivation by Zdbm. Previous studies have identified sequences within the amino terminus of Zta that appear to play an important role in transcriptional activation (13, 22, 34). In our previous characterization of Zta transactivation, we employed a series of 26 amino acid internal deletions (13). To gain insights into the mechanism of Zdbm transactivation, the same series of internal deletions was generated for Zdbm1 and the ability of these deletion mutants to transactivate −221ZpCAT was assessed (Fig. 5). From this analysis it appeared that Zdbm1 transactivation has sequence requirements that are strikingly similar to those for Zta (13). Deletion of residues 27 to 53 reduced Zta activity to ca. 3% of that of the wild-type protein, and this same deletion in Zdbm1 reduced activity to ca. 1% of that of the intact Zdbm1. Deletion of residues 52 to 78 also significantly reduced the activities of both Zta and Zdbm1 (17 and 7%, respectively). Furthermore, deletion of residues 77 to 103 had little effect on transactivation by Zta or Zdbm1. However, while deletion of residues 102 to 128 and 127 to 153 had little effect on Zta transactivation, these deletions significantly diminished Zdbm1 activity.

Immunoblot analysis, performed with a fraction of the transfected cells used for the activity analysis, demonstrated that all of the deletion mutants were expressed at a lower level than the full-length protein, and Zdbm1(d102/128) was consistently expressed at a lower level than the other mutants (Fig. 5B). All of the expression vectors described in this report were engineered such that the SV40 large T antigen nuclear localization signal was present at the carboxyl terminus of Zta or Zdbm sequences in an attempt to ensure their localization to the nucleus. Immunofluorescence staining of transiently transfected HeLa cells demonstrated that the localization of Zdbm1, Zdbm1(d27/53), Zdbm1(d53/78), and Zdbm1(d77/103) was exclusively nuclear. However, the staining pattern observed with Zdbm1(d102/128) and Zdbm1(d127/153) was diffuse and largely cytoplasmic with only weak nuclear fluorescence (data not shown). Therefore, it is unclear whether residues 102 to 153 are important for Zdbm1 transactivation. Notwithstanding this ambiguity, it appears that the region from residue 27 to 78 is critical for both Zta and Zdbm1 function, suggesting that the mechanism(s) involved in Zdbm1 and Zta transactivation is likely to be similar.

Promoter specificity of Zdbm1 transactivation. To examine the specificity of Zdbm1 transactivation, a panel of promoters was tested for responsiveness to Zta and Zdbm1 (Fig. 6). While several promoters were transactivated by Zdbm1, of those assayed none was nearly as responsive as Zp. No activation of the SV40 early promoter/enhancer (SVp/e) or the Rous sarcoma virus (RSV) long terminal repeat was observed with Zdbm1. However, Zta did transactivate both SVp/eCAT and RSVCAT, an effect which may be elicited through binding of Zta to AP-1 sites in these promoters. A reporter containing the EBV Cp and Wp latency-associated promoters, as well as the minimal adenovirus E1B promoter, also exhibited little responsiveness to Zdbm1. On the other hand, discernable activation of the EBV BRLF1 (Rp), tumor necrosis factor alpha (TNFp), and beta interferon (INFp) promoters by Zdbm1 was observed. These results demonstrate that Zdbm1 transactivation is not promiscuous, indicating that specific interactions are required and that these can occur only on a subset of naturally occurring promoters. In addition, while Zdbm1 can apparently activate other promoters, the architecture of Zp is such that it is extremely responsive to Zdbm.

The CREB/AP-1 (ZII) domain of Zp contributes to Zdbm1 transactivation. We have previously identified *cis* elements in Zp that are likely to play a role in the activation of Zp expression during induction of the lytic cycle (15, 16) (Fig. 7B). In addition to two Zta binding sites, ZIIIA and ZIIIB, Zp

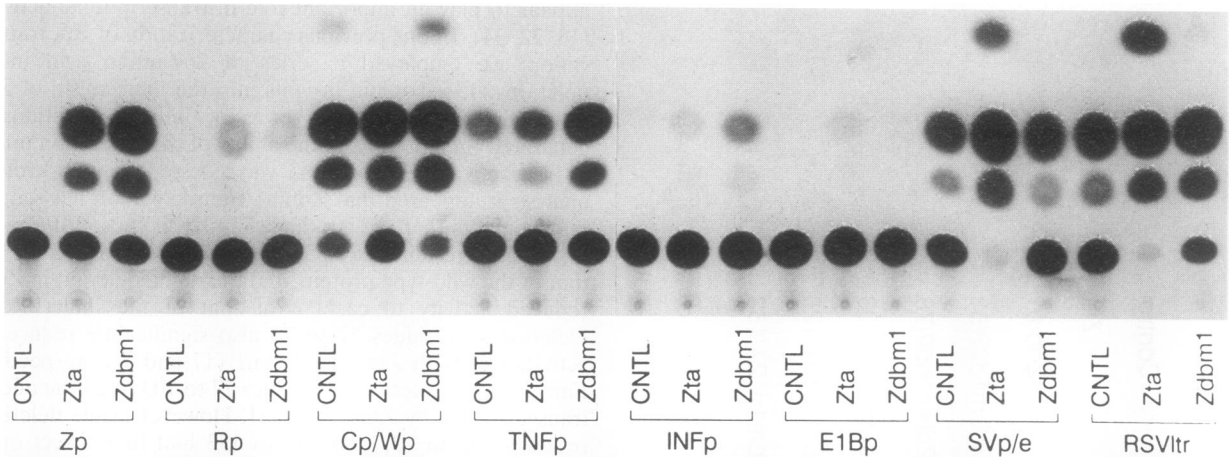

FIG. 6. Zta and Zdbm1 transactivation of various cellular and viral promoters. The promoters employed in the analysis were as follows: Zp, the EBV BZLF1 promoter (−221 to +14 bp); Rp, the EBV BRLF1 promoter (−386 to +4 bp); Cp/Wp, a region of the EBV genome containing two EBV latency-associated promoters in their normal physiological orientation (CW1CAT) (50); TNFp, tumor necrosis factor alpha promoter (−600 to +87 bp) (23); INFp, beta interferon promoter (−105 to +72 bp); E1Bp, the minimal adenovirus E1B promoter (35); SVp/e, the SV40 early promoter and enhancer (pSV2CAT); and RSVltr, the RSV long terminal repeat region (pRSVCAT). It should be noted that although the activity of the Cp/Wp reporter in the experiment was in the nonlinear range, other experiments employing less extract confirmed that Zdbm1 only weakly activates Cp/Wp.

contains several elements that bind cellular factors. There are four AT-rich elements (ZI family) which have similar sequences and from binding competition experiments appear to bind the same or related cellular factors (15). A distinct element, ZII, present at ca. −65 bp, is a CRE-like element to which c-Jun/c-Fos (15) and C/EBP (31) have been shown to interact.

To map elements that are important for Zdbm transactivation, a Zp deletion series was assayed for responsiveness to Zdbm1 (Fig. 7). Figure 7B shows a schematic representation of

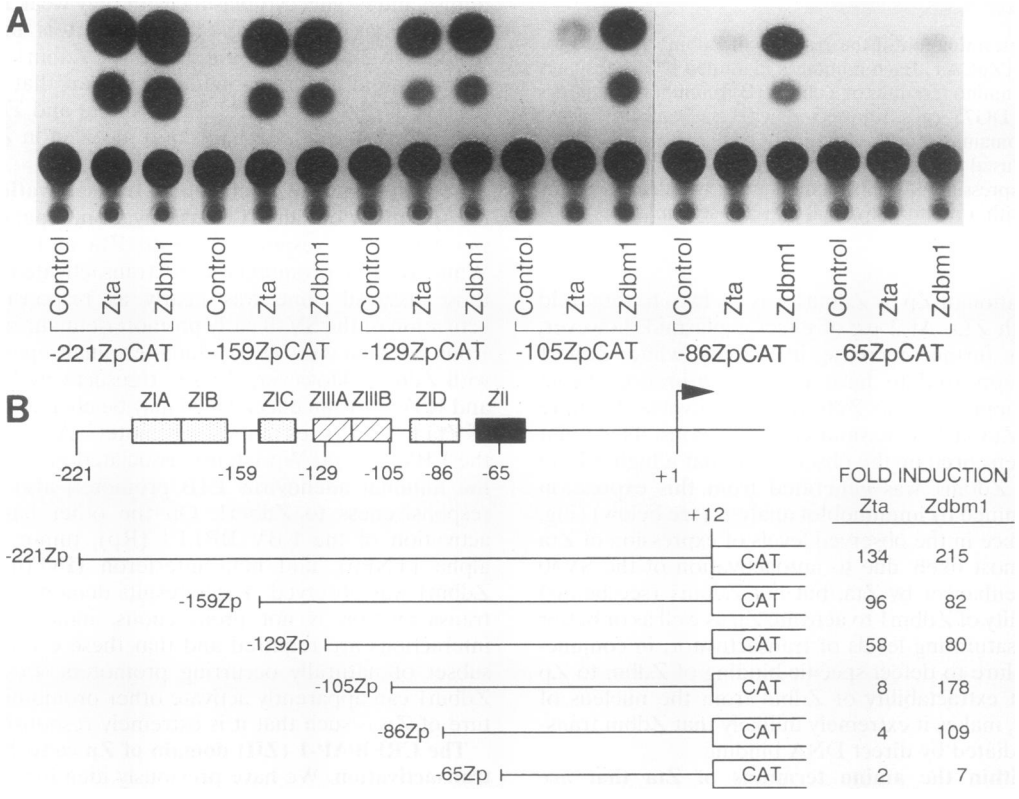

FIG. 7. Mapping the region of Zp mediating Zdbm1 transactivation. (A) CAT assays of Zta and Zdbm1 transactivation of nested Zp deletions in DG75 cells. (B) Schematic illustration of the nested Zp deletions with respect to the previously characterized promoter elements (15, 16) and summary of the CAT activities shown in panel A.

the Zp deletion series with respect to the previously identified promoter elements. Each reporter was cotransfected with a control expression plasmid or Zta or Zdbm1 expression vectors. An approximately threefold decrease in Zdbm1 activation was observed when the region between −221 and −159 bp, containing the ZIA and ZIB elements, was deleted (Fig. 7A). No significant change was observed when the sequences between −159 and −129 bp were deleted, which eliminated the ZIC element. However, deletion of the sequences from −129 to −105 bp consistently resulted in an approximately twofold increase in Zdbm1 transactivation. As expected, since the deletion from −129 to −105 bp removes the two Zta binding sites, it caused a dramatic decrease in Zta transactivation. The fact that Zdbm1 transactivation increased with this deletion underscores the lack of dependence of Zdbm transactivation on the Zta binding sites present in Zp. Deletion of sequences between −105 and −86 bp resulted in an approximately twofold decrease in Zdbm1 activation, while deletion of sequences between −86 and −65 bp (which ablates the ZII domain) resulted in a nearly complete loss of Zdbm1 transactivation. These data suggested that the ZII domain plays a role in the activation of transcription by Zdbm1. It should be noted that similar results were obtained when the luciferase reporter gene was cloned in place of the CAT gene, demonstrating that Zdbm-mediated transactivation of Zp is not reporter gene dependent (data not shown).

To confirm the role of the ZII domain in mediating Zdbm1 transactivation, the ZII domain was removed from the context of other Zp sequences and cloned upstream of a minimal β-globin promoter [(1xZII)BGCAT]. Little activation of the parent reporter plasmid containing only the β-globin TATA element was observed with either Zta or Zdbm1 (Fig. 8A). In addition, Zta only weakly transactivated the (1xZII)BGCAT reporter plasmid. However, Zdbm1 transactivated the (1xZII)BGCAT construct ca. 40-fold. Conversely, a (1xZIIIB)BGCAT reporter plasmid was activated by Zta (30-fold), but not by Zdbm1, again underscoring the altered specificity exhibited by Zdbm1.

As discussed above, we have shown that Zdbm do not appear to bind to any *cis* elements present in Zp. To further confirm that these mutants do not bind to the ZII response element, EMSA analysis was performed with in vitro-translated Zta, Zdbm1, and Zdbm2 and a ^{32}P-labeled double-stranded oligonucleotide containing the ZII domain (Fig. 8B). For comparison, binding of Zta, Zdbm1, and Zdbm2 to a double-stranded oligonucleotide containing the ZIIIB Zta binding site was also assessed. As we reported previously, Zta, but not Zdbm1 or Zdbm2, bound well to the ZIIIB site (47). In addition, low-level binding of Zta to the probe containing the ZII element was observed, consistent with the observed low-level transactivation of the (1xZII)BGCAT reporter by Zta. However, no binding of Zdbm1 or Zdbm2 to the ZII domain was observed.

Tandem copies of ZI elements from Zp support Zdbm1 transactivation. Although the ZII domain can mediate Zdbm1 transactivation, we observed that mutation of this element, in the context of Zp sequences from −221 to +14 bp, did not abrogate Zdbm1 transactivation (data not shown). This suggested that other elements in Zp also mediate Zdbm transactivation. In addition, deletion of promoter sequences between −221 and −159 bp and between −105 and −86 bp also appeared to diminish activation by Zdbm1 (Fig. 7). Therefore, we assessed the possible involvement of the ZI elements in mediating Zdbm transactivation. For this analysis one or three copies of either the ZIB or the ZIC element were cloned upstream of a minimal β-globin promoter CAT reporter construct. As shown in Fig. 9, the reporter constructs containing one copy of either ZIB or ZIC were not detectably responsive to Zdbm1. However, reporters containing three copies of ZIB or ZIC were activated 25- and 55-fold, respectively. As stated above, Zp contains at least four ZI elements, suggesting that the presence of multiple ZI elements also plays a role in Zdbm transactivation of Zp. The combination of the ZII domain and multiple ZI domains may account for the highly responsive nature of Zp compared with those of the other promoters surveyed.

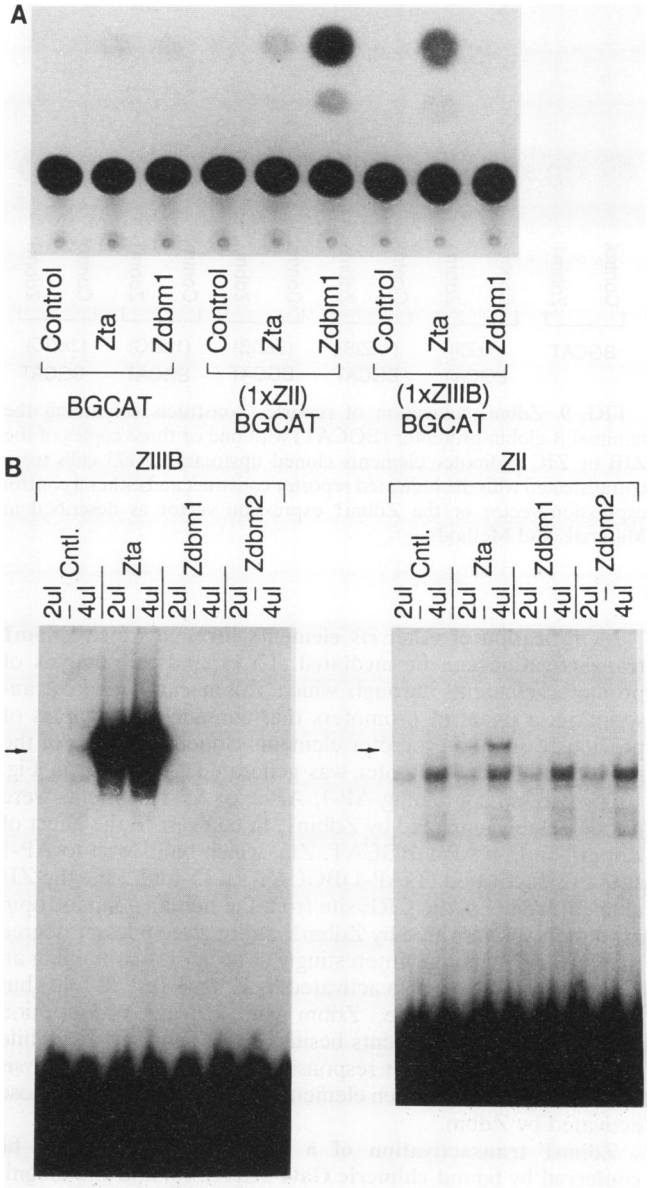

FIG. 8. (A) Zdbm1 transactivation can be mediated by the ZII domain of Zp. Shown are results of transactivation of a minimal β-globin promoter-driven CAT construct (BGCAT) or derivatives containing one copy of the ZII or ZIIIB elements, by Zta and Zdbm1. (B) Zdbm do not exhibit any specific binding in vitro to the ZII domain. Shown are results of EMSA of Zta, Zdbm1, and Zdbm2 binding to double-stranded oligonucleotide probes containing the ZIIIB or ZII element. Two or 4 μl of in vitro-translated proteins, generated in a wheat germ in vitro translation system, was incubated with the appropriate ^{32}P-labeled oligonucleotide. Cntl., control extract from unprogrammed wheat germ lysate.

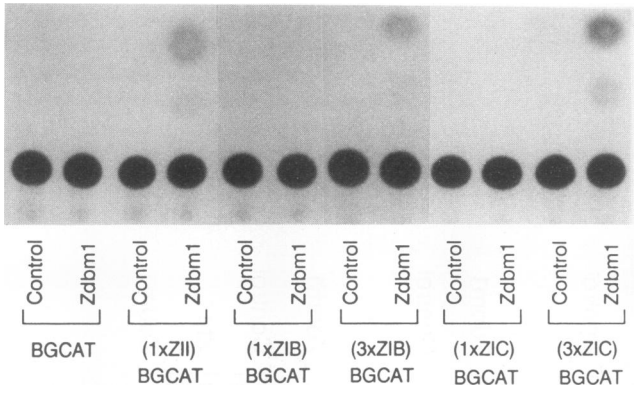

FIG. 9. Zdbm1 activation of reporter constructs containing the minimal β-globin promoter (BGCAT) with one or three copies of the ZIB or ZIC promoter elements cloned upstream. DG75 cells were cotransfected with the indicated reporter construct and either a control expression vector or the Zdbm1 expression vector as described in Materials and Methods.

Identification of other *cis* elements through which Zdbm1 transactivation can be mediated. To expand the analysis of promoter elements through which Zdbm can activate transcription, a panel of promoters that contain single copies of previously defined promoter elements cloned upstream of the minimal β-globin promoter was generated. As shown in Fig. 10, promoters containing AP-1, AP-2, or SP-1 elements were not detectably activated by Zdbm1. In contrast to the effect of Zdbm1 on (1×AP-1)BGCAT, Zta, which binds well to AP-1 sites (12), activated (1xAP-1)BGCAT ca. 15-fold. Like the ZII element from Zp, the CRE site from the human gonadotropin promoter was activated by Zdbm1, although to a lesser degree than the ZII element. Interestingly, a promoter containing an NF-κB element was also activated by Zdbm1 (ca. 20-fold) but not by Zta. Therefore, Zdbm can activate transcription through promoter elements besides those found in Zp, while other elements were not responsive. In addition, this analysis also distinguishes between elements activated by Zta and those activated by Zdbm.

Zdbm1 transactivation of a minimal promoter can be conferred by bound chimeric Gal4 activators, and the magnitude of this effect appears to be activation domain dependent. Taken together, the data presented above suggest that Zdbm transactivation is dependent on synergy between the DNA-binding mutant and a transactivator bound to a *cis* element upstream of the TATA box. Notably, Zta exhibits strong homosynergy (6, 13), suggesting that a bound Zta should mediate Zdbm transactivation. However, this possibility cannot be assessed with intact Zta, since presumably Zta-Zdbm1 heterodimers would be formed. To alleviate this problem, a Gal4-Zta chimeric transactivator in which the transactivation domain of Zta (residues 1 to 167) was fused to the DNA-binding domain of Gal4 (residues 1 to 147) was generated. As shown in Fig. 11, a reporter construct containing a single Gal4 binding site cloned upstream of the minimal β-globin promoter was not detectably activated by Zdbm1 in the absence of the Gal4-Zta chimera. However, addition of the Gal4-Zta expression vector led to significant Zdbm1 transactivation, and this transactivation was of a magnitude equivalent to that of Zdbm1 transactivation of the (1xZII)BGCAT reporter construct.

When a Gal4-VP16 chimeric transactivator was employed in place of Gal4-Zta, a lower level of Zdbm1 transactivation was

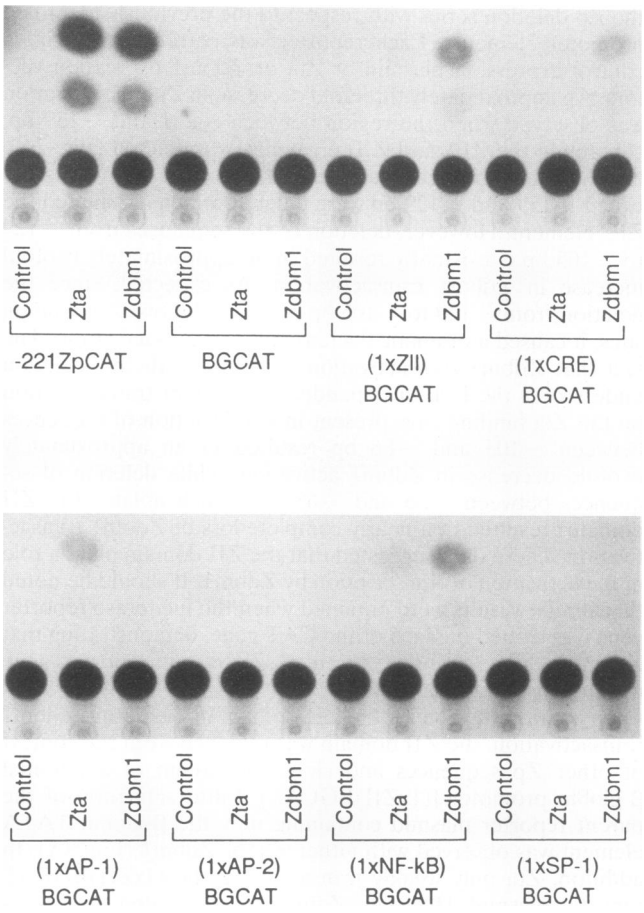

FIG. 10. Assessment of the ability of known promoter elements to mediate activation by Zta or Zdbm1. DG75 cells were cotransfected with the indicated reporter plasmids and a control expression vector, the Zta expression vector, or the Zdbm1 expression vector as described in Materials and Methods. The sequences of the oligonucleotides containing known promoter elements, which were used to generate the indicated reporter constructs, are given in Materials and Methods. BGCAT, minimal β-globin promoter-driven CAT construct.

observed, suggesting that Zdbm1 transactivation displays specificity through the activation domain of factors bound to upstream *cis* elements. Evidence that Gal4-VP16 was functional in this system was apparent from the activity of this chimera, compared with that of Gal4-Zta, on the (1xGal4)(1xZII)BGCAT reporter construct. Both chimeric proteins were able to synergize with the cellular factor(s) bound to the ZII domain. However, this effect was approximately fourfold greater with the Gal4-VP16 chimera. In addition, cotransfection of Zdbm1 resulted in a significant enhancement in activity with the Gal4-Zta chimera but only a modest increase in activity with the Gal4-VP16 chimera.

