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Identification of a Glucocorticoid-Responsive Element in Epstein-Barr Virus

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Immortalization of B lymphocytes by Epstein-Barr virus (EBV) is complex and poorly understood. However, some evidence suggests that glucocorticoids influence this process. We identified a glucocorticoid-responsive element in the *Bam*HI C fragment of EBV which we call ES-1. In glucocorticoid-treated cells, ES-1 enhanced chloramphenicol acetyltransferase gene expression from the herpes simplex virus thymidine kinase promoter, as well as the EBV *Bam*-C promoter, from which several latent viral gene products are transcribed. By Northern blot analysis, glucocorticoid treatment enhanced transcription from the *Bam*-C promoter in Jijoye cells, a Burkitt's lymphoma cell line. In addition, the DNA-binding domain of the glucocorticoid receptor bound specifically to the ES-1 region. These glucocorticoid effects on the *Bam*-C promoter region may provide some insight into the process of EBV immortalization.

Epstein-Barr virus (EBV), a human herpesvirus, is the etiologic agent of mononucleosis and is associated with two forms of malignant disease, Burkitt's lymphoma and nasopharyngeal carcinoma (11, 25). When EBV infects B lymphocytes in vitro, cell immortalization occurs and viral DNA is latently maintained as multiple copies of extrachromosomal, circular plasmids (31). Several EBV proteins expