Multivalent Sequence Recognition by Epstein-Barr Virus Zta Requires Cysteine 171 and an Extension of the Canonical B-ZIP Domain▽

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Epstein-Barr virus (EBV) immediate-early protein Zta is a member of the basic-leucine zipper (B-ZIP) family of DNA binding proteins that has an unusual capacity to recognize multiple DNA recognition sites, including AP-1 and C/EBP binding sites. To better understand the structure and function of Zta, we have mutagenized cysteine residues within or adjacent to the B-ZIP domain. We found that serine substitution for cysteine 171 (C171S), which lies outside and amino terminal to the B-ZIP basic region, completely abrogates Zta capacity to initiate lytic cycle replication. C171S disrupted Zta transcription activation function of several EBV lytic cycle promoters, including the BMRF1 gene (EA-D) and the other lytic activator, Rta. Overexpression of Rta could not rescue the C171S defect for transcription reactivation or viral DNA replication. Zta C171S was defective for binding to these promoters in vivo, as measured by chromatin immunoprecipitation assay. Purified Zta C171S bound AP-1 sites similar to wild-type Zta, but it was incapable of binding several degenerate Zta sites, including a consensus C/EBP site. Zta truncation mutations reveal that residues N terminal to the B-ZIP (amino acids 156 to 178) confer C/EBP binding capacity to the otherwise AP-1-restricted DNA recognition function. Comparison among viral orthologues of Zta suggest that a conserved N-terminal extension of the consensus B-ZIP domain is required for this multivalent DNA recognition capacity of Zta and is essential for viral reactivation.

Epstein-Barr virus (EBV) is a human lymphotropic herpesvirus that contributes to the etiology of several lymphoid and epithelial malignancies (reviewed in references 22 and 32). EBV exists predominantly as a latent episome in B lymphocytes, but it periodically enters a lytic replication cycle to produce progeny viral particles. Lytic cycle reactivation can occur spontaneously or may be induced by various signaling pathways linked to cell stress response and B-cell differentiation (reviewed in reference 4). Lytic cycle replication and associated gene products may contribute directly and indirectly to EBV pathogenesis. In immunosuppressed individuals, increased lytic infection has been directly linked with oral hairy leukoplakia and indirectly linked with risk of non-Hodgkins lymphomas (16, 24). Detection of lytic antigens correlates with risk of EBV-associated nasopharyngeal carcinoma in areas where the virus is endemic (13). Recent evidence also indicates that lytic cycle gene products or viral replication is required for EBV-associated tumorigenesis in mouse models (18, 19). Together, these findings suggest that lytic amplification of infectious virus or viral gene products expressed during latency contribute to EBV-associated pathogenesis.

The EBV lytic cycle can be initiated by ectopic expression of the immediate-early protein Zta (also referred to as BZLF1, ZEBRA, and EB1) (11, 12). Zta is a member of the basic leucine zipper (B-ZIP) family of DNA binding proteins with sequence similarity to C/EBP, c-jun, and c-*fos* (23). Zta binds multiple recognition sites, including AP-1 and C/EBP recognition sites, and activates transcription of both viral and cellular

genes (10, 20, 28, 29). Zta functions as a DNA-bound transcription activator that can recruit cellular general transcription factors and coactivators to target promoters through an amino-terminal activation domain (14). Zta also functions as a lytic cycle replication factor by recruiting viral replication proteins to the origin of lytic replication (OriLyt) (26, 27, 33). Zta also modulates cellular functions, including transcription activation of cellular genes encoding transforming growth factor β (10) and fatty acid synthase (25), inhibition of cell cycle progression (8, 9), and the disruption of the PML/ND10 (1, 5). Viruses lacking Zta are incapable of lytic cycle gene expression or DNA replication, indicating that Zta is essential for virus viability (15).

The Zta B-ZIP domain is essential for the multivalent DNA sequence recognition as well as for mediating interactions with numerous host cell proteins (1, 3, 17, 21, 36–39). In addition to recognizing diverse DNA sequences, Zta can recognize cytosine-methylated DNA sequences with higher affinity than unmethylated DNA (7). Recently, the three-dimensional structure of the Zta B-ZIP domain (amino acids 175 to 245) complexed to DNA was solved by X-ray crystallography (31). Important new insights were provided from this study, including the discovery of a novel hairpin-like fold in the C-terminal zipper region and a structural explanation for the diverse sequence recognition capability of Zta. However, some of the DNA binding properties of Zta were not apparent in the X-ray structure. For example, S186 plays a key role in the recognition of methylated cytosine (6), but this residue was mutated to alanine to improve increased resolution of the crystal structure. Similarly, the redox-sensitive cysteine 189 (C189), which we have shown is important for lytic cycle replication (35) and which can affect DNA binding specificity (34), was mutated to serine (31) in the solved structure. Consequently, the contri-
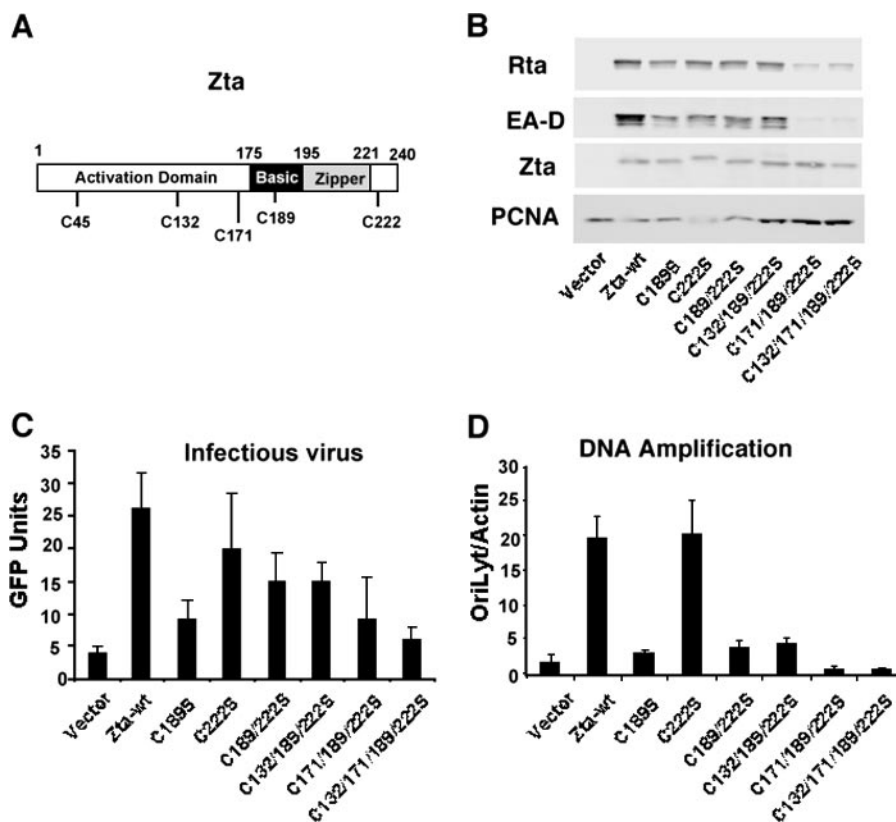
FIG. 1. Identification of Zta cysteine residues required for the reactivation function. A) Schematic of Zta functional domains and the position of all cysteine residues. B) Western blot of ZKO-293 cells transfected with Zta wt or cysteines replaced by serines at the positions indicated. Western blots were probed with antibodies to Rta, EA-D, Zta, or the control cellular protein PCNA. C) Production of infectious green fluorescent protein (GFP)-positive virus was measured from the supernatants of ZKO-293 cells transfected with wt Zta or cysteine substitution mutants as indicated. Infectious virus was monitored by fluorescent-activated cell sorter analysis of GFP-positive Raji cells. D) Viral DNA amplification was measured after transfection of ZKO-293 cells with wt Zta or cysteine substitution mutants and real-time PCR of the EBV OriLyt region relative to actin DNA.

