Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10115–10119, November 1993
Medical Sciences

Serine-173 of the Epstein–Barr virus ZEBRA protein is required for DNA binding and is a target for casein kinase II phosphorylation

JOHN L. KOLMAN*, NAOMI TAYLOR*, DANIEL R. MARSHAK†, AND GEORGE MILLER*‡§

Departments of *Molecular Biophysics and Biochemistry, ‡Pediatrics, and Epidemiology and Public Health, Yale University, New Haven, CT 06510; and †Cold Spring Harbor Laboratory, W. M. Keck Structural Biology Laboratory, Cold Spring Harbor, NY 11724

Communicated by Dorothy M. Horstmann, July 13, 1993 (received for review April 20, 1993)

ABSTRACT **An Epstein–Barr virus-encoded protein, ZEBRA, mediates the switch from latency to the viral lytic life cycle. ZEBRA's domain structure and DNA binding specificity resemble that of cellular transcriptional activators such as c-Fos/c-Jun. We show that ZEBRA, like c-Jun, is phosphorylated by casein kinase II (CKII). The principal site of phosphorylation is serine-173 (S173), five amino acids upstream of the basic DNA recognition domain. CKII phosphorylation abrogated ZEBRA's capacity to bind its target DNA sequences. S173 is a functional component of ZEBRA's DNA binding domain, since mutation of S173 to alanine (S173A) reduced DNA binding *in vitro* to 10% of wild-type levels. Transcriptional activation of a native viral promoter *in vivo* by mutant S173A was also reduced markedly. Reversible phosphorylation of S173 is likely to be an important means of regulating ZEBRA's activity *in vivo*.**

Epstein–Barr virus (EBV) can be switched from latency into the viral lytic cycle by expression of a virally encoded protein, ZEBRA (1, 2). ZEBRA binds DNA and activates transcription from promoters of early viral lytic cycle genes (3, 4). Several other proteins that regulate EBV gene expression are encoded among these early lytic cycle genes (5, 6). The C terminus of ZEBRA encodes a DNA binding domain composed of basic amino acids (basic domain) followed by a protein homodimerization domain. This organization is similar to the basic zipper (bZIP) motif found in many transcriptional activators (7–9) (Fig. 1*A*). ZEBRA is homologous to bZIP proteins of the c-Fos/c-Jun family in the basic DNA recognition domain. Furthermore, ZEBRA and c-Fos/c-Jun share the ability to bind a heptamer AP-1 consensus site (10). ZEBRA also binds other heptamer DNA sequences (ZEBRA response elements; ZREs) not bound by c-Fos/c-Jun (4, 15–17).

Cellular kinases such as casein kinase II (CKII) regulate the activity of many nuclear DNA binding proteins (18–21). CKII, a ubiquitous protein kinase present in both the cytoplasm and nucleus of eukaryotic cells, catalyzes the phosphorylation of serine or threonine residues N-terminal to clusters of acidic amino acids (22, 23). Although CKII activity can be detected in resting cells, it is elevated in cells treated with mitogens (24–26). Thus, CKII may play a role in signal transduction and regulation of cell division.

CKII can exert positive or negative effects on DNA binding by cellular nuclear proteins that participate in the transcriptional response to mitogens or DNA damage. For example, CKII phosphorylation inhibits DNA binding by the protooncoprotein c-Myb but enhances the DNA binding activity of serum response factor (SRF) (27, 28). The protooncoprotein c-Myc and its *in vivo* heterodimer mate, Max, are both phosphorylated by CKII (12, 29) (Fig. 1*B*). Phosphorylated Max homodimers fail to bind DNA *in vitro*; however, phosphorylated Myc/Max heterodimers do bind DNA (13). c-Jun can be phosphorylated by CKII, although the effect of this modification on DNA binding is not clear (11, 30). In addition to DNA binding, other properties of transcription factors such as nuclear localization, transactivation, or transrepression are influenced by phosphorylation by CKII (21).

We determined whether the activity of ZEBRA, a viral immediate early transcriptional activator, is influenced by phosphorylation. We found that phosphorylation of ZEBRA by CKII inhibited DNA binding. ZEBRA contains two potential CKII acceptor sites, serine-167 and -173 (S167 and S173), in an acidic domain N-terminal to the basic DNA recognition domain (10) (Fig. 1*B*). By examining proteins mutated at these potential acceptor sites for their ability to be phosphorylated by CKII *in vitro*, we mapped the principal phosphorylation site to S173. We found that ZEBRA mutants in which S173 was mutated to alanine (S173A) were markedly inhibited in their ability to bind DNA and to activate transcription of a ZEBRA-inducible viral early gene.

METHODS AND MATERIALS

Plasmids. Plasmid constructs expressing full-length or deleted ZEBRA as TrpE fusion proteins in *Escherichia coli* have been described (17, 31). ZEBRA mutants S167A and S173A were constructed by PCR-based mutagenesis (32). To make S167A, the oligonucleotide 5′-GAATCGCATTCCTC-CAGCGCTTCTGG-3′ (single point mutation underlined) and an oligonucleotide in vector sequences were used to prime the wild-type ZEBRA cDNA. The reaction product was cut at both ends with *Bsm* I and ligated to a *Bsm* I cut TrpE–ZEBRA expression construct. Similarly, the mutant S173A was constructed by using 5′-TATTTCTAGTTCAG-CATCGC-3′, cutting the PCR product and the TrpE–ZEBRA expression construct with *Nhe* I and *Rma* I and ligating. ZEBRA S167A/S173A was constructed by swapping the *Bsm* I fragment from ZEBRA S167A into ZEBRA S173A. ZEBRA M187–189 has basic amino acids RKC in the DNA recognition domain replaced with EES (17). ZEBRA coding sequences were transferred by using convenient polylinker restriction sites to the pSP64 or pSP65 expression vectors for *in vitro* transcription and to the cytomegalovirus virus eukaryotic expression vector pHD1013 for use in B-cell transfections (33).

Protein Expression. TrpE fusion proteins were expressed in *E. coli* AG1 (31). ZEBRA protein was also expressed in *E.*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EBV, Epstein–Barr virus; ZRE, ZEBRA response element; CKII, casein kinase II; SRF, serum response factor; EMSA, electrophoretic mobility-shift assay; PP2A, protein phosphatase 2A; CAT, chloramphenicol acetyltransferase.

§To whom reprint requests should be sent.

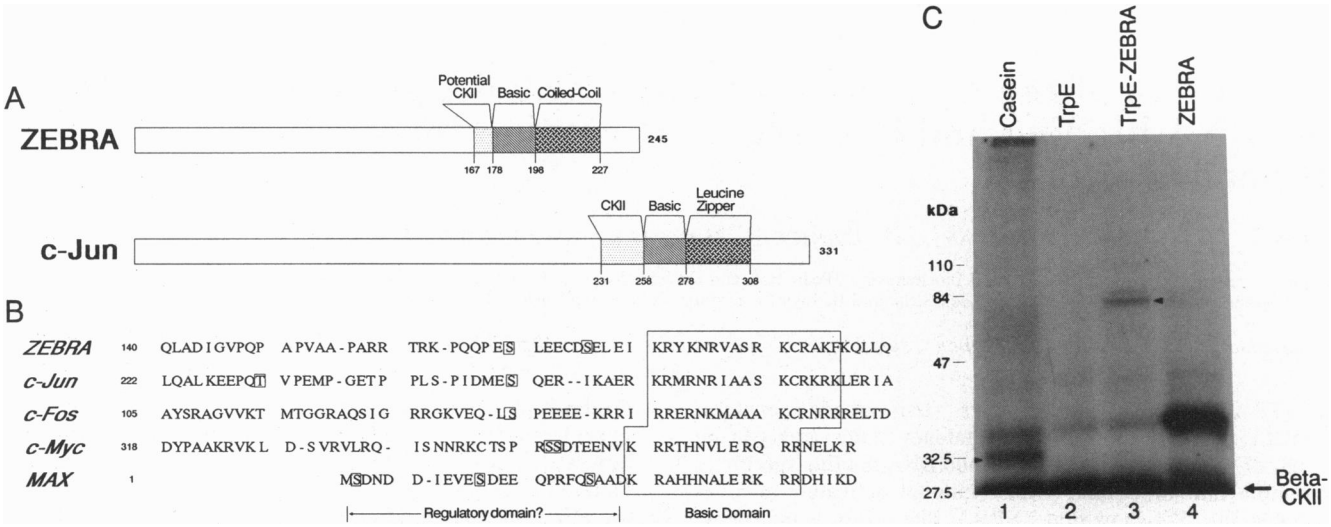

FIG. 1. Phosphorylation of ZEBRA. (*A*) Schematic representation of three related domains of ZEBRA and c-Jun: an acidic domain with known or potential sites for phosphorylation by CKII; the basic domain; and the dimerization domains, coiled-coil and leucine zipper. (*B*) Amino acid sequences N-terminal to the basic domains of ZEBRA and several human immediate early genes. Potential CKII acceptor sites are boxed in ZEBRA (10). Known CKII phosphorylation sites are boxed in c-Jun (11), c-Myc (12), and Max (13). c-Fos may be phosphorylated by $p34^{cdc2}$ kinase at S133 (14). (*C*) Phosphorylation of ZEBRA by casein kinase II as described in *Results*. Casein and TrpE proteins were used as controls. Phosphorylated species are indicated by arrowheads. Autophosphorylation of the β subunit of CKII in each lane is indicated by Beta-CKII.

coli from the tac expression vector pRW76, purified, and judged to be 90% pure by Coomassie blue staining of SDS/polyacrylamide gel (34). *In vitro* transcribed/translated ZEBRA derivatives radiolabeled with [^{35}S]cysteine and [^{35}S]methionine (Trans-35; ICN) were made in wheat germ extracts (Promega). Protein/protein cross-links were performed with 3 μl of labeled translation product diluted in 0.004% glutaraldehyde for 30 min.

Protein Phosphorylations. Cell extracts containing 300 ng of fusion protein or purified ZEBRA protein (100 ng) were phosphorylated in CKII buffer (75 mM KCl/5 mM $MgCl_2$/10 mM Hepes, pH 7.9/1 mM EGTA/0.5 mM dithiothreitol) in the presence of 10 μCi of [γ-^{32}P]ATP (1 Ci = 37 GBq) and CKII purified from bovine liver [88 pmol of casein phosphorylated in 10 min in the presence of 0.1 mM ATP (24)]. Samples were incubated at 37°C for 15 min.

Electrophoretic Mobility-Shift Assays (EMSAs). EMSAs were performed in 30 μl of CKII buffer using 100 ng of purified ZEBRA protein or cell extract containing 300 ng of ZEBRA fusion protein, with or without 0.33 μM ATP (Pharmacia) and CKII. Some extracts were then exposed to 5–10 units of protein phosphatase 2A (PP2A; in 50 mM Tris·HCl, pH 7.5/0.1% bovine serum albumin) for an additional 15 min. Samples were analyzed by EMSA with 0.4 pM double-stranded AP-1, ZIIIB, or double oligonucleotides (17) that had been radiolabeled by standard methods (32). Conversion to bound oligonucleotide was quantitated by scintillation counting.

B-Cell Gene Transfer and Chloramphenicol Acetyltransferase (CAT) Assays. Transcriptional activation was measured in the EBV-negative Burkitt lymphoma cell line BJAB (34). Ten micrograms of reporter CAT construct, either EABSCAT [BMRF1 promoter −333 to +1 (35)] or Z3CAT (34), was transfected with various amounts of activator equalized to 3.6 μg with pHD1013. Conversion to acetylated chloramphenicol was quantitated by scintillation counting. Transfections were repeated at least three times. Activator constructs were also transfected into the EBV-positive cell line Raji and assayed for induction of the endogenous viral BMRF1 transcript (36). The abundance of the transcripts was measured by densitometry.

RESULTS

CKII Phosphorylates ZEBRA *in Vitro*. *E. coli* protein preparations containing TrpE–ZEBRA fusion protein or purified ZEBRA expressed as a nonfusion protein were incubated with purified CKII and [γ-^{32}P]ATP. The phosphorylation products were analyzed by SDS/PAGE followed by autoradiography (24). Fig. 1*C* shows that both TrpE–ZEBRA and purified ZEBRA proteins were phosphorylated, as well as casein, which served as a positive control. In addition, the 28-kDa β subunit of CKII was autophosphorylated in all reactions. TrpE protein was not phosphorylated by CKII. Radiolabeled phosphate was not incorporated into ZEBRA protein when [γ-^{32}P]ATP was added without CKII (Fig. 2*A*, lane 1).

Mapping the Residues of ZEBRA Phosphorylated by CKII *in Vitro*. A panel of ZEBRA deletion mutants expressed as TrpE fusion proteins in *E. coli* were assayed for the ability to serve as substrates for CKII phosphorylation (17). The mutant proteins Z(141–245), Z(1–110 + 160–245), and Z(1–198) were all phosphorylated (data not shown). This analysis localized the region recognized by CKII to amino acids 160–198. There are three serines in this region: S167, S173, and S186 (Fig. 1*B*). However, S186 is in the center of the basic DNA binding domain and is not associated with the previously described CKII consensus sequences (22).

The principal target for CKII phosphorylation was determined by examining ZEBRA proteins mutated at S167 and S173. The serines at positions 167 and 173 were changed to alanines singly (ZEBRA S167A, ZEBRA S173A) and together (ZEBRA S167A/S173A) (Fig. 2). Fig. 2*A* shows that full-length ZEBRA and the ZEBRA S167A mutant were strongly phosphorylated, whereas ZEBRA S173A and the double mutant were weakly labeled. Therefore, the primary site of ZEBRA phosphorylated by CKII is S173. The low level of phosphorylation of ZEBRA S173A suggests that S167 may be a secondary target for CKII.

CKII Phosphorylation of ZEBRA *in Vitro* Is Inhibited by Mutation of the Basic Domain but Not by Mutation of the Dimerization Domain. S173 is located immediately upstream of the basic and homodimerization domains required for DNA binding. To determine whether CKII phosphorylation was dependent on structures in either of these two domains,

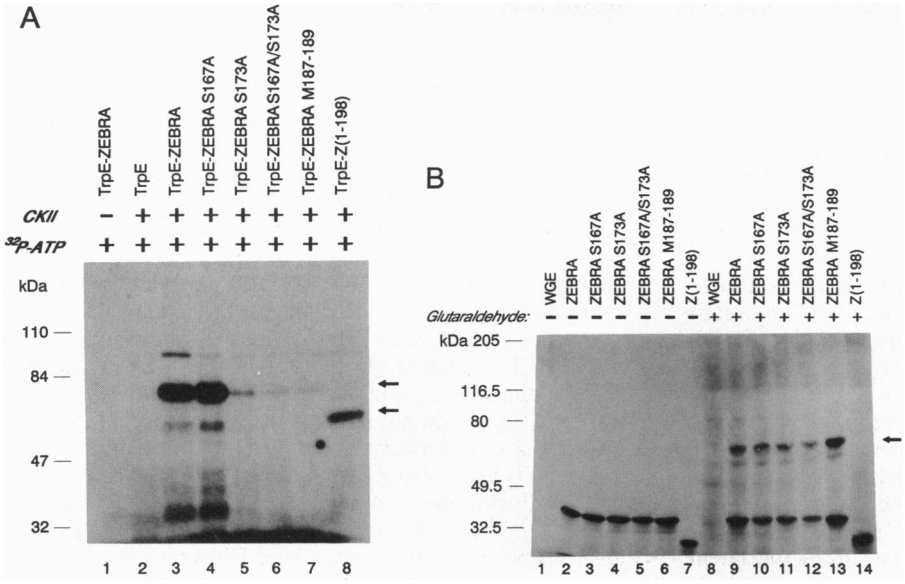

FIG. 2. CKII phosphorylation of wild-type and mutant ZEBRA proteins. (*A*) Extracts of *E. coli* normalized for TrpE fusion protein content by SDS/PAGE and Coomassie blue staining (data not shown) were assayed for phosphorylation by CKII. Phosphorylated ZEBRA proteins are indicated with arrows at 70 kDa in lanes 3–7 and at 65 kDa in lane 8. No specifically phosphorylated species were detected in the absence of CKII (lane 1) or in the absence of ZEBRA sequences (lane 2). (*B*) Wild-type and mutant ZEBRA proteins were translated and radiolabeled *in vitro* in a wheat germ extract (WGE); the products are ≈36 kDa (lanes 2–6) or ≈31 kDa (lane 7); an aliquot was crosslinked with glutaraldehyde (lanes 8–14). Crosslinked products are indicated by an arrow.

additional ZEBRA mutants were tested in CKII assays. There was marked reduction in phosphorylation of a ZEBRA mutant in which two basic residues have been changed to acidic ones (M187–189; Fig. 2*A*, lane 7). A ZEBRA mutant lacking the dimerization domain [Z(1–198)] was strongly phosphorylated (Fig. 2*A*, lane 8). Cross-linking of *in vitro* translated proteins indicated that all the ZEBRA mutants dimerized in solution except Z(1–198) (Fig. 2*B*). Thus, sequences in the basic domain but not in the dimerization region are required for CKII phosphorylation.

CKII Phosphorylation Inhibits Specific DNA Binding by ZEBRA. Purified ZEBRA protein or *E. coli* extract containing TrpE–ZEBRA fusion protein were preincubated with CKII and ATP before binding to a ^{32}P-labeled oligonucleotide containing a ZRE and analyzed by EMSA. Equivalent results were obtained with both expressed forms of ZEBRA (Fig. 3). Untreated ZEBRA bound an oligonucleotide containing an AP-1 site as did ZEBRA preincubated with CKII or ATP alone (Fig. 3, lanes 1–3). However, binding to the AP-1 oligonucleotide was almost completely inhibited when the ZEBRA proteins were preincubated with both ATP and CKII (lanes 4). Similar results were obtained with an oligonucleotide containing a ZIIIB site (data not shown).

The inhibition of ZEBRA's ability to bind DNA after CKII phosphorylation was reversible. Both preparations of ZEBRA that had been phosphorylated by CKII were treated with PP2A and then analyzed for DNA binding activity (37). PP2A treatment partially restored DNA binding activity (lanes 5).

ZEBRA Mutation S173A Markedly Reduces DNA Binding Affinity *in Vitro*. *E. coli* extracts containing equal amounts of wild-type or serine point-mutant TrpE–ZEBRA fusion proteins were tested for DNA binding activity. In Fig. 4, two oligonucleotides were used as probes in EMSAs: ZIIIB, which contains a ZRE heptamer present in the viral ZEBRA promoter; double, which consists of two ZREs (ZIIIA and ZIIIB) oriented as they appear in the same promoter (4). Wild-type ZEBRA formed a single shifted band with the ZIIIB probe and two shifted bands with double (lanes 2 and

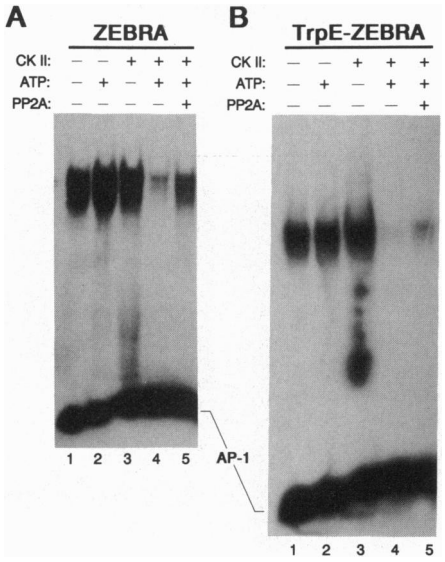

FIG. 3. CKII-mediated phosphorylation inhibits DNA binding by ZEBRA. Purified ZEBRA protein (*A*) and TrpE–ZEBRA fusion protein extract (*B*) were tested for their ability to bind a radiolabeled oligonucleotide containing an AP-1 heptamer sequence by EMSA. ZEBRA proteins were untreated (lane 1), incubated with ATP alone (lane 2), CKII alone (lane 3), or ATP and CKII (lane 4). Treatment of CKII phosphorylated proteins with PP2A is shown in lane 5.

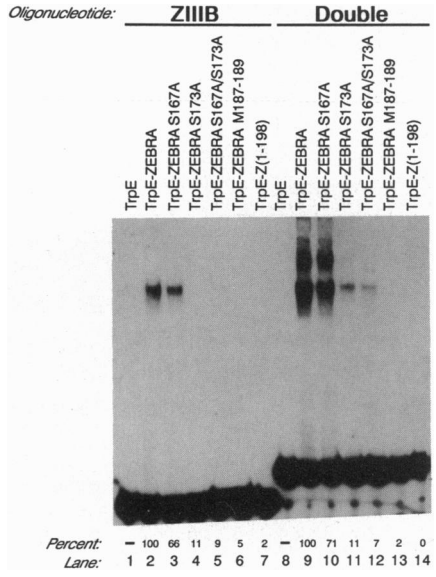

FIG. 4. DNA binding activity of mutant ZEBRA proteins. Binding of the ZIIIB and double oligonucleotides was examined by EMSA. Binding activity of each mutant was expressed as percentage of labeled oligonucleotide bound relative to wild type.

9), presumably representing single and double occupancy of the oligonucleotide by ZEBRA dimers. Binding by ZEBRA S167A was reduced to 70% of wild-type activity (lanes 3 and 10). Mutants S173A and S167A/S173A were markedly reduced in their capacity to bind both probes, to ≈10% of wild-type levels (lanes 4, 5, 11, and 12). Only trace binding activity was measured with the mutants, which alter the basic domain (M187–189) or delete the dimerization domain [Z(1–198)]. The five ZEBRA mutants showed the same relative reduced affinity for all ZREs tested (data not shown).

Serine Point Mutants of ZEBRA Have Reduced Transactivation Activity *in Vivo*. The effect of S173 and S167 mutations on the capacity of ZEBRA to activate transcription *in vivo* was tested in two assays. In the first assay, wild-type or mutant ZEBRA expression constructs were examined for their ability to activate a cotransfected reporter plasmid in EBV-negative human B cells (BJAB). The reporter contained the promoter of a ZEBRA-responsive early lytic cycle gene, BMRF1, linked to CAT (35). In the second assay, we studied the ability of wild-type and mutant ZEBRA proteins to activate transcription of the BMRF1 gene from the latent EBV genome in Raji cells.

Using reporter gene assays, mutant S167A was ≈33% as active as wild-type ZEBRA, whereas S173A was ≈11% as active as wild type (averages of data from Fig. 5*A*; data not shown). Increasing the amount of activator plasmid in the cotransfection assay compensated for the mutant phenotypes. Mutants S167A and S173A were, respectively, 22% and 4% as active as wild type in stimulating transcription of BMRF1 from the latent genome in Raji cells (Fig. 5*B*). In both assays the double-mutant S167A/S173A, although severely impaired relative to wild type, was more active than the single S173A mutant. All three mutants and wild-type ZEBRA partitioned to the nucleus to the same extent (data not shown).

DISCUSSION

Phosphorylation of ZEBRA by CKII. This work reports the functional consequence of posttranslational modification of ZEBRA. We define several requirements for phosphorylation of ZEBRA by CKII and show that phosphorylation of ZEBRA *in vitro* inhibits DNA binding. ZEBRA contains two potential CKII acceptor sites at S167 and S173. Although these sites are similar, SLEE and SELE, only the latter was strongly phosphorylated *in vitro*. The preference for S173 may result from features surrounding the acceptor site; S173 has acidic residues both N-terminal and C-terminal of the serine and is proximal to the α-helix secondary structure in the basic domain. Sequences within the basic domain of ZEBRA are important for CKII phosphorylation since mutating residues 187–189, located 15–17 amino acids downstream of the acceptor site, abrogated phosphorylation *in vitro*. CKII-mediated phosphorylation of ZEBRA does not require dimer formation.

These studies do not address the phosphorylation state of ZEBRA *in vivo*; however, since CKII is a ubiquitous protein kinase, it is likely that ZEBRA serves as a substrate for CKII *in vivo*. Furthermore, other cellular kinases may react with ZEBRA *in vivo* (38). What are the possible functions of a ZEBRA protein that does not bind DNA as a result of CKII phosphorylation? Mutant forms of ZEBRA that do not bind DNA *in vitro* have been found to activate transcription of the BZLF1 promoter (E. K. Flemington and S. H. Speck, personal communication; J.L.K., N.T., and G.M., unpublished data); therefore, *in vivo* phosphorylated ZEBRA that does not bind DNA might continue to stimulate its own transcription. The ZEBRA protein has a long half-life *in vivo* (39). The half-life of the protein might be altered by phosphorylation. Furthermore, a modified form of ZEBRA that does not bind DNA may be recruited into a late gene transcription complex, perhaps by association with late viral proteins (40).

Role of S173 and S167 in DNA Binding and Transcriptional Activation. Our studies provide the observation that S173 of ZEBRA, the principal target for CKII phosphorylation, is an intrinsic component of ZEBRA's DNA binding domain. Mutation of S173 to alanine resulted in a 90% diminution in DNA binding activity relative to wild type. Thus, a serine hydroxyl moiety at position 173 may be necessary to establish a conformation of ZEBRA permissive for DNA binding.

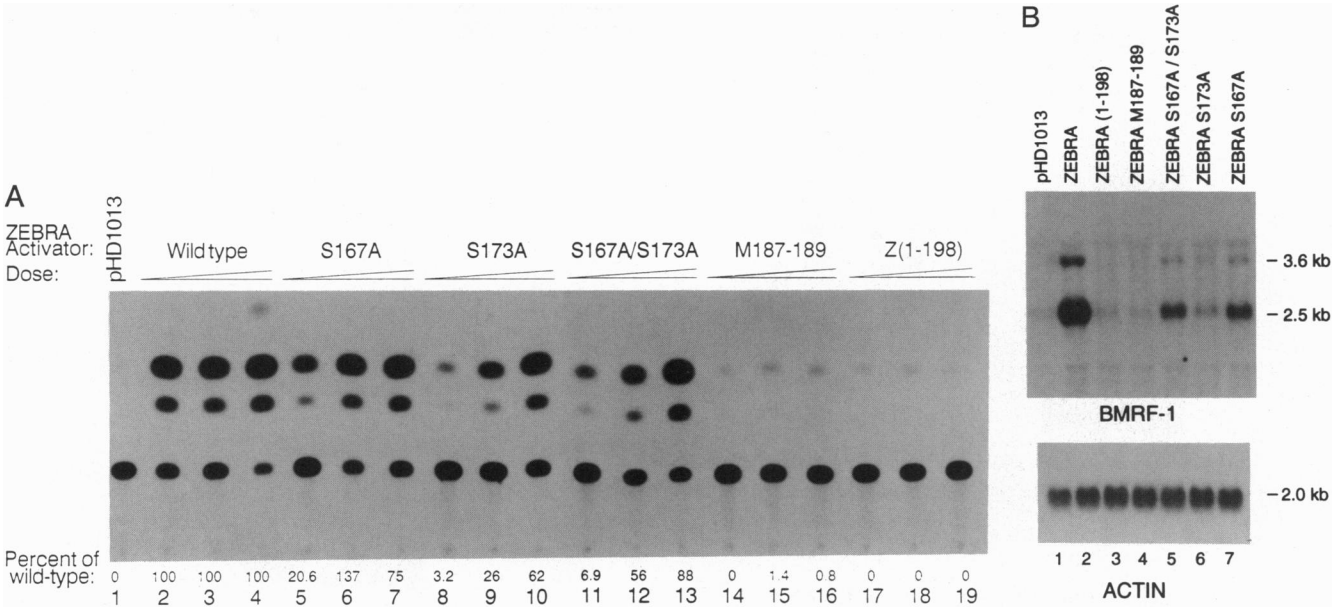

FIG. 5. Transcriptional activation by wild-type and mutant ZEBRA proteins in human B cells. (*A*) Activation of a CAT reporter plasmid containing the promoter of the EBV BMRF1 gene in BJAB cells. Three input amounts of activator plasmid DNA (0.4, 1.2, and 3.6 μg) were tested. Transcriptional activity is expressed as percentage acetylation relative to wild-type ZEBRA. (*B*) Activation of transcription of the BMRF1 gene from the latent EBV genome. Raji cells were transfected with 2.5 μg of plasmids expressing wild-type or mutant ZEBRA proteins. BMRF1-specific mRNAs expressed 24 hr after transfection were detected by Northern blotting.

Phosphorylation of S173 may disrupt DNA binding and, therefore, transactivation by shifting ZEBRA into a conformation that restricts DNA binding. ZEBRA differs from Oct-1 and SRF, whose DNA binding activities are also regulated by CKII phosphorylation (41, 42). Serine-to-alanine substitutions in the phosphorylation sites of Oct-1 and SRF do not impair their capacity to bind DNA.

The decreased ability of ZEBRA mutant S173A to activate transcription of an EBV early gene *in vivo* correlated with its impaired capacity to bind DNA. ZEBRA S167A was also affected in its capacity to bind DNA and activate transcription although to a lesser extent than ZEBRA S173A. Compared to mutant S173A, the double-mutant S167A/S173A was more active *in vivo*, even though it was as deficient as S173A in DNA binding. These results suggest that mutations of S167 and S173 may affect some property of ZEBRA other than DNA binding. One explanation for these observations is that ZEBRA may contact other proteins in order to activate transcription and disrupt latency. Modification of either S167 or S173 may alter the three-dimensional structure of the ZEBRA protein in a region that contacts the ancillary factor. Alternatively, mutation or phosphorylation of S167 or S173 may influence the stability and half-life of the ZEBRA protein.

Analogy Between ZEBRA and Cellular Immediate Early Genes. Stimulation of quiescent cells, in the absence of *de novo* protein synthesis, is accompanied by transcription of cellular early response or immediate early genes. Among these genes are nuclear proteins, such as c-Myc, c-Fos, and c-Jun, which are believed to play a role in regulating subsequent events in the cell cycle (18). ZEBRA has several features in common with the cellular immediate early genes. Its transcription is activated by external stimuli in the absence of protein synthesis (43, 44) and may be regulated by SRF, a DNA binding protein that modulates c-Fos transcription (45). Also, the DNA binding specificity of ZEBRA overlaps that of c-Fos/c-Jun (4, 15–17).

Our study demonstrates that ZEBRA, like cellular immediate early genes, has functional CKII phosphorylation sites N-terminal to its DNA binding domain. Moreover, CKII phosphorylation of ZEBRA inhibits DNA binding. The general structure N-terminal kinase site/DNA binding domain (kbZIP) appears to be a conserved motif by which the activity of a DNA binding protein can be modulated by kinases and phosphatases in response to mitogenic and other external stimuli. The biologic role of ZEBRA as an immediate early gene in the EBV lytic life cycle may be analogous to immediate early genes that initiate the cell cycle. The virus may respond to external stimuli and changes in patterns of cell growth via changes in the phosphorylation state of ZEBRA.

We thank E. Flemington and S. Speck for the ZEBRA M187–189 mutant, M. Carey for purified ZEBRA protein, Clark Huang for the cytomegalovirus immediate early expression vector (pHD1013), and Jonathan Chernoff for the PP2A. Special thanks to Lyndle Gradoville and Elizabeth Grogan for help with Raji transfections and IFAs, respectively. This work was supported by American Cancer Society Grant MV-548L and National Institutes of Health Grants CA12055, CA52228, CA16038, AI22959, and CA13106.

