JOURNAL OF VIROLOGY, Mar. 1990, p. 1227–1232
0022-538X/90/031227-06$02.00/0

Vol. 64, No. 3

Autoregulation of Epstein-Barr Virus Putative Lytic Switch Gene BZLF1

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Received 9 October 1989/Accepted 1 December 1989

Expression of the Epstein-Barr virus (EBV) BZLF1 gene in latently infected lymphocytes is sufficient to trigger the viral lytic cycle. As shown in the accompanying report (E. Flemington and S. H. Speck, J. Virol. 64:1217–1226, 1990), the promoter for the BZLF1 gene (Zp) contains two distinct types of elements (ZI and ZII [an AP-1-like domain]) which are responsive to the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), an inducer of the viral lytic cycle. Although Zp can be activated with TPA in an EBV-negative Burkitt's lymphoma cell line (Ramos), its activity is considerably lower than in EBV-positive cell lines which can be induced with TPA. Here we show that the protein product of the BZLF1 gene (ZEBRA) can transactivate its own promoter by a mechanism which involves direct binding to a region distinct from the ZI and ZII element. Moreover, we show that this region is composed of two distinct ZEBRA-binding–transactivation domains. Interestingly, these two domains are not homologous, and while one domain (ZIIIA) is similar to previously described ZEBRA-binding domains, the second (ZIIIB) is a higher-affinity site which bears no detectable homology to the consensus ZEBRA recognition sequence. We also show that transactivation is independent of the otherwise essential ZII domain, suggesting that ZEBRA binding may functionally replace or supercede the need for a functional ZII domain. This observation supports a model for activation of the lytic cycle whereby synthesis of a critical level of ZEBRA signals commitment to BZLF1 transcription and initiation of the lytic cascade.

Among the viral proteins which have been identified as being expressed during the onset of the Epstein-Barr virus (EBV) lytic cycle, the BZLF1 gene product (ZEBRA) is unique in its ability to disrupt latency when introduced into latently infected lymphocytes (3). The ability of a single viral antigen to trigger the lytic cycle appears to be analogous to that of bacteriophage lambda and the expression of *cro* (14). ZEBRA has been shown to activate transcription of the EBV BHLF1 and BHRF1 genes, which give rise to two abundant early lytic viral transcripts (2, 9, 10, 12, 13, 17–19), and has also recently been shown to bind and transactivate the promoter for the BSLF2/BMLF1 gene (5, 19). It has been shown by Farrell et al. (5) that two regions of ZEBRA share sequence similarity with c-*fos*. One of these regions of homology corresponds to the region of c-*fos* which is similar to the DNA-binding domains of the c-*jun* and GCN4 proteins (20). Indeed, the BZLF1 protein was shown to bind to a region of the BSLF2/BMLF1 promoter containing a consensus AP-1 site (5).

Recently it was shown that the viral lytic cycle can be synchronously induced by treatment of the EBV-infected Burkitt's lymphoma cell line Akata with anti-immunoglobulin (17). In the presence of the protein synthesis inhibitor cycloheximide, only transcription of the BZLF1 and BRLF1 genes can be induced by treatment with anti-immunoglobulin. The time course of BZLF1 transcription revealed that expression peaks at 2 to 4 h postinduction, and the message was not detectable by 6 h. Thus, transcription of the BZLF1 gene appears to be activated for a short time followed by abrupt down regulation.

It has recently been demonstrated that ZEBRA can transactivate the BZLF1 promoter in HeLa cells (19). Here we demonstrate autoregulation of the BZLF1 gene in B lymphocytes and map the sequences involved in transactivation of Zp by ZEBRA. Moreover, we show that transactivation occurs via direct binding to two juxtaposed but distinct ZEBRA-binding domains. Similar to the results obtained with ZEBRA binding to the promoter for the BSLF2/BMLF1 gene (5), one of these ZEBRA-binding–transactivation domains (ZIIIA) shares homology with the AP-1 recognition sequence. Moreover, mutation of this domain has a negative effect on 12-*O*-tetradecanoylphorbol-13-acetate (TPA) inducibility and ZEBRA transactivation. In contrast, the second ZEBRA-binding–transactivation domain bears no detectable homology to previously described ZEBRA-binding domains, and mutation of this domain dramatically decreased ZEBRA transactivation but did not affect the TPA inducibility of Zp. In addition, we show that although initial activation of BZLF1 transcription by TPA requires the ZII domain, in the presence of ZEBRA, this site can be mutated without significantly affecting Zp activity.