DISCUSSION

In this paper we describe the ability of DNA-binding mutants of a transcriptional activator, Zta, to activate transcription independent of specific targeting to its DNA recognition sequence. The initial observation that Zdbm retain the ability to transactivate Zp suggested several possibilities: (i) Zdbm homodimers are able to bind specifically to DNA (perhaps with altered site recognition) with sufficient affinity in vivo to

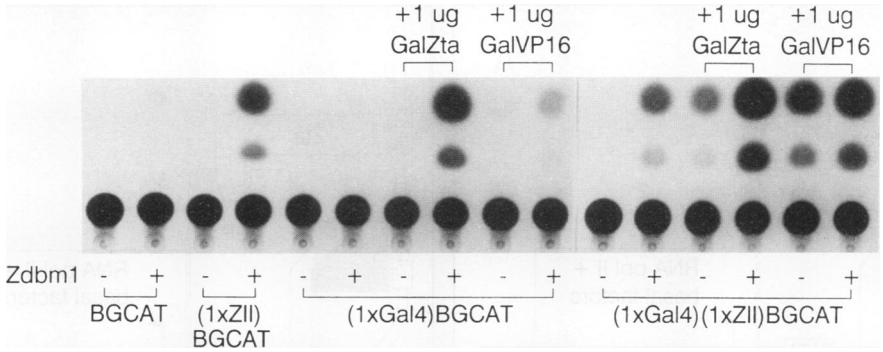

FIG. 11. Zdbm1 transactivation can be mediated through a bound Gal4-Zta chimera that does not contain the DNA-binding or dimerization domain of Zta. The DG75 cell line was cotransfected with 2 μg of the indicated reporter plasmid and 2 μg of either a control expression plasmid (−) or the Zdbm1 expression plasmid (+), as described in Materials and Methods. In addition, when indicated, 1 μg of GalZta (Gal4 DNA-binding domain [residues 1 to 147] linked to the Zta activation domain [residues 1 to 167]) or GalVP16 (Gal4 DNA-binding domain linked to the herpesvirus VP16 activation domain [44]) expression plasmids were transfected along with the indicated plasmids. BGCAT, minimal β-globin promoter-driven CAT construct.

account for the observed transactivation of Zp; (ii) Zdbm are able to dimerize with a cellular transcription factor, and these heterodimers transactivate Zp through binding to a specific recognition sequence(s) in Zp; or (iii) Zdbm do not bind directly to DNA but rather transactivate Zp through protein-protein interactions with specific transcription factors bound to *cis* elements in Zp. The data presented here appear to largely rule out the first two possibilities. With regard to the first possibility, the EMSA data clearly demonstrate that all three Zdbm assayed have a greatly reduced affinity for binding to either Zta recognition sequences or Zp. However, Zdbm1 is at least as good a transactivator of Zp as Zta at subsaturating transactivator concentrations. Thus, if direct binding of Zdbm1 homodimers to Zp were required for transactivation, then the affinity of Zdbm1 binding would need to be equivalent to that observed with Zta. In addition, Zdbm are readily extracted from the nucleus under very low salt conditions, while the wild-type protein remains avidly associated with genomic DNA, further substantiating the in vitro DNA-binding data. The latter experiment also makes it unlikely that Zdbm transactivation is mediated via heterodimerization with a cellular transcription factor, leading to the formation of a DNA-binding-competent complex, since such an interaction should have given rise to a significant fraction of mutant protein which was not easily extracted from the nucleus. Furthermore, the ability of Zdbm1 transactivation to be mediated through bound Gal4 chimeric transactivators indicates that this activity is unlikely to arise through heterodimerization, since Gal4 has none of the structural hallmarks of a bZIP transactivator.

The cis requirements for Zdbm activation are clearly different from those of Zta, since activation of Zp by Zdbm1 is not dependent upon the Zta recognition sequences, ZIIIA and ZIIIB, but instead requires promoter elements that bind cellular factors. Conversely, Zta functions poorly on Zdbm-responsive elements (e.g., ZII and NF-κB) but as expected functions well on Zta recognition sequences (e.g., ZIIIB and AP-1). The inability of Zdbm to activate through Zta binding sites is expected, but it is less clear why Zta fails to activate through Zdbm response elements. This likely reflects the fact that localization of Zta in the cell is dictated primarily by high-affinity interactions with specific DNA recognition sites which dominate over localization through weaker protein-protein interactions. That these interactions differ greatly in affinity clearly appears to be the case, since binding of Zta to its DNA recognition sequences is readily demonstrated, while the interaction of Zta with cellular proteins has been difficult to demonstrate. Furthermore, since the Zta DNA recognition sequence is relatively degenerate (7, 16, 17, 33, 42, 47), it is expected that there are many Zta binding sites in the human genome. This would mean that there should be little unbound Zta in the nucleus, and therefore DNA-binding-independent transactivation by Zta would be precluded. As shown in Fig. 3A, while nearly all the Zta expressed in transiently transfected cells was tightly associated with genomic DNA and could not be extracted under low-salt conditions, Zdbm1 and Zdbm2 were readily extracted from genomic DNA under low-salt conditions.

One possible model to explain Zdbm transactivation of Zp involves protein-protein interactions of Zdbm with factors bound to upstream *cis* elements (Fig. 12A). Previous studies have provided several examples of direct interactions between transcription factors. For example, the herpes simplex virus VP-16 protein has been shown to transactivate through interactions with the DNA-binding factor Oct-1 (21, 41). A mutant form of the GC-box-binding protein SP-1, defective for DNA binding, was shown to activate promoters bearing a bound SP-1 molecule (43). In the latter study, it was shown that SP-1 can form homooligomeric complexes, and it was suggested that the SP-1 mutant was able to transactivate through tethering with nonmutant DNA-bound SP-1. It should be noted, however, that our attempts to demonstrate an interaction between Zdbm1 and the cellular factor(s) interacting with the ZII domain have been negative to date. This suggests that these interactions are either of very low affinity or that they do not occur.

As discussed above, Lieberman and Berk (34) have demonstrated that Zta can form a stable interaction with TBP in vitro, and they mapped the region of Zta that is required for this interaction to residues 25 to 86. We have shown here that residues 28 to 53 are essential for Zdbm transactivation. This suggests another possible mechanism to explain Zdbm transactivation involving targeting of Zdbm to promoters through direct interactions with TBP (Fig. 12B). It is also possible that Zta interacts with other components of the basal complex which could in fact add additional stabilizing interactions. As such, a Zdbm might function as a coactivator and supply a function(s) that is necessary for activation by specific transactivators. While the coactivator (i.e., the Zdbm) or the transactivator alone functions poorly, together they are able to efficiently activate transcription. Interestingly, the concerted