bution of these critical residues to Zta structure and function remain elusive. In the studies presented here, we further explore the role of cysteine residues in Zta function and present evidence that a second cysteine at position 171 (C171) is essential for reactivation and for sequence recognition of C/EBP-like sites. These findings suggest that amino acid residues amino terminal to the consensus B-ZIP domain contribute to sequence recognition by a mechanism not revealed in the crystal structure, but they are nevertheless essential for lytic reactivation.

MATERIALS AND METHODS

Point mutations in Zta were generated by PCR mutagenesis using the Quickchange site-directed mutagenesis kit (Stratagene). Full-length Zta proteins with cysteine substitution mutations were cloned into the BamHI site of a pQE8 bacterial expression vector (QIAGEN). Zta truncation mutations N156 and N166 were cloned into the NheI-HinDIII sites of pET28a. Zta N178 was cloned into the BamHI site of pQE8. Zta wild type (wt), C171S, and C132S were cloned as EcoRI-Sal1 fragments in p3XFLAG-myc-CMV24 vector (Sigma) for mammalian cell expression. Luciferase plasmids for Zp, Rp, Hp, and Mp have been described previously (14). 3× AP-1/SV-LUC and 3× C/EBP/SV-LUC were generated by cloning the 3× AP-1 oligonucleotide (5′GTACCACTGACTCAT CACTGACTCATCACTGACTCATG) or the 3× C/EBP oligonucleotide (5′G TACCATTGCGCAATCATTGCGCAATCATTGCGCAATG) into the Asp1/ Nhe1 site of a pGL2 promoter (Invitrogen) which contains the simian virus 40 early promoter region upstream of the luciferase gene. Rta expression vector

pRTS15 was a gift from S. D. Hayward and contains the BRLF1 open reading frame in a derivative of pSG5 (Stratagene). All of the methods for this study have been described previously (35). Sequences of oligonucleotide probes for electrophoretic mobility shift assay (EMSA) have been published previously (31, 35).

RESULTS AND DISCUSSION

Identification of cysteine residues essential for lytic cycle replication. A series of cysteine-to-serine mutations were introduced into Zta (Fig. 1A). Previous studies demonstrated that combinations of these cysteines were responsible for redox-sensitive DNA binding but that no single cysteine could completely account for this activity (35). To further explore the molecular biology of these cysteine residues in mediating Zta functions, we assayed single cysteine-to-serine mutations at positions 189 and 222 as well as combined mutations at positions 189 and 222 (designated 189/222), 132/189/222, 171/189/ 222, and 132/171/189/222. These cysteine substitution mutations were expressed as FLAG-tagged proteins in ZKO-293 cells to determine their ability to stimulate viral reactivation and lytic gene expression. ZKO-293 cells contain a bacmid with the EBV genome lacking the Zta coding sequence, and they are therefore well suited for Zta complementation studies. We found that C189S, C222S, C189/222S, and C132/189/222S ac-
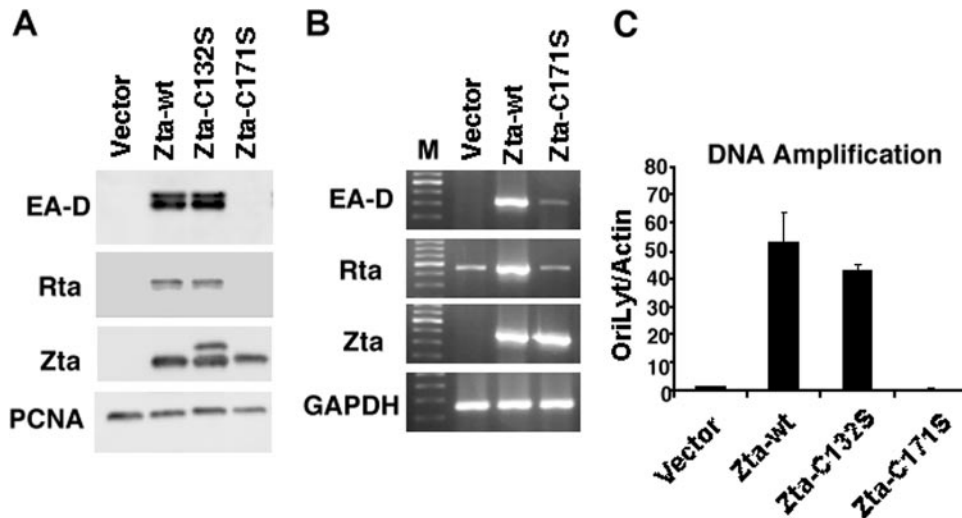
FIG. 2. C171S fails to activate early genes and lytic replication. A) Zta wt, C132S, C171S, or control expression vector was transfected into ZKO-293 cells and assayed by Western blotting for expression of Zta, Rta, EA-D (BMRF1), or control PCNA. B) Reverse transcription-PCR analysis of mRNA for EA-D, Rta, Zta, or cellular GAPDH control-derived ZKO-293 cells transfected with control expression vector or expression vectors for Zta wt or C171S at 24 h posttransfection. C) EBV DNA amplification was measured by real-time PCR after transfection of ZKO-293 cells with vector, Zta wt, C132S, or C171S.

tivated EA-D and Rta in a manner indistinguishable from that of wild-type Zta (Fig. 1B). In contrast, C171/189/222S and C132/171/189/222S were significantly reduced for EA-D and Rta transcription activation (Fig. 1B). These Zta mutants were then tested for their ability to stimulate production of infectious virus (Fig. 1C) and amplification of EBV genome DNA (Fig. 1D). We found the C189S was defective for production of infectious virus and DNA amplification, consistent with our previous report that this cysteine residue was critical for viral lytic cycle replication (35). We also observed that C171/189/222S and C132/171/189/222S were reduced for production of infectious virus (Fig. 1C) and completely defective for amplification of genomic DNA (Fig. 1D). These findings indicate that combinations of mutations containing C171S were highly defective for transcription activation of some viral early genes and were incapable of lytic cycle DNA replication.

Cysteine 171 is required for viral reactivation. To determine if single-serine substitution mutations at C132 or C171 reduced Zta activity, we expressed C132S and C171S in ZKO-293 cells (Fig. 2A) and assayed by Western blot for activation of early lytic antigens EA-D and Rta. We found that C132S behaved essentially like wild-type Zta, while C171S was defective for activation of EA-D and Rta (Fig. 2A). This defect of C171S was further validated by measuring the mRNA levels by reverse transcription-PCR (Fig. 2B). C171S was incapable of stimulating EA-D or Rta mRNA levels despite identical levels of Zta and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control mRNA in each reaction. We next tested whether C132S or C171S could stimulate DNA amplification of latently infected EBV in ZKO-293 cells (Fig. 2C). We found that C132S stimulated viral DNA amplification at a level similar to that of wt Zta (~40- to 50-fold relative to cellular actin DNA), while C171S was incapable of stimulating viral DNA amplification (Fig. 2C). Both C132S and C171S were expressed to similar levels as wt Zta, as demonstrated by the Western blot

shown in Fig. 2A (Zta panel). These findings indicate that C171S is incapable of stimulating viral reactivation of gene expression and lytic replication.

C171 is required for transcription activation of viral early promoters. To further understand the mechanistic basis for the defect in C171S reactivation function, we assayed the ability of C171S and C132S to stimulate transcription activation of four lytic cycle promoters using a luciferase reporter assay (Fig. 3). We found that C171S was defective for activation of the BMRF1 (Mp), BHLF1 (Hp), BRLF1 (Rp), and BZLF1 (Zp) early region promoters, while C132S behaved indistinguishably from wild-type Zta. In particular, Zta C171S stimulated the Mp and Hp promoters to ~14% of wt Zta and stimulated the Rp and Zp promoters to ~25- to 30% of the wt. Expression levels for Zta wt, C132S, and C171S were nearly identical, indicating that these differences in transcription activity were not attributable to differences in protein expression levels. These findings indicate that C171S is defective for transcription activation of all of the viral early promoters tested but may also have more pronounced defects at some subset of these promoters.

We next determined whether Zta C171S was defective for binding to the target promoter regions of EBV DNA in vivo using real-time PCR analysis of chromatin-immunoprecipitated (ChIP) DNA (Fig. 4A to D). We found that C171S was substantially reduced for binding at all promoters tested relative to wt Zta. Binding at BHLF1p and BHRF1p was reduced approximately six- to sevenfold relative to wild-type Zta (Fig. 4A and B), while binding at BMRF1p and BRLF1p was reduced only approximately twofold relative to wt Zta (Fig. 4C and D). These findings suggest that C171S impairs Zta DNA binding capabilities but has more penetrating effects on the DNA binding at some sites (e.g., BHLF1) relative to other sites (BMRF1p).

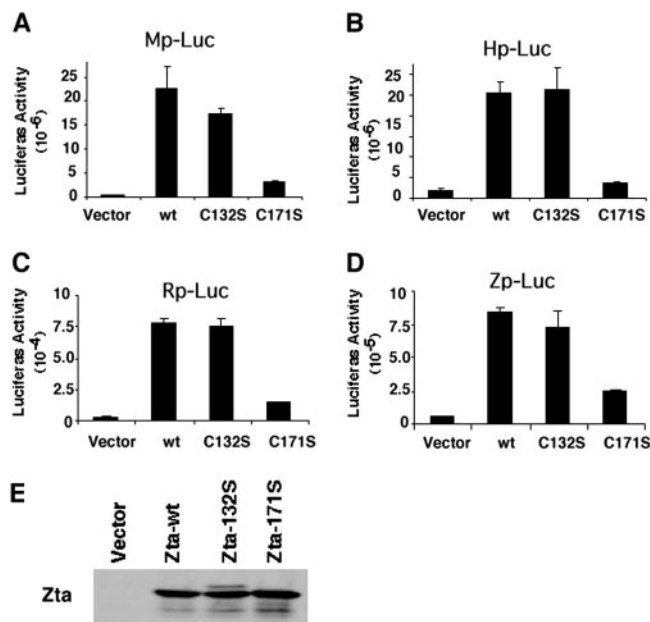Since reactivation is strongly dependent on Rta coexpres-

FIG. 3. C171S is defective for transcription activation of viral promoters. 293 cells were transfected with vector, Zta wt, C132S, or C171S and assayed for activation of luciferase reporter plasmids for EBV promoters BMRF1(Mp)-Luc (A), BHLF1(Hp)-Luc (B), BRLF1(Rp)-Luc (C), or BZLF1(Zp)-Luc (D). (E) A Western blot of Zta proteins after a typical transfection assay.

sion, and since C171S is defective for transcription activation of Rta through the BRLF1 promoter, we tested whether overexpression of Rta could rescue the C171S defect. Others have found that Rta could rescue the reactivation defects in other Zta mutations, especially the S186A mutation that is defective for binding methylated DNA at the BRLF1 promoter (2). An Rta expression vector was cotransfected with or without Zta wt, C132S, or C171S expression vectors and assayed for Zta, Rta, and EA-D expression (Fig. 4E), as well as for viral DNA amplification (Fig. 4F) in ZKO-293 cells. Short exposures of Western blots revealed that EA-D levels were elevated by cotransfection of Rta with Zta wt and Zta C131S but not significantly in the presence of Zta C171S (Fig. 4E, middle panel). Longer exposure times indicate that Rta coexpression does stimulate EA-D expression in the presence of C171S but to a lesser extent than that of C131S or wt Zta (Fig. 4E, top panel). We also noted that Rta levels were consistently lower in the presence of Zta C171S, suggesting that stabilizing interactions between Zta and Rta may also be affected by mutations in Zta. Expression levels of Zta were nearly identical for wt, C132S, and C171S, as were control levels for cellular PCNA. We next asked whether the addition of Rta was sufficient to rescue the defect in C171S in lytic DNA replication (Fig. 4F). We found that Rta cotransfection had a slight stimulation on the DNA replication of C132S but had no detectable effect on C171S, which was indistinguishable from vector control, while Zta wt stimulated viral DNA ~70-fold. We conclude from these studies that Rta cotransfection cannot rescue the defect of C171S on lytic DNA replication or EA-D transcription activation. These findings indicate that the defect in Zta C171S
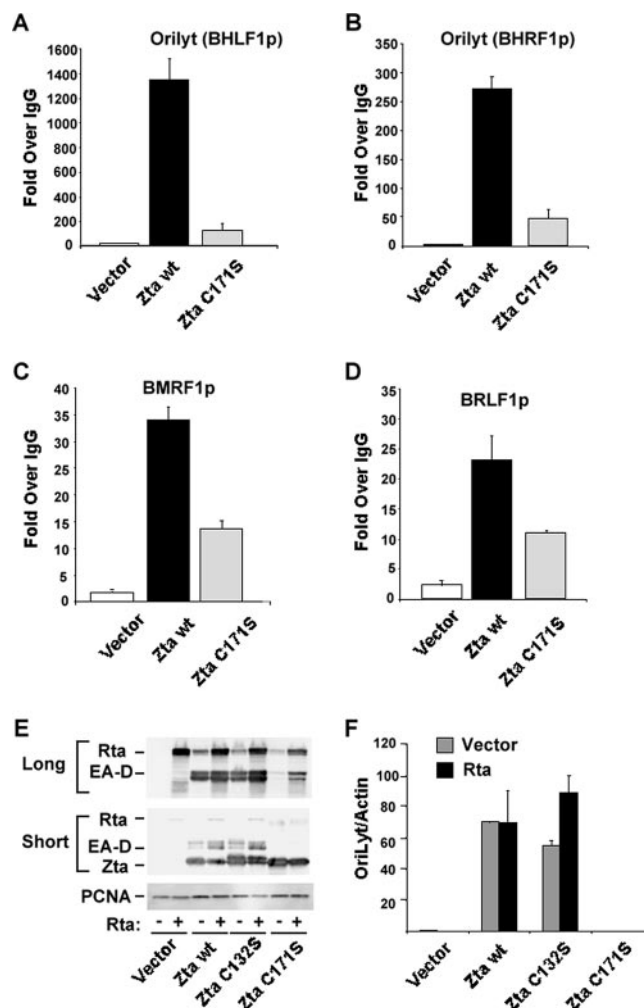

FIG. 4. C171S is defective for binding to viral promoters in vivo. (A to D) Chromatin immunoprecipitation assays were performed with antibody to Zta or control immunoglobulin G (IgG) after transfection of ZKO-293 cells with vector, wt Zta, or C171S. ChIP DNA was assayed at BHLF1p (A), BHRF1p (B), BMRF1p (C), or BRLF1p (D) and quantitated by real-time PCR for DNA recovered by Zta-specific antibody relative to the IgG control. (E) Western blot analysis of ZKO-293 cells transfected with (+) or without (−) Rta expression vector and either vector, Zta wt, Zta C132S, or Zta C171S. Long exposure times for chemiluminescence development are indicated for detection with Rta- and EA-D-specific antibodies (top panel), and short exposures are shown for Rta, EA-D, and Zta (middle panel). Levels of control cellular protein PCNA are indicated in the lower panel. (F) Viral lytic replication was measured by real-time PCR of viral DNA relative to cellular actin DNA in ZKO-293 cells transfected with Zta wt, C132S, or C171S cotransfected with (black) or without (gray) Rta expression plasmid.

is not limited to the expression of Rta through the transcription activation of the BRLF1 promoter.