MATERIALS AND METHODS

Cell culture, transfections, and chloramphenicol acetyltransferase assays. Cells were grown at 37°C in RPMI 1640 medium (GIBCO Laboratories) containing 10% newborn calf serum as previously described (15). Transfections and chloramphenicol acetyltransferase assays were performed exactly as described in the accompanying report (6), except that in addition to the reporter plasmid, cells were cotransfected with 2 μg of either control plasmid pSV40 or BZLF1 expression vector pSV40-BZLF1. Descriptions of these plasmids are given below.

Plasmid construction. The ZpCAT constructs used were derived from those described in the accompanying report (6). Because of background transactivation of the vector in

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which the original ZpCAT constructs were cloned, all ZpCAT constructs were transferred to a pUC-based *cat* vector. This vector showed no ability to be transactivated by ZEBRA. In addition, two new ZpCAT constructs were generated. The −105MII construct was made by performing site-directed mutagenesis (7) on the −105ZpCAT construct. The mutation in this construct is identical to the mutation in the MII construct (see Fig. 3 and reference 6). The control plasmid used for cotransfections (pSV40) was generated by cloning a 520-base-pair (bp) *Acc*I-*Hin*dIII fragment containing the simian virus 40 promoter and enhancer from pSV2CAT (8) into the *Kpn*I-*Hin*dIII sites of the Bluescript (Stratagene) vector. The BZLF1 expression plasmid was constructed by inserting a 1,230-bp *Nae*I-*Bam*HI fragment from the EBV *Bam*HI Z fragment, containing the entire BZLF1 gene, into the *Eco*RV-*Bam*HI sites of pSV40 (pSV40-BZLF1). pATH2-BZLF1 was generated by cloning the entire open reading frame from a full-length BZLF1 cDNA clone (1) in frame with the *trpE* gene in the procaryotic expression vector pATH2 (4).

Extract preparation and binding assays. Nuclear extracts were prepared as described in the accompanying report (6). For TPA-induced B95-8 extracts, TPA was added to a final concentration of 20 ng/ml 36 h before harvesting of the cells.

Bacterial extracts containing the ZEBRA-TrpE fusion protein or TrpE were prepared essentially as previously described (15). Briefly, DH5 cells containing either pATH2 or pATH2-BZLF1 were grown overnight in L broth. Bacteria from 10 ml of the overnight culture were pelleted and suspended in 100 ml of M9CA minimal medium plus 10 μg of thiamine B1 per ml. These cultures were incubated for 1 h at 37°C with vigorous shaking followed by addition of indoleacrylic acid to a final concentration of 5 μg/ml. After further incubation for 4 h, the cells were harvested, suspended in TEN buffer (50 mM Tris [pH 7.5], 0.5 mM EDTA, 0.3 M NaCl), and lysed by sonication, and the insoluble fraction was washed three times with TEN buffer. The protein pellets were subsequently solubilized in 8 M urea plus 0.5% 2-mercaptoethanol, and the fusion proteins were renatured by dialysis against 50 volumes of buffer D (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.9], 20% (vol/vol) glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol) plus 0.05% Nonidet P40 for 12 h with three changes. DNase I footprinting was performed as previously described (11).

RESULTS

The BZLF1 protein product transactivates its own promoter. To study the effect of ZEBRA on Zp, the BZLF1 gene was cloned downstream of the simian virus 40 early promoter-enhancer (pSV40-BZLF1). This plasmid was cotransfected into the EBV-negative cell line Ramos with a plasmid containing Zp (−221 to +12) linked to the bacterial *cat* gene (−221ZpCAT). Cotransfection of the −221ZpCAT plasmid with pSV40-BZLF1 resulted in a 14-fold increase in activity over the control (Table 1; Fig. 1 and 2), while the effect of TPA alone on −221ZpCAT resulted in approximately 23-fold activation. Furthermore, TPA treatment in conjunction with cotransfection of the pSV40-BZLF1 plasmid yielded a 140-fold increase in the activity of −221ZpCAT. The superinducibility of Zp in the presence of both TPA and ZEBRA suggests a synergism between these two reagents. However, since the BZLF1 gene is driven by a TPA-responsive simian virus 40 promoter-enhancer, this synergism may be in part due to an increased level of ZEBRA in cells treated with TPA. With regard to this point, cloning of either of the ZEBRA response elements ZIIIA or ZIIIB (see below) upstream of the beta-globin promoter confers ZEBRA transactivation which is unaffected by the addition of TPA (data not shown). Therefore, it is unlikely that the synergism observed with TPA and ZEBRA is due to increased levels of ZEBRA in TPA-treated cells.

TABLE 1. Effects of specific mutations in Zp on ZEBRA transactivation[a]

Plasmid	Mean ± SE CAT activity			
	Control	With TPA	With ZEBRA	With TPA and ZEBRA
−221CAT	0.04 ± 0.007	1.0 ± 0.08	0.6 ± 0.02	5.6 ± 0.42
MIICAT	0.04 ± 0.004	0.16 ± 0.06	0.6 ± 0.03	4.7 ± 0.81
MIIIACAT	0.02 ± 0.002	0.3 ± 0.04	0.1 ± 0.01	0.8 ± 0.07
MIIIBCAT	0.03 ± 0.002	0.7 ± 0.06	0.09 ± 0.01	0.9 ± 0.04

[a] Chloramphenicol acetyltransferase (CAT) assays of extracts prepared from Ramos cells 36 h posttransfection with the indicated plasmid (6; Fig. 1). The assays were quantitated by liquid scintillation counting, and the activities relative to −221ZpCAT (with TPA) are given. These data represent the averages of three separate experiments.

ZEBRA transactivation domains map to a region distinct from the ZII (AP-1-like) domain. Initial mapping of the *cis*-acting element(s) involved in ZEBRA transactivation was performed by cotransfection of a series of ZpCAT deletion mutants with the pSV40-BZLF1 plasmid (Fig. 1 and 2). Deletion of sequences between −221 and −159 bp did not diminish transactivation but rather resulted in a slight increase. Deletion of sequences between −159 and −129 bp resulted in a decrease in transactivatibility from 14-fold to 8-fold, and a more pronounced decline in transactivation was observed when the sequences from −129 to −105 bp were deleted (a drop from eightfold to twofold). Further deletions (−86Zp and −65Zp) showed little change in transactivation. These data suggest that ZEBRA transactivation occurs via a *cis*-acting element(s) located between −159 and −105 bp.

Since ZEBRA has been shown to be a DNA-binding protein (5), we assayed for direct binding of ZEBRA to this region. ZEBRA was generated as a bacterial fusion protein by cloning of a full-length BZLF1 cDNA (1) into the pATH2 expression vector (4) in frame with the bacterial *trpE* gene (pATH2.BZLF1). Extracts were prepared from induced bacteria harboring either this plasmid or the control plasmid (pATH2). Following crude purification and renaturation (16), these extracts were used to assay for ZEBRA binding to Zp (Fig. 3). ZEBRA footprints a region spanning from approximately −125 to −104 bp (ZIIIB domain) (Fig. 3 and 4). In contrast to the results obtained by Farrell et al. (5) for binding to the promoter of the EBV BSLF2/BMLF1 gene, there appeared to be no sequence within the ZIIIB domain with discernible homology to an AP-1 binding site. Also shown in Fig. 3 is a comparison of nuclear extracts prepared from either TPA-induced or uninduced B95.8 cells (an EBV-infected marmoset cell line which can readily be induced into the lytic cycle with TPA). Extracts from TPA-treated B95.8 cells revealed a footprint not detected with the nuclear extract from untreated B95.8 cells. This footprint maps to the same region as the ZEBRA footprint, suggesting that endogenously produced ZEBRA is responsible for this protection. No other TPA-specific footprints were detected.

Immediately upstream from ZIIIB lies a sequence (TGAGCCA) with considerable homology to the previously described ZEBRA-binding domain (TGAGTCA) (Fig. 4). Increasing the amount of ZEBRA fusion protein in the