1. Chevallier-Greco, A., Manet, E., Chavrier, P., Mosnier, C., Daillie, J. & Sergeant, A. (1986) *EMBO J.* **5**, 3243–3249.
2. Countryman, J. & Miller, G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4085–4089.
3. Lieberman, P. M., Hardwick, J. M. & Hayward, S. D. (1989) *J. Virol.* **63**, 3040–3050.
4. Flemington, E. & Speck, S. H. (1990) *J. Virol.* **64**, 1227–1232.
5. Kenney, S., Kamine, J., Holley-Guthrie, E., Mar, E. C., Lin, J. C., Markovitz, D. & Pagano, J. (1989) *J. Virol.* **63**, 3870–3877.
6. Hardwick, J. M., Lieberman, P. M. & Hayward, S. D. (1988) *J. Virol.* **62**, 2274–2284.
7. Lieberman, P. M. & Berk, A. J. (1990) *J. Virol.* **64**, 2560–2568.
8. Flemington, E. & Speck, S. H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9459–9463.
9. Vinson, C. R., Sigler, P. B. & McKnight, S. L. (1989) *Science* **246**, 911–916.
10. Farrell, P. J., Rowe, D. T., Rooney, C. M. & Kouzarides, T. (1989) *EMBO J.* **8**, 127–132.
11. Lin, A., Frost, J., Deng, T., Smeal, T., Al-Alawi, N., Kikkawa, U., Hunter, T., Brenner, D. & Karin, M. (1992) *Cell* **70**, 777–789.
12. Lüscher, B., Kuenzel, E. A., Krebs, E. & Eisenman, R. N. (1989) *EMBO J.* **8**, 1111–1119.
13. Berberich, S. J. & Cole, M. D. (1992) *Genes Dev.* **6**, 166–176.
14. Abate, C., Marshak, D. R. & Curran, T. (1991) *Oncogene* **6**, 2179–2185.
15. Lieberman, P. M., Hardwick, J. M., Sample, J., Hayward, G. S. & Hayward, S. D. (1990) *J. Virol.* **64**, 1143–1155.
16. Packham, G., Economou, A., Rooney, C. M., Rowe, D. T. & Farrell, P. J. (1990) *J. Virol.* **64**, 2110–2116.
17. Taylor, N., Flemington, E., Kolman, J. L., Baumann, R. P., Speck, S. H. & Miller, G. (1991) *J. Virol.* **65**, 4033–4041.
18. Karin, M. (1991) *Curr. Opin. Cell Biol.* **3**, 467–473.
19. Meisner, H. & Czech, M. P. (1991) *Curr. Opin. Cell Biol.* **3**, 474–483.
20. Edelman, A. M., Blumenthal, D. A. & Krebs, E. G. (1987) *Annu. Rev. Biochem.* **56**, 567–613.
21. Hunter, T. & Karin, M. (1992) *Cell* **70**, 375–387.
22. Kuenzel, E. A., Mulligan, J. A., Sommercorn, J. & Krebs, E. G. (1987) *J. Biol. Chem.* **262**, 9136–9140.
23. Carroll, D., Santoro, N. & Marshak, D. R. (1988) *Cold Spring Harbor Symp. Quant. Biol.* **53**, 91–95.
24. Carroll, D. & Marshak, D. (1989) *J. Biol. Chem.* **264**, 7345–7348.
25. Sommercorn, J., Mulligan, J. A., Lozeman, F. J. & Krebs, E. G. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8834–8838.
26. Pinna, L. (1990) *Biochim. Biophys. Acta* **1054**, 267–284.
27. Lüscher, B., Christenson, E., Litchfield, D. W., Krebs, E. G. & Eisenman, R. N. (1990) *Nature (London)* **344**, 517–521.
28. Manak, J. R., Bisschop, N. D., Kris, R. M. & Prywes, R. (1990) *Genes Dev.* **4**, 955–967.
29. Blackwood, E. M., Lüscher, B. & Eisenman, R. N. (1992) *Genes Dev.* **6**, 71–80.
30. Baker, S. J., Kerppola, T. K., Luk, D., Vandenberg, M. T., Marshak, D. R., Curran, T. & Abate, C. (1992) *Mol. Cell. Biol.* **12**, 4694–4705.
31. Taylor, N., Countryman, J., Rooney, C., Katz, D. & Miller, G. (1989) *J. Virol.* **63**, 1721–1728.
32. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1987) *Current Protocols in Molecular Biology* (Wiley, New York).
33. Davis, M. G. & Huang, E. S. (1988) *Biotechnol. Appl. Biochem.* **10**, 6–12.
34. Carey, M., Kolman, J. L., Katz, D. A., Gradoville, L., Barberis, L. & Miller, G. (1992) *J. Virol.* **66**, 4803–4813.
35. Holley-Guthrie, E., Quinlivan, E. B., Mar, E.-C. & Kenney, S. (1990) *J. Virol.* **64**, 3753–3759.
36. Baumann, R., Grogan, E., Ptashne, M. & Miller, G. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4436–4440.
37. Cohen, P. & Cohen, P. T. W. (1989) *J. Biol. Chem.* **264**, 21435–21438.
38. Daibata, M., Humphreys, R. E. & Sairenji, T. (1992) *Virology* **188**, 916–920.
39. Taylor, N. (1991) Ph.D. thesis (Yale Univ., New Haven, CT).
40. Katz, D. A., Baumann, R. P., Sun, R., Kolman, J. L., Taylor, N. & Miller, G. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 378–382.
41. Segil, N., Boseman-Roberts, S. & Heintz, N. (1991) *Science* **254**, 1814–1816.
42. Manak, J. R. & Prywes, R. (1991) *Mol. Cell. Biol.* **11**, 3652–3659.
43. Flemington, E. K., Goldfeld, A. E. & Speck, S. H. (1991) *J. Virol.* **65**, 7073–7077.
44. Mellinghoff, I., Diabata, M., Humphreys, R. E., Mulder, C., Takada, K. & Sairenji, T. (1991) *Virology* **185**, 922–928.
45. Montalvo, E. A., Shi, Y., Shenk, T. E. & Levine, A. J. (1991) *J. Virol.* **65**, 3647–3655.