C171 is required for binding to C/EBP sites but not AP-1 sites. The transcription activation defects at several different promoters (Fig. 3) and the failure of Zta C171S to bind BHLF1 and BHRF1 promoter regions by ChIP assay (Fig. 4A to D) suggest that C171S has a primary defect in DNA binding at some, but not necessarily all, recognition sites. Therefore, we assayed the DNA binding activity of purified Zta proteins
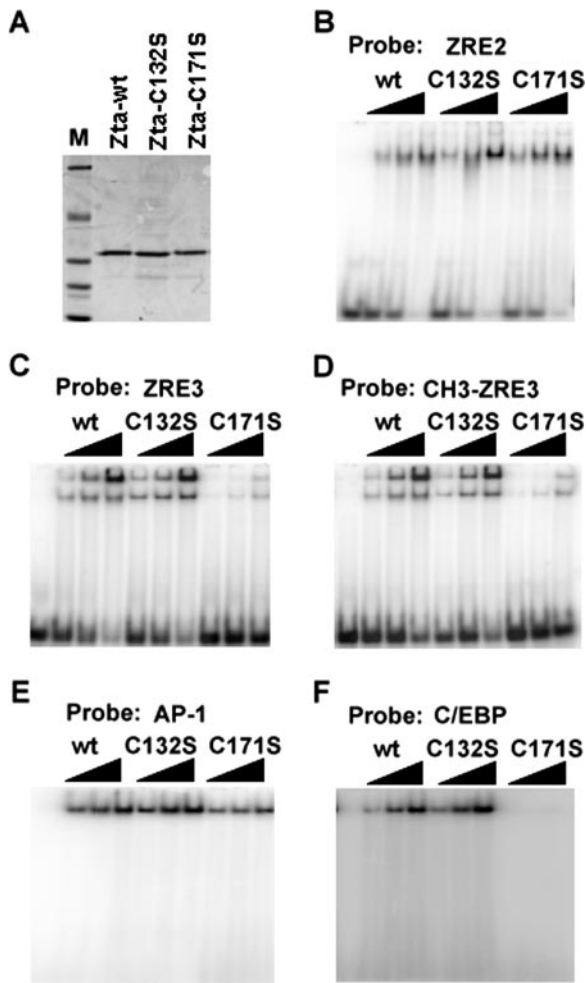
FIG. 5. C171S is defective for DNA binding to C/EBP recognition sites. A) Purified recombinant Zta wt, C132S, or C171S was stained with Coomassie brilliant blue. B to F) DNA binding was monitored by EMSA for Zta wt (lanes 2 to 4), C132S (lanes 5 to 7), or C171S (lanes 8 to 10) at concentrations of 10, 30, and 90 ng of Zta protein. Radiolabeled oligonucleotide probes were Rp-ZRE2 (B), Rp-ZRE3 (C), methylated Rp-ZRE3 (D), 11-bp AP-1 (E), and 11-bp C/EBP (F) as indicated.
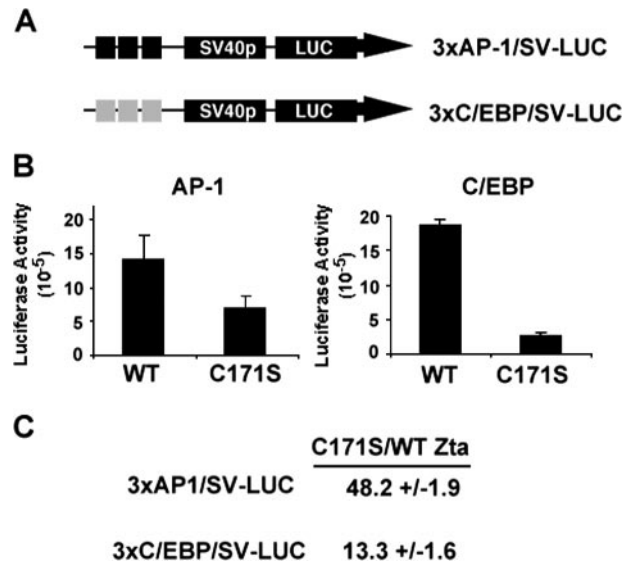

FIG. 6. C171S is defective for transcription activation of C/EBP site-containing promoters. A) Schematic description of the 3× AP-1/SV-LUC and 3× C/EBP/SV-LUC reporter constructs. B) Luciferase assays with 3× AP-1/SV-LUC (left panel) or 3× C/EBP/SV-LUC (right panel) cotransfected with either Zta wt or C171S as indicated. C) Quantification of the ratio of Zta C171S relative to wt Zta on the 3× AP-1- or the 3× C/EBP-containing reporter plasmids.

for several different Zta recognition sites in vitro using electrophoretic mobility shift assay (EMSA). C171S, C132S, and wt Zta were expressed and purified from *Escherichia coli* and adjusted to identical protein concentrations (Fig. 5A). These proteins were assayed for DNA binding to several Zta recognition sequences. We found that C171S bound the BRLF1 ZRE2 site with affinity nearly identical to that of wild-type Zta and C132S (Fig. 5B). In contrast, Zta C171S was highly defective for binding the more degenerate recognition site ZRE3, also derived from the BRLF1 promoter, relative to Zta wt or C132S (Fig. 5C). A similar defect in Zta C171S was observed when tested for binding to the cytosine-methylated form of these ZRE3 sites (Fig. 5D), with no significant change in wt or C132S Zta. These findings suggest that C171S limits the variety of sites recognized by Zta. To further explore this possibility, we compared C171S with wt and C132S Zta for binding to 11-bp probes that contain only the AP-1 or C/EBP recognition

sites as described in the crystallographic studies (31). We found that C171S bound the AP-1 site at levels similar to those of Zta wt and C132S (Fig. 5E). In striking contrast, C171S was incapable of binding the C/EBP site probe, while the wt and C132S Zta proteins bound with similar affinity to that of the AP-1 site (Fig. 5F). These findings indicate that C171S abrogates the C/EBP site recognition capability of Zta but has limited effect on the AP-1 binding activity.

To determine if this difference in DNA binding recognition correlated with transcription activation function, we generated reporter constructs with either 3× AP-1 sites or 3× C/EBP sites in the pGL2 promoter construct, which places these test sequences immediately upstream of the simian virus early promoter driving the luciferase gene (Fig. 6A). We compared wt and C171S Zta expression vectors for their ability to stimulate transcription from either 3× AP-1/SV-LUC or 3× C/EBP/SV-LUC in 293 cells. We found that Zta C171S stimulated the AP-1-containing construct to levels ~48% of that of wt Zta (Fig. 6B and C). In contrast, C171S stimulated the C/EBP-containing construct to only ~13% of that of wt Zta (Fig. 6B and C). These findings indicate that C171S was approximately threefold more defective for transcription activation of the C/EBP-containing promoter than the AP-1-containing promoter. This activation correlates well with the difference in DNA binding activity (Fig. 5E and F) and further supports our model that C171S affects the DNA recognition capabilities of Zta.