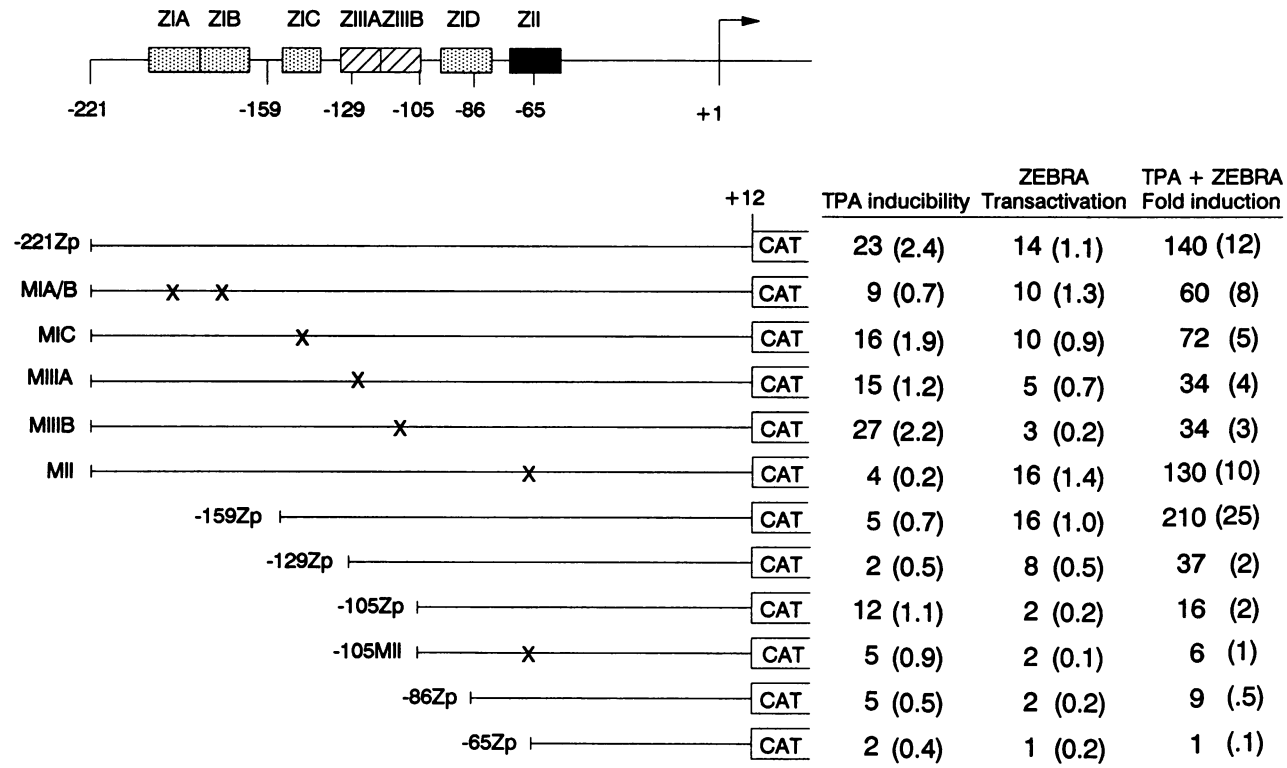

FIG. 1. TPA induction and ZEBRA transactivation of ZpCAT constructs. ZEBRA transactivation values were calculated from the activities of non-TPA-treated cells. Site-directed mutations are indicated by an X, and specific base changes are shown in Fig. 4. The data were generated in three separate experiments (standard errors are shown in parentheses). A scatter plot of these data is shown in Fig. 2. CAT, Chloramphenicol acetyltransferase.

DNase I protection assay revealed an additional footprint which spans this sequence (ZIIIA) (Fig. 5). These data are consistent with the functional analysis obtained with the Zp deletions (Fig. 1 and 2). As discussed above, transactivation was essentially abrogated by the −105Zp deletion, in which both of these domains are deleted. Deletion of sequences up to −129 bp also had an effect on ZEBRA transactivation. This may be due to encroachment into this second ZEBRA recognition sequence and/or it may reflect deletion of the ZIC domain (see below).

Site-directed mutation of individual ZEBRA-binding domains in Zp diminishes both binding and transactivation by ZEBRA. Site-directed mutagenesis was used to mutate specifically the core ZEBRA-binding domains (MIIIA and MIIIB; Fig. 4). Binding of ZEBRA was abrogated by mutation of either the ZIIIA or ZIIIB core-binding domain (Fig. 5), while binding to the adjacent domain was not affected. This finding indicates that ZEBRA binding ZIIIA and ZIIIB is not cooperative. In addition, ZEBRA appears to bind to the ZIIIB domain with a higher affinity than to the ZIIIA site. Similarly, a significant diminution was observed when these mutants were assayed for the ability to be transactivated by ZEBRA (Fig. 1; Table 1). Mutation of ZIIIA resulted in a reduction in ZEBRA transactivation from 14-fold to 5-fold, while mutation of ZIIIB resulted in a more significant reduction (from 14-fold to 3-fold). Importantly, the MIIIB mutant plasmid is fully responsive to TPA, clearly distinguishing this transactivation domain from elements of Zp involved in TPA induction. Mutation of the AP-1-like ZEBRA-binding domain (ZIIIA), however, did result in a reduction in TPA inducibility (from 23-fold to 15-fold). Therefore, these two domains appear to be distinct not only with respect to their nucleotide sequences but also with respect to their functional behavior.