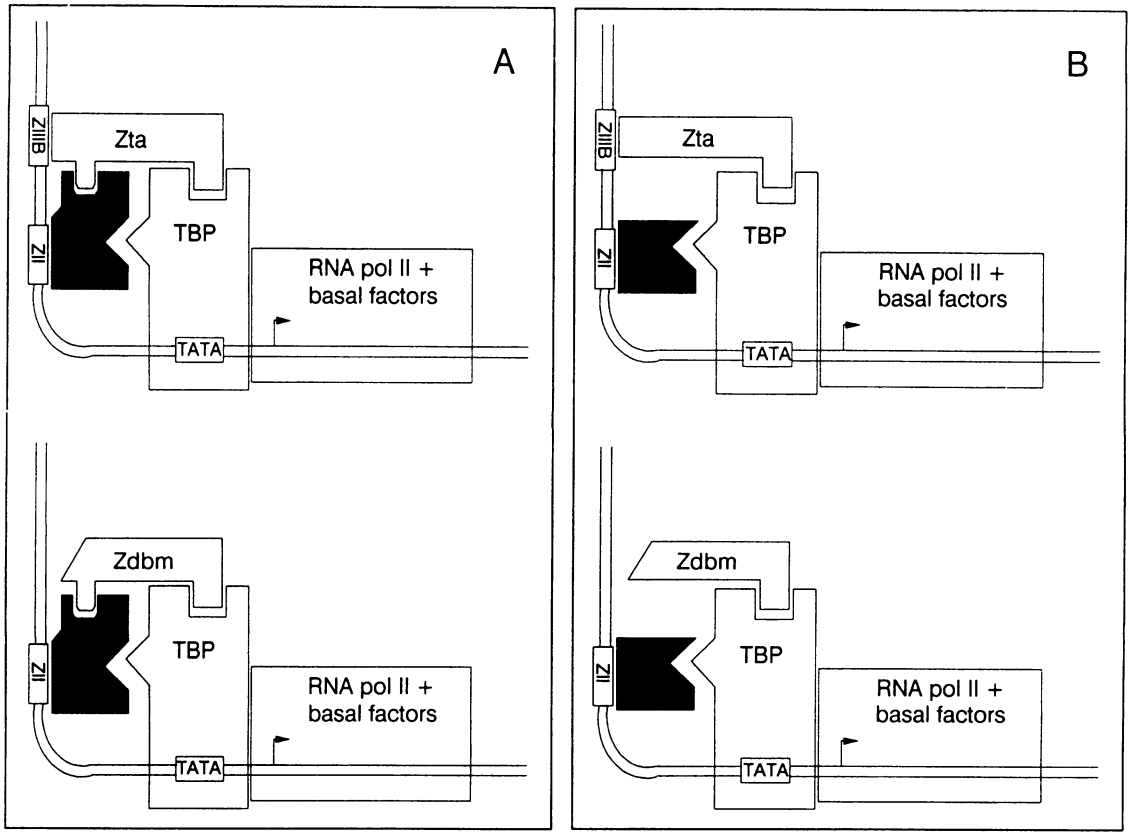

FIG. 12. Schematic representations of possible mechanisms by which Zta and Zdbm activate transcription. (A) In this model Zta is predicted to make interactions with both the TATA-box-binding factor (TBP) and the cellular factor which binds to the octamer AP-1/CREB site (ZII domain) present in Zp. DNA-binding-independent transactivation by Zdbm might then be facilitated through tethering to the cellular factor bound to the ZII domain. (B) An alternative model in which Zta and Zdbm function as coactivators. In this case, the Zdbm is directed to the promoter through interactions with TBP. Specificity is elicited by either the availability of the appropriate TBP-promoter complex which can bind Zdbm or the ability of transcription factors bound to upstream *cis* elements to synergize with the signal provided by Zdbm. pol, polymerase.

effect of Zdbm and the ZII or ZI elements is strikingly similar to the heterosynergistic effect observed between these elements and Zta. We have previously observed a high degree of synergy between the ZI elements in Zp and Zta (16) and we have observed a high level of heterosynergy between Zta and ZII binding factors employing a minimal promoter containing one Zta binding site and one ZII element (19). Therefore, the ability of a Zdbm to function as a coactivator could be related to the ability of Zta to synergize with specific upstream bound transcription factors. One level of specificity then could be explained by a preferential ability to synergize with a subset of transcription factors. Importantly, upstream bound transcription factors might also exert negative effects on Zdbm transactivation by interacting with the basal machinery in a similar way, thereby precluding the association of Zdbm with the promoter. We consistently find that highly active promoters with large arrays of promoter and enhancer elements (e.g., SVp/e and RSV long terminal repeat) are not activated by Zdbm. Even though these promoters might have elements that can synergize with Zdbm, it might also be predicted that these promoters have bound factors that preclude the association of Zdbm with the promoter.

Finally, a third possibility not addressed by the models presented in Fig. 12 is that the activation of Zp by Zdbm could involve derepression of the promoter. It has not been ruled out that the extremely low basal level exhibited by Zp is achieved by an active repression of the promoter. If this is the case, then activation of Zp by Zdbm might involve displacement of such a repressor(s), resulting in activation of transcription from Zp. Although a deletional analysis of Zp did not indicate the presence of a site(s) for repression (15), such a site(s) may be coincident with a positive element(s), thereby masking the effect of derepression.

While we have employed an artificial system to study the effect of non-DNA-binding forms of Zta by genetically altering the ability of Zta to bind DNA, several lines of evidence suggest that a DNA-binding-deficient form of Zta could play a role in the EBV viral lytic program. First, Zdbm1 transactivation exhibits promoter specificity, and Zp appears to have evolved to be particularly responsive to Zdbm. Second, Zdbm1 activates Zp at least as well as Zta, even at low effector concentrations. Third, Lau et al. (32) have identified an alternatively spliced BZLF1 RNA species in which exon 2, which encodes the DNA-binding domain of Zta, is absent. Fourth, specific protein modifications have previously been shown to abrogate Zta DNA binding. With respect to the latter point, Zta contains a cysteine residue at position 189 in the DNA-binding domain, oxidation of which generates a non-DNA-binding form of Zta (3). In addition, phosphorylation of the serine residue at amino acid 173 of Zta by casein kinase II abrogates DNA binding (30). These data raise the possibilities that a non-DNA-binding form(s) of Zta is expressed at specific

times in the viral life cycle and has a productive role in the viral life cycle.

Modulation of Zta DNA-binding activity by oxidation and phosphorylation is particularly interesting, since these events can occur through enzymatic processes. DNA binding by c-Jun has been shown to be affected by oxidation, and Abate et al. (1) identified a reducing activity in nuclear extracts. Phosphorylation by creatine kinase II has been shown to inhibit DNA binding of c-Myb (38) and c-Jun (5). Moreover, Lin et al. (36) have provided evidence that phosphorylation of c-Jun by creatine kinase II occurs in vivo. Regulation of these processes by external stimuli is likely to play a role in regulating the activity of promoters containing recognition sequences for these factors. The implications of regulating Zta binding activity in vivo would be more complicated. Negative regulation of Zta DNA-binding activity could not only decrease the activation of some promoters containing Zta recognition sequences but might also result in the activation of other promoters independent of Zta recognition sequences. Moreover, regulating the DNA-binding activity of other transcription factors likewise may not only impact the activity of their specific DNA-binding elements but may also impart activities that are manifested through distinct sets of *cis* elements.

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REFERENCES

1. **Abate, C., L. Patel, F. J. Rauscher III, and T. Curran.** 1990. Redox regulation of FOS and JUN DNA-binding activity in vitro. Science **249:**1157–1161.
2. **Baer, R., A. T. Bankier, M. D. Biggin, P. L. Deininger, P. J. Farrell, T. J. Gibson, G. Hatfull, G. S. Hudson, S. C. Satchwell, C. Seguin, P. S. Tuffnell, and B. G. Barrell.** 1984. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature (London) **310:**207.
3. **Bannister, Z. J., A. Cook, and T. Kouzarides.** 1991. In vitro DNA binding activity of Fos/Jun and BZLF1 but not C/EBP is affected by redox changes. Oncogene **6:**1243–1250.
4. **Bauer, G., P. Hofler, and H. zur Hausen.** 1982. Epstein-Barr virus induction by a serum factor. I. Induction and cooperation with additional inducers. J. Virol. **121:**184–194.
5. **Boyle, W. J., T. Smeal, L. H. K. Defize, P. Angel, J. R. Woodgett, M. Karin, and T. Hunter.** 1991. Activation of protein kinase C decreases phosphorylation of c-jun at sites that negatively regulate its DNA-binding activity. Cell **64:**573–584.
6. **Carey, M., J. Kolman, D. A. Katz, L. Gradoville, L. Barberis, and G. Miller.** 1992. Transcriptional synergy by the Epstein-Barr virus transactivator ZEBRA. J. Virol. **66:**4803–4813.
7. **Chang, Y.-N., D. L.-Y. Dong, G. S. Hayward, and S. D. Hayward.** 1990. The Epstein-Barr virus Zta transactivator: a member of the bZIP family with unique DNA-binding specificity and dimerization domain that lacks the characteristic heptad leucine zipper motif. J. Virol. **64:**3368–3369.
8. **Chevallier-Greco, A., E. Manet, P. Chavrier, C. Mosnier, J. Daillie, and A. Sergeant.** 1986. Both Epstein-Barr virus (EBV)-encoded trans-acting factors, EB1 and EB2, are required to activate transcription from an EBV early promoter. EMBO J. **5:**3243–3249.
9. **Cohen, L. K., S. H. Speck, B. E. Roberts, and J. L. Strominger.** 1984. Identification and mapping of polypeptides encoded by the P3HR-1 strain of Epstein-Barr virus. Proc. Natl. Acad. Sci. USA **81:**4183–4187.
10. **Countryman, J., H. Jenson, R. Seibel, H. Wolf, and G. Miller.** 1987. Polymorphic proteins encoded within BZLF1 of defective and standard Epstein-Barr viruses disrupt latency. J. Virol. **61:** 3672–3679.
11. **Countryman, J., and G. Miller.** 1985. Activation of expression of latent Epstein-Barr herpesvirus after gene transfer with a small cloned subfragment of heterogeneous viral DNA. Proc. Natl. Acad. Sci. USA **82:**4085–4089.
12. **Farrell, P. J., D. T. Rowe, C. M. Rooney, and T. Kouzarides.** 1989. Epstein-Barr virus BZLF1 trans-activator specifically binds to a consensus ZP-1 site and is related to c-fos. EMBO J. **8:**127–132.
13. **Flemington, E. K., A. M. Borras, J. P. Lytle, and S. H. Speck.** 1992. Characterization of the Epstein-Barr virus BZLF1 protein transactivation domain. J. Virol. **66:**922–929.
14. **Flemington, E. K., A. E. Goldfeld, and S. H. Speck.** 1991. Efficient transcription of the Epstein-Barr virus immediate-early BZLF1 and BRLF1 genes requires protein synthesis. J. Virol. **65:**7073–7077.
15. **Flemington, E. K., and S. H. Speck.** 1990. Identification of phorbol ester response elements in the promoter of the Epstein-Barr virus putative lytic switch gene BZLF1. J. Virol. **64:**1217–1226.
16. **Flemington, E. K., and S. H. Speck.** 1990. Autoregulation of Epstein-Barr virus putative lytic switch gene BZLF1. J. Virol. **64:**1227–1232.
17. **Flemington, E. K., and S. H. Speck.** 1990. Epstein-Barr virus BZLF1 *trans* activator induces the promoter of a cellular cognate gene, c-*fos*. J. Virol. **64:**4549–4552.
18. **Flemington, E. K., and S. H. Speck.** 1990. Evidence for coiled-coil dimer formation by an Epstein-Barr virus transactivator that lacks a heptad repeat of leucine residues. Proc. Natl. Acad. Sci. USA **87:**9459–9463.
19. **Flemington, E. K., and S. H. Speck.** Unpublished data.
20. **Foss, K., and W. H. McClain.** 1987. Rapid site-specific mutagenesis in plasmids. Gene **59:**285–290.
21. **Gerster, T., and R. G. Roeder.** 1988. A herpes virus transactivating protein interacts with transcription factor OTF-1 and other cellular proteins. Proc. Natl. Acad. Sci. USA **85:**6347–6351.
22. **Giot, J.-F., I. Mikaelian, M. Buisson, E. Banet, I. Joab, J.-C. Nicolas, and Z. Sergeant.** 1991. Transcriptional interference between the EBV transcription factors EB1 and R: both DNA binding and activation domains of EB1 are required. Nucleic Acids Res. **19:**1251–1258.
23. **Goldfeld, A. E., C. Doyle, and T. Maniatis.** 1990. Human tumor necrosis factor alpha gene regulation by virus and lipopolysaccharide. Proc. Natl. Acad. Sci. USA **87:**9769–9773.
24. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
25. **Grogan, E., H. Jenson, J. Countryman, L. Heston, L. Gradoville, and G. Miller.** 1987. Transfection of a rearranged viral DNA fragment WZhet, stably converts latent Epstein-Barr viral infection to productive infection in lymphoid cells. Proc. Natl. Acad. Sci. USA **84:**1332–1336.
26. **Hardwick, J. M., P. M. Lieberman, and S. D. Hayward.** 1988. A new Epstein-Barr virus transactivator, R, induces expression of cytoplasmic early antigen. J. Virol. **62:**2274–2284.
27. **Hummel, M., and E. Kieff.** 1982. Mapping of polypeptides encoded by the Epstein-Barr virus genome in productive infection. Proc. Natl. Acad. Sci. USA **79:**5698–5702.
28. **Kallin, B., J. Luka, and G. Klein.** 1979. Immunochemical characterization of Epstein-Barr virus-associated early and late antigens in *n*-butyrate-treated P3HR-1 cells. J. Virol. **32:**710–716.
29. **Knutson, J. C., and B. Sugden.** 1989. Immortalization of B lymphocytes by Epstein-Barr virus: what does the virus contribute to the cell. Adv. Viral Oncol. **8:**151–172.
30. **Kolman, J. L., N. Taylor, D. R. Marshak, and G. Miller.** 1993. Serine-173 of the Epstein-Barr virus ZEBRA proteins is required for DNA-binding and is a target for casein kinase II phosphorylation. Proc. Natl. Acad. Sci. USA **90:**10115–10119.
31. **Kouzarides, T., G. Packham, A. Cook, and P. Farrell.** 1991. The BZLF1 protein of EBV has a coiled coil dimerisation domain without a heptad leucine repeat but with homology to the C/EBP leucine zipper. Oncogene **6:**195–204.
32. **Lau, R., G. Packham, and P. J. Farrell.** 1992. Differential splicing

of Epstein-Barr virus immediate-early RNA. J. Virol. **66**:6233–6236.

33. **Lieberman, P., and A. Berk.** 1990. In vitro transcriptional activation, dimerization, and DNA-binding specificity of the Epstein-Barr virus Zta protein. J. Virol. **64**:2560–2568.
34. **Lieberman, P. M., and A. J. Berk.** 1991. The Zta trans-activator protein stabilizes TFIID association with promoter DNA by direct protein-protein interaction. Genes Dev. **5**:2441–2454.
35. **Lillie, J. W., and M. R. Green.** 1989. Transcription activation by the adenovirus E1a protein. Nature (London) **338**:39–44.
36. **Lin, A., J. Frost, T. Deng, T. Smeal, N. Al-Alawi, U. Kikkawa, T. Hunter, D. Brenner, and M. Karin.** 1992. Casein kinase II is a negative regulator of c-Jun DNA binding and AP-1 activity. Cell **70**:777–789.
37. **Luka, J., B. Kallin, and G. Klein.** 1979. Induction of the Epstein-Barr virus (EBV) cycle in latently infected cells by n-butyrate. Virology **94**:228–231.
38. **Luscher, B., E. Christenson, D. Litchfield, E. G. Krebs, and R. Eisenman.** 1990. Myb DNA binding inhibited by phosphorylation at a site deleted during oncogenic activation. Nature (London) **344**:517–522.
39. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
40. **Miller, G., M. Rabson, and L. Heston.** 1984. Epstein-Barr virus with heterogeneous DNA disrupts latency. J. Virol. **50**:174–182.
41. **O'Hare, P., and C. R. Goding.** 1988. Herpes simplex virus regulatory elements and the immunoglobulin octamer domain bind a common factor and are both targets for virion transactivation. Cell **52**:435–445.
42. **Packham, G., A. Economou, C. M. Rooney, D. T. Rowe, and P. J. Farrell.** 1990. Structure and function of the Epstein-Barr virus BZLF1 protein. J. Virol. **64**:2110–2116.
43. **Pascal, E., and R. Tjian.** 1991. Different activation domains of Sp1 govern formation of multimers and mediate transcriptional synergism. Genes Dev. **5**:1646–1656.
44. **Sadowski, I., J. Ma, S. Triezenberg, and M. Ptashne.** 1988. GAL4-VP-16 is an unusually potent transcriptional activator. Nature (London) **335**:563–565.
45. **Speck, S. H., and J. L. Strominger.** 1989. Transcription of Epstein-Barr virus in latently infected, growth transformed lymphocytes. Adv. Viral Oncol. **8**:133–150.
46. **Takada, K., and Y. Ono.** 1989. Synchronous and sequential activation of latently infected Epstein-Barr virus genomes. J. Virol. **63**:445–449.
47. **Taylor, N., E. K. Flemington, J. L. Kolman, R. P. Baumann, S. H. Speck, and G. Miller.** 1991. ZEBRA and a Fos-GCN4 chimeric protein differ in their DNA-binding specificities for sites is the Epstein-Barr virus BZLF1 promoter. J. Virol. **65**:4033–4041.
48. **Tovey, M., G. Lenoir, and J. Lours-Begon.** 1978. Activation of latent Epstein-Barr virus by antibody to human IgM. Nature (London) **276**:270–272.
49. **Urier, G., M. Buisson, P. Chambard, and A. Sergeant.** 1989. The Epstein-Barr virus early protein EB1 activates transcription from different responsive elements including AP-1 binding sites. EMBO J. **8**:1447–1453.
50. **Woisetschlaeger, M., J. L. Strominger, and S. H. Speck.** 1989. Mutually exclusive use of viral promoters in Epstein-Barr virus latently infected lymphocytes. Proc. Natl. Acad. Sci. USA **86**:6498–6502.
51. **zur Hausen, H., F. O'Neil, and U. Freese.** 1978. Persisting oncogenic herpesvirus induced by the tumor promoter TPA. Nature (London) **272**:373–375.