Evidence for a conserved amino-terminal extension of the B-ZIP basic region required for multiple-sequence recognition. A comparison of Zta proteins from the related cercopithecine herpesvirus 15 and callitrichine herpesvirus 3 revealed a strong conservation of the C171 residue as well as an
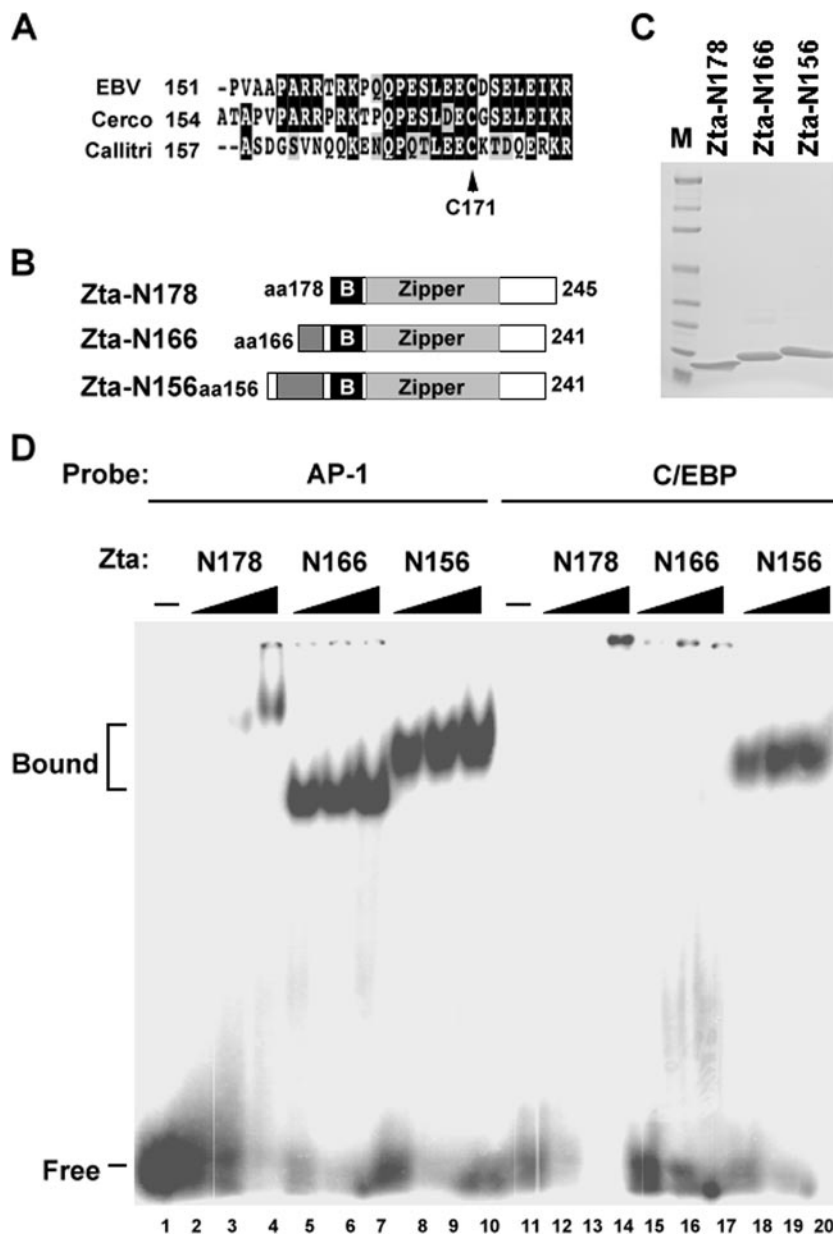
FIG. 7. Conserved motif N terminal to the basic homology region confers DNA binding to C/EBP sites. A) Alignment of Zta proteins from EBV (amino acids [aa] 151 to 179), cercopithecine (Cerco) herpesvirus 15 (aa 154 to 182), and callitrichine (Callitri) herpesvirus 3 (aa 157 to 185). B) Schematic of Zta truncation mutants starting at amino acids 178 (Zta-N178), 166 (Zta-N166), or 156 (Zta-N156) containing portions of the conserved amino-terminal extended basic domain. C) Coomassie brilliant blue staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis containing Zta-N178, Zta-N166, and Zta-N156. D) EMSA with 11-bp probes for AP-1 (lanes 1 to 10) or C/EBP (lanes 11 to 20). Zta protein N178, N166, or N156 was added at 10, 30, or 90 ng.

additional stretch of residues extending to amino acid 156 of Zta (Fig. 7A). To test if this additional stretch of conserved amino acids contribute to DNA binding recognition, we compared Zta proteins with N termini initiating at amino acid 156 (N156), 166 (N166), or 178 (N178) (Fig. 7B). These proteins were expressed and purified from *E. coli* and adjusted for protein concentration by Coomassie staining (Fig. 7C), and they were then assayed for their ability to bind to the AP-1 or C/EBP 11-mer probes (Fig. 7D). We found that the shortest derivative, N178, bound to AP-1 weakly and did not bind to the

C/EBP probe. The N166 truncation bound to the AP-1 probe with high affinity (lanes 5 to 7) but did not bind detectably to the C/EBP probe (lanes 15 to 17). In contrast, the N156 truncation bound with high affinity to AP-1 (lanes 8 to 10) and nearly similar affinity to the C/EBP probe (lanes 18 to 20). These findings indicate that the amino acids N terminal to the canonical basic region of the B-ZIP domain stimulate binding to a C/EBP recognition site. Since the N156 truncation did not bind C/EBP with the same affinity as the AP-1 site but the full-length protein bound both sites with equal affinity (Fig. 5E

and F), it is likely that additional N-terminal amino acids contribute further to the recognition of C/EBP and other non-AP-1 consensus sites found throughout the viral and cellular genome.