The ZII domain is unnecessary in the presence of ZEBRA. Mutational analysis suggests that the ZI domains play a role in achieving full ZEBRA transactivation. When either the ZIA and ZIB elements or the ZIC element was mutated (MIA-MIB and MIC, respectively), transactivation by ZEBRA was reduced (Fig. 1 and 2). Moreover, the reduction in Zp activity observed in the presence of both ZEBRA and TPA was even more pronounced. The latter result is consistent with the notion that these domains also play an important role in the TPA inducibility of Zp. The results obtained with MIA/B were, however, not corroborated by the effect of deletion of the region from −221 to −159 bp, which contains ZIA and ZIB. Indeed, deletion of these sequences resulted in an increase in transactivation (Fig. 1 and 2). Thus, the sequences between −221 and −159 bp may also contain *cis*-acting elements which diminish ZEBRA transactivation as well as sequences which positively affect transactivation. The effect of deletion of the ZIC domain was not clear, since the −129Zp deletion, as discussed above, deletes not only the ZIC domain but also part of the ZIIIA ZEBRA-binding domain.

Unlike mutation of the ZI domains, mutation of ZII did not have a deleterious effect on ZEBRA transactivation (Table 1; Fig. 1). Mutation of ZII (MII) had little effect on ZEBRA transactivation of Zp (14- to 16-fold), and the MII mutant exhibited essentially the same superinducibility with TPA and ZEBRA (−221Zp, 140-fold; MII, 130-fold). Moreover, the activity of MII in the presence of ZEBRA and TPA

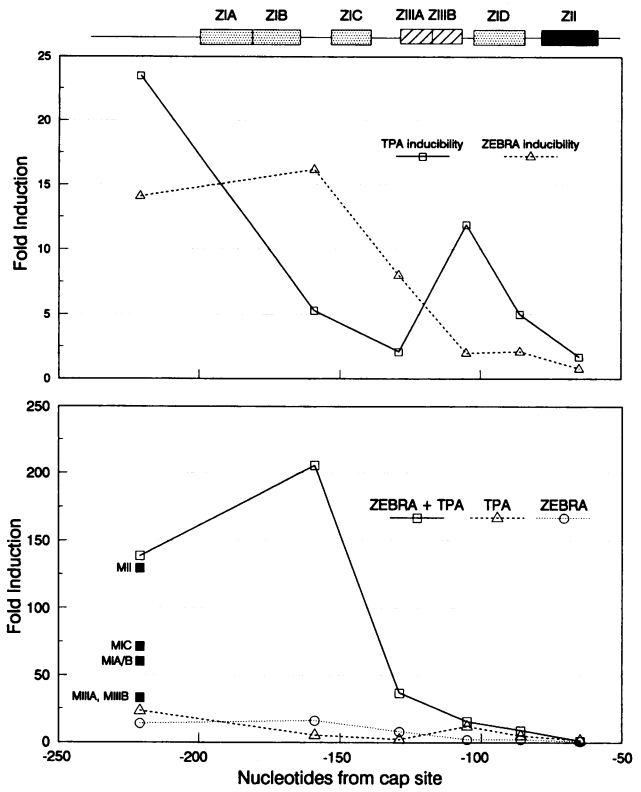

FIG. 2. TPA inducibility and ZEBRA transactivation of ZpCAT deletion constructs. Solid squares denote induction of the indicated site-directed mutants with TPA-BZLF1 (lower panel).

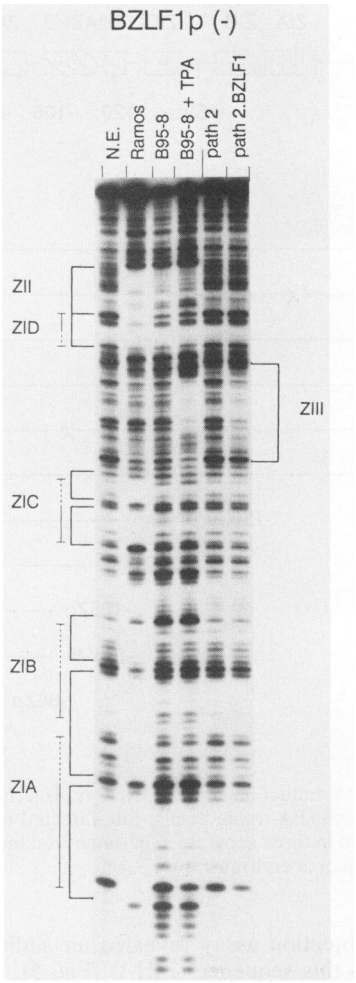

FIG. 3. BZLF1 protein (ZEBRA) binds directly to its own promoter. A partially purified bacterial extract containing a TrpE-ZEBRA fusion protein was generated as described in Materials and Methods. One-microliter volumes of partially purified TrpE-BZLF1 extracts (pATH2-BZLF1) or control extracts (pATH2) were used in binding reactions. Footprint analysis of nuclear extracts prepared from Ramos (an EBV-negative BL-cell line) and B95-8 (an EBV-infected marmoset cell line) cells were performed by using 80 μg of nuclear protein. ZIA, ZIB, ZIC, ZID, and ZII refer to regions protected by cellular factors (6), and ZIII refers to the ZEBRA-binding domain. The promoter fragment used for footprinting was labeled on the antisense strand. N.E., No extract.

approaches the activity of the unmutated promoter (−221Zp) in the presence of ZEBRA and TPA (Table 1). Therefore, the ZII domain does not appear to be involved in the synergism between ZEBRA transactivation and TPA induction. As discussed in the accompanying report (6), in the absence of ZEBRA, mutation of ZII had the most marked effect on TPA-inducible activity. Indeed, mutation of the ZII domain decreased TPA-inducible activity to between 8 and 16% of that of the unmutated promoter activity. In the presence of ZEBRA, the TPA-inducible activity of Zp was largely unaffected by mutation of the ZII domain, suggesting that in the presence of ZEBRA the contribution of the ZII domain to the overall activity of Zp is nominal.

Although unlikely, it is possible that mutation of the ZII domain altered ZEBRA transactivatability. As discussed above, ZEBRA has been shown to bind to an AP-1 site (5), and although ZEBRA does not appear to bind to the ZII domain (a putative AP-1 site), the mutation introduced into the ZII domain may have transformed it into a recognition sequence to which ZEBRA can bind and transactivate Zp. This might compensate for the loss of normal ZII function. To test this possibility, a mutant promoter was constructed in which the ZEBRA transactivation domains were deleted and the ZII domain was mutated as in the MII mutant (−105MII). A comparison of −105Zp with −105MII (Fig. 1) showed that mutation of ZII results in the expected loss of TPA inducibility while transactivation was unaffected. We therefore conclude that transactivation by ZEBRA largely compensates for mutation of the ZII domain by interaction through the ZIII domains.

DISCUSSION

In this study, we mapped two ZEBRA-binding-transactivation domains within Zp (ZIIIA and ZIIIB). The DNase I footprint analysis shown here revealed that ZEBRA protects two domains within Zp. It has been reported (5) that ZEBRA binds to a site within the EBV BSLF2/BMLF1 promoter region which contains a consensus AP-1 recognition sequence. Binding of ZEBRA to AP-1-like sites has also been observed in the promoter regions of the EBV BHLF1 and BHRF1 genes (D. S. Hayward, personal communication). We have demonstrated here that the BZLF1 promoter contains an AP-1-like ZEBRA-binding-transactivation domain. At the core of this sequence is a sequence with 6-of-7-bp homology to a consensus AP-1 site (−129 TGAG CCA −123 bp versus TGAGTCA). Moreover, mutation of this domain has a significant effect on TPA inducibility.

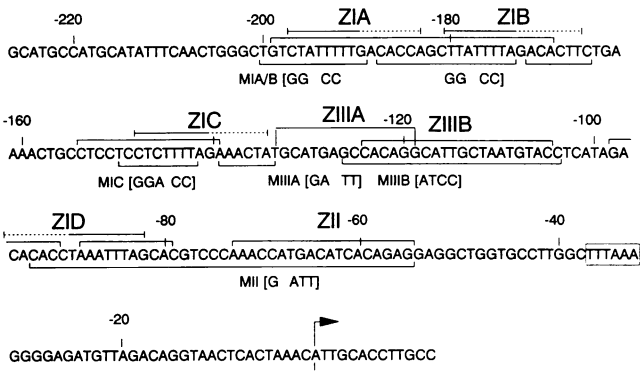

FIG. 4. Sequence of Zp and locations of footprinted domains. Overbrackets indicate sense (+) strand footprints, and underbrackets indicate antisense (−) strand footprints. ZI repeat sequences are indicated by overbars (solid regions indicate A+T-rich domains). Specific base changes introduced in the site-directed mutations are shown below the corresponding sequences. ZIA, ZIB, ZIC, ZID, and ZII refer to regions protected by cellular factors (6).

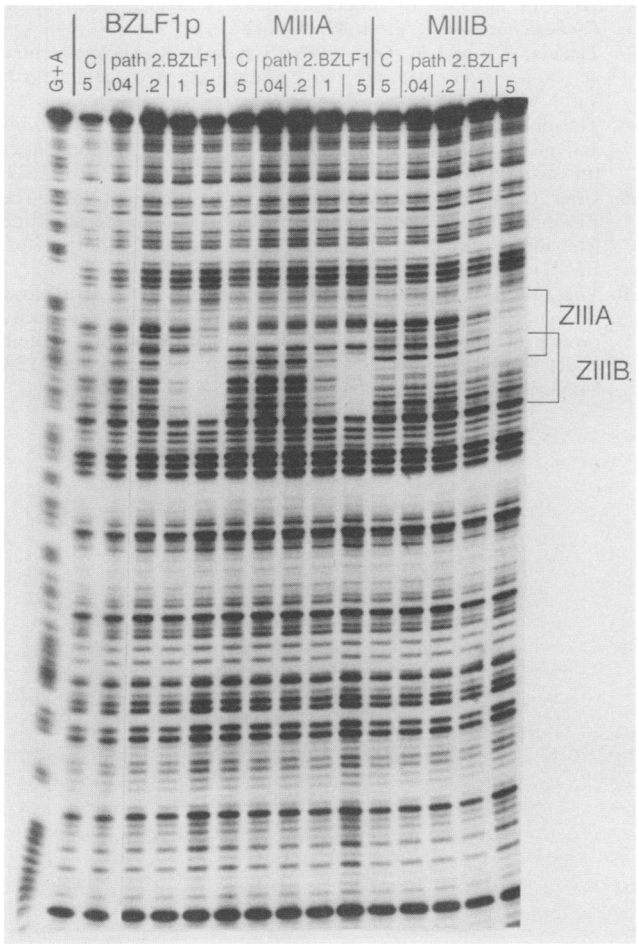

FIG. 5. Mutation of ZIIIA or ZIIIB abrogates ZEBRA binding. Zp, MIIIA, or MIIIB was labeled on the sense strand, and increasing amounts of fusion protein were added to binding reactions (0.04, 0.2, 1, or 5 μl). For the control lane (C), 5 μl of TrpE extract was used. The specific nucleotide changes introduced into the ZIIIA or ZIIIB domain are shown in Fig. 4.

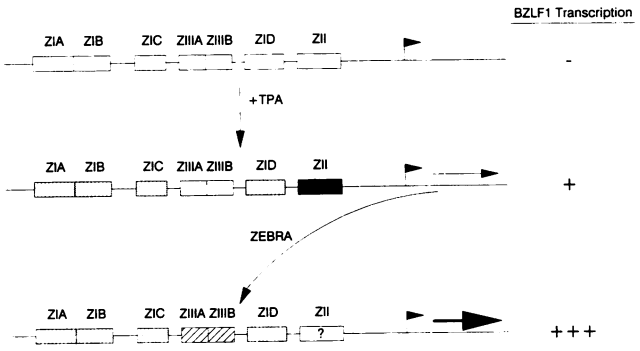

FIG. 6. Proposed model for induction of BZLF1 transcription during onset of the viral lytic cycle. Initial activation of Zp by TPA occurs through the ZI and ZII domains, leading to low-level BZLF1 transcription. Synthesis of a critical level of ZEBRA allows transactivation by ZEBRA at the ZIII domain which, in conjunction with TPA activation at the ZI domains, leads to high-level BZLF1 transcription.

Within the ZIIIB domain, there are no recognizable AP-1-binding sequences. The distinction between the elements involved in TPA induction and the ZIIIB domain was further demonstrated by site-directed mutagenesis, which affected ZEBRA binding and transactivation but did not decrease TPA inducibility. These data suggest that ZEBRA can bind to sequences other than AP-1 sites, indicating that there is some degree of degeneracy in ZEBRA-binding specificity. We do not know the reason for the apparent distinction between the ZIIIB domain and ZIIIA or other previously identified ZEBRA transactivation sites. Interestingly, ZIIIB appears to be a high-affinity ZEBRA-binding site (Fig. 5; unpublished data).

Urier et al. (19) reported previously that ZEBRA transactivation can occur only if the *cis*-acting transactivation domain is near the CAP site. Therefore, the presence of two *cis*-acting elements, one of which appears to be a high-affinity site, may be a means by which initial transactivation of BZLF1 transcription can occur at lower ZEBRA concentrations. This might ensure the appropriate temporal sequence of early events in the initiation of the viral lytic cycle. Interestingly, deletion of sequences between −129 and −105 results in a significant increase in activity in the absence of the ZEBRA transactivator (6). This deletion excises the entire ZIIIB ZEBRA recognition sequence in addition to a few bases of the flanking sequence. These data suggest the presence of a negative element near the ZIIIB domain and therefore the possibility that ZEBRA may function in part to displace a putative repressor protein. This, however, requires further investigation.

On the basis of the results presented here, we propose a two-step model for Zp activation (Fig. 6). Initial induction of Zp by TPA involves activation of the TPA response elements (ZI and ZII domains), which leads to low-level Zp activity, and this results in production of correspondingly low levels of ZEBRA. If the initial activation signal is sufficient to allow synthesis of adequate levels of ZEBRA, an autoactivation loop is established which leads to high-level ZEBRA expression and thereby activation of other early viral promoters. Although the data presented here suggest that factors binding to the ZI domains are important for both TPA inducibility and ZEBRA transactivation, mutation of the ZII domain has little effect on Zp activity in the presence of ZEBRA. Therefore, ZEBRA binding to the ZIIIA and ZIIIB domains appears to functionally supercede

the requirement for a functional ZII domain. This would then obviate the requirement for a sustained TPA signal at the ZII AP-1-like domain.

ACKNOWLEDGMENTS

We thank Paul Farrell for the generous gift of BZLF1 cDNA, and we thank Mary-Ann Sells, Mike Krangel, and Carolyn Doyle for critical reading of the manuscript.

This work was supported by Public Health Service grant 5 R01 CA43143 from the National Institutes of Health, a Leukemia Society special fellowship to S.H.S., and a National Institutes of Health/ National Research Service award grant (1 F32 CA 08482-01) to E.F.

LITERATURE CITED

1. **Biggin, M., M. Bodescot, M. Perricaudet, and P. Farrell.** 1987. Epstein-Barr virus gene expression in P3HR1-superinfected Raji cell. J. Virol. **61**:3120–3132.
2. **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.
3. **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.
4. **Dieckmann, C. L., and A. Tzagoloff.** 1985. Assembly of the mitochondrial membrane system. J. Biol. Chem. **260**:1513–1520.
5. **Farrell, P. J., D. T. Rowe, C. M. Rooney, and T. Kouzarides.** 1989. Epstein-Barr virus BZLF1 trans-activator specifically binds to a consensus AP-1 site and is related to c-fos. EMBO J. **8**:127–132.
6. **Flemington, E., and S. H. Speck.** 1990. Identification of phorbol ester response elements in the promoter of Epstein-Barr virus putative lytic switch gene BZLF1. J. Virol. **64**:1217–1226.
7. **Foss, K., and W. H. McClain.** 1987. Rapid site-specific mutagenesis in plasmids. Gene **59**:285–290.
8. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferse in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
9. **Hardwick, J. M., P. M. Lieberman, and S. D. Hayward.** 1988. A new Epstein-Barr virus transactivator, R, induces expression of a cytoplasmic early antigen. J. Virol. **62**:2274–2284.
10. **Kenney, S., J. Kamine, E. Holley-Guthrie, J.-C. Lin, E.-C. Mar, and J. Pagano.** 1989. The Epstein-Barr virus (EBV) BZLF1 immediate-early gene product differentially affects latent versus productive EBV promoters. J. Virol. **63**:1729–1736.
11. **Lee, W., P. Mitchell, and R. Tjian.** 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell **49**:741–752.
12. **Lieberman, P. M., J. M. Hardwick, and S. D. Hayward.** 1989. Responsiveness of the Epstein-Barr virus *Not*I repeat promoter to the Z transactivator is mediated in a cell-type-specific manner by two independent signal regions. J. Virol. **63**:3040–3050.
13. **Lieberman, P. M., P. O'Hare, G. S. Hayward, and S. D. Hayward.** 1986. Promiscuous *trans* activation of gene expression by an Epstein-Barr virus-encoded early nuclear protein. J. Virol. **60**:140–148.
14. **Ptashne, M.** 1986. A genetic switch: gene control and phage lambda. Cell Press, Cambridge.
15. **Speck, S. H., and J. L. Strominger.** 1985. Analysis of the transcript encoding the latent Epstein-Barr virus nuclear antigen I: a potentially polycistronic message generated by long-range splicing of several exons. Proc. Natl. Acad. Sci. USA **82**: 8305–8309.
16. **Spindler, K. R., D. S. E. Rosser, and A. J. Berk.** 1984. Analysis of adenovirus transforming proteins from early regions 1A and 1B with antisera to inducible fusion antigens produced in *Escherichia coli*. J. Virol. **49**:132–141.
17. **Takada, K., and Y. Ono.** 1989. Synchronous and sequential activation of latently infected Epstein-Barr virus genomes. J. Virol. **63**:445–449.
18. **Takada, K., N. Shimizu, S. Sakuma, and Y. Ono.** 1986. *trans* activation of the latent Epstein-Barr virus (EBV) genome after transfection of the EBV DNA fragment. J. Virol. **57**:1016–1022.
19. **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.
20. **Vogt, P. K., T. J. Bos, and R. F. Doolittle.** 1987. Homology between the DNA-binding domain of the GCN4 regulatory protein of yeast and the carboxyl-terminal region of a protein coded for by the oncogene jun. Proc. Natl. Acad. Sci. USA **84**:3316–3319.