Conclusions. In this work, we found that serine substitution for cysteine 171 disrupted the transcription activation and lytic cycle replication function of Zta (Fig. 2). C171S was defective for binding to viral target sequences at several viral promoters in vivo using ChIP assay (Fig. 4A to D), and purified recombinant C171S was defective for binding to a subset of target sites in vitro (Fig. 5). C171S in the context of full-length Zta protein bound to AP-1-like sites with nearly equal affinity to wt Zta but did not bind to several non-AP-1 consensus sites or to a consensus C/EBP site. Truncation mutations revealed that amino acids N terminal to the B-ZIP homology domain contribute to the C/EBP site recognition more significantly than to the AP-1 site. X-ray crystallography studies with a Zta truncation protein starting at amino acid 175 showed detectable but reduced binding to C/EBP sites relative to AP-1 sites (31). These structural studies are essentially consistent with our findings, although we now propose that amino acids N terminal to the basic region contribute significantly to multiple-sequence recognition. Additional structural studies with Zta peptides extended in the amino-terminal direction may be informative and may reveal a novel stabilization domain similar to that observed at the C-terminal side of the zipper/dimerization domain.

In addition to these structural implications, our data also indicate that multiple-sequence recognition by Zta is required for lytic cycle reactivation. While we cannot rule out that C171S eliminates some essential protein-protein interactions as well, our data are most consistent with the interpretation that multiple-site recognition properties of Zta are essential for completion of lytic cycle gene expression and DNA replication. Finally, it should be noted that regions amino terminal to other basic homology regions have been described, including the "cap 'n' collar" motif of the MAF family of proteins, which contribute to DNA recognition during the oxidative stress response (30). We suspect that the region N terminal to the basic homology domain of Zta represents a conserved motif among Zta family members that is essential for multivalent DNA site recognition and other interactions required for lytic cycle activation.

ACKNOWLEDGMENTS

REFERENCES

1. **Adamson, A. L., and S. Kenney.** 2001. Epstein-Barr virus immediate-early protein BZLF1 is SUMO-1 modified and disrupts promyelocytic leukemia bodies. J. Virol. **75:**2388–2399.
2. **Adamson, A. L., and S. C. Kenney.** 1998. Rescue of the Epstein-Barr virus BZLF1 mutant, Z(S186A), early gene activation defect by the BRLF1 gene product. Virology **251:**187–197.
3. **Aho, S., M. Buisson, T. Pajunen, Y. W. Ryoo, J. F. Giot, H. Gruffat, A. Sergeant, and J. Uitto.** 2000. Ubinuclein, a novel nuclear protein interacting with cellular and viral transcription factors. J. Cell Biol. **148:**1165–1176.
4. **Amon, W., and P. J. Farrell.** 2005. Reactivation of Epstein-Barr virus from latency. Rev. Med. Virol. **15:**149–156.
5. **Bell, P., P. M. Lieberman, and G. G. Maul.** 2000. Lytic but not latent replication of Epstein-Barr virus is associated with PML and induces sequential release of nuclear domain 10 proteins. J. Virol. **74:**11800–11810.
6. **Bhende, P. M., W. T. Seaman, H. J. Delecluse, and S. C. Kenney.** 2005. BZLF1 activation of the methylated form of the BRLF1 immediate-early promoter is regulated by BZLF1 residue 186. J. Virol. **79:**7338–7348.
7. **Bhende, P. M., W. T. Seaman, H. J. Delecluse, and S. C. Kenney.** 2004. The EBV lytic switch protein, Z, preferentially binds to and activates the methylated viral genome. Nat. Genet. **36:**1099–1104.
8. **Cayrol, C., and E. Flemington.** 1996. G0/G1 growth arrest mediated by a region encompassing the basic leucine zipper (bZIP) domain of the Epstein-Barr virus transactivator Zta. J. Biol. Chem. **271:**31799–31802.
9. **Cayrol, C., and E. K. Flemington.** 1996. The Epstein-Barr virus bZIP transcription factor Zta causes G0/G1 cell cycle arrest through induction of cyclin-dependent kinase inhibitors. EMBO J. **15:**2748–2759.
10. **Cayrol, C., and E. K. Flemington.** 1995. Identification of cellular target genes of the Epstein-Barr virus transactivator Zta: activation of transforming growth factor βigh3 (TGF-βigh3) and TGF-β1. J. Virol. **69:**4206–4212.
11. **Chevallier, G. 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.
12. **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.
13. **Dardari, R., M. Khyatti, A. Benider, H. Jouhadi, A. Kahlain, C. Cochet, A. Mansouri, B. El Gueddari, A. Benslimane, and I. Joab.** 2000. Antibodies to the Epstein-Barr virus transactivator protein (ZEBRA) as a valuable biomarker in young patients with nasopharyngeal carcinoma. Int. J. Cancer **86:**71–75.
14. **Deng, Z., C.-J. Chen, D. Zerby, H.-J. Delecluse, and P. M. Lieberman.** 2001. Identification of acidic and aromatic residues in the Zta activation domain essential for Epstein-Barr virus reactivation. J. Virol. **75:**10334–10347.
15. **Feederle, R., M. Kost, M. Baumann, A. Janz, E. Drouet, W. Hammerschmidt, and H. J. Delecluse.** 2000. The Epstein-Barr virus lytic program is controlled by the co-operative functions of two transactivators. EMBO J. **19:**3080–3089.
16. **Greenspan, J. S., D. Greenspan, E. T. Lennette, D. I. Abrams, M. A. Conant, V. Petersen, and U. K. Freese.** 1985. Replication of Epstein-Barr virus within the epithelial cells of oral "hairy" leukoplakia, an AIDS-associated lesion. N. Engl. J. Med. **313:**1564–1571.
17. **Gutsch, D. E., E. A. Holley-Guthrie, Q. Zhang, B. Stein, M. A. Blanar, A. S. Baldwin, and S. C. Kenney.** 1994. The bZIP transactivator of Epstein-Barr virus, BZLF1, functionally and physically interacts with the p65 subunit of NF-κB. Mol. Cell. Biol. **14:**1939–1948.
18. **Hong, G. K., M. L. Gulley, W. H. Feng, H. J. Delecluse, E. Holley-Guthrie, and S. C. Kenney.** 2005. Epstein-Barr virus lytic infection contributes to lymphoproliferative disease in a SCID mouse model. J. Virol. **79:**13993–14003.
19. **Hong, G. K., P. Kumar, L. Wang, B. Damania, M. L. Gulley, H. J. Delecluse, P. J. Polverini, and S. C. Kenney.** 2005. Epstein-Barr virus lytic infection is required for efficient production of the angiogenesis factor vascular endothelial growth factor in lymphoblastoid cell lines. J. Virol. **79:**13984–13992.
20. **Kenney, S., J. Kamine, E. Holley-Guthrie, J. C. Lin, E. C. Mar, and J. Pagano.** 1988. The Epstein-Barr virus (EBV) BZLF1 immediate-early gene product differentially affects latent versus productive EBV promoters. J. Virol. **63:**1729–1736.
21. **Kenney, S. C., E. Holley-Guthrie, E. B. Quinlivan, D. Gutsch, Q. Zhang, T. Bender, J. F. Giot, and A. Sergeant.** 1992. The cellular oncogene c-myb can interact synergistically with the Epstein-Barr virus BZLF1 transactivator in lymphoid cells. Mol. Cell. Biol. **12:**136–146.
22. **Kieff, E.** 1996. Epstein-Barr virus and its replication, p. 2343–2396. In D. Knipe and P. M. Howley (ed.), Fields virology, 3rd ed., vol. 2. Lippincott-Raven Publishers, Philadelphia, Pa.
23. **Kouzarides, T., G. Packham, A. Cook, and P. J. 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.
24. **Lau, R., J. Middeldorp, and P. J. Farrell.** 1993. Epstein-Barr virus gene expression in oral hairy leukoplakia. Virology **195:**463–474.
25. **Li, Y., J. Webster-Cyriaque, C. C. Tomlinson, M. Yohe, and S. Kenney.** 2004. Fatty acid synthase expression is induced by the Epstein-Barr virus immediate-early protein BRLF1 and is required for lytic viral gene expression. J. Virol. **78:**4197–4206.
26. **Liao, G., J. Huang, E. D. Fixman, and S. D. Hayward.** 2005. The Epstein-Barr virus replication protein BBLF2/3 provides an origin-tethering function through interaction with the zinc finger DNA binding protein ZBRK1 and the KAP-1 corepressor. J. Virol. **79:**245–256.
27. **Liao, G., F. Y. Wu, and S. D. Hayward.** 2001. Interaction with the Epstein-Barr virus helicase targets Zta to DNA replication compartments. J. Virol. **75:**8792–8802.
28. **Lieberman, P. M., and A. J. Berk.** 1990. In vitro transcriptional activation, dimerization, and DNA-binding specificity of the Epstein-Barr virus Zta protein. J. Virol. **64:**2560–2568.
29. **Lieberman, P. M., J. M. Hardwick, J. Sample, G. S. Hayward, and S. D.**

Hayward. 1990. The Zta transactivator involved in induction of lytic cycle gene expression in Epstein-Barr virus-infected lymphocytes binds to both AP-1 and ZRE sites in target promoter and enhancer regions. J. Virol. **64:**1143–1155.

30. **Motohashi, H., T. O'Connor, F. Katsuoka, J. D. Engel, and M. Yamamoto.** 2002. Integration and diversity of the regulatory network composed of Maf and CNC families of transcription factors. Gene **294:**1–12.

31. **Petosa, C., P. Morand, F. Baudin, M. Moulin, J. B. Artero, and C. W. Muller.** 2006. Structural basis of lytic cycle activation by the Epstein-Barr virus ZEBRA protein. Mol. Cell **21:**565–572.

32. **Rickinson, A. B., and E. Kieff.** 1996. Epstein-Barr Virus, p. 2397–2446. *In* D. Knipe and P. M. Howley (ed.), Fields virology, 3rd ed., vol. 2. Lippincott-Raven Publishers, Philadelphia, Pa.

33. **Sarisky, R. T., Z. Gao, P. M. Lieberman, E. D. Fixman, G. S. Hayward, and S. D. Hayward.** 1996. A replication function associated with the activation domain of the Epstein-Barr virus Zta transactivator. J. Virol. **70:**8340–8347.

34. **Schelcher, C., S. Valencia, H. J. Delecluse, M. Hicks, and A. J. Sinclair.** 2005. Mutation of a single amino acid residue in the basic region of the Epstein-Barr virus (EBV) lytic cycle switch protein Zta (BZLF1) prevents reactivation of EBV from latency. J. Virol. **79:**13822–13828.

35. **Wang, P., L. Day, J. Dheekollu, and P. M. Lieberman.** 2005. A redox-

sensitive cysteine in Zta is required for Epstein-Barr virus lytic cycle DNA replication. J. Virol. **79:**13298–13309.

36. **Wu, F. Y., H. Chen, S. E. Wang, C. M. ApRhys, G. Liao, M. Fujimuro, C. J. Farrell, J. Huang, S. D. Hayward, and G. S. Hayward.** 2003. CCAAT/enhancer binding protein alpha interacts with ZTA and mediates ZTA-induced p21(CIP-1) accumulation and G(1) cell cycle arrest during the Epstein-Barr virus lytic cycle. J. Virol. **77:**1481–1500.

37. **Wu, F. Y., S. E. Wang, H. Chen, L. Wang, S. D. Hayward, and G. S. Hayward.** 2004. CCAAT/enhancer binding protein alpha binds to the Epstein-Barr virus (EBV) ZTA protein through oligomeric interactions and contributes to cooperative transcriptional activation of the ZTA promoter through direct binding to the ZII and ZIIIB motifs during induction of the EBV lytic cycle. J. Virol. **78:**4847–4865.

38. **Zerby, D., C.-J. Chen, E. Poon, D. Lee, R. Shiekhattar, and P. M. Lieberman.** 1999. The amino-terminal C/H1 domain of CREB binding protein mediates Zta transcription activation of latent Epstein-Barr virus. Mol. Cell. Biol. **19:**1617–1626.

39. **Zhang, Q., D. Gutsch, and S. Kenney.** 1994. Functional and physical interaction between p53 and BZLF1: implications for Epstein-Barr virus latency. Mol. Cell. Biol. **14:**1929–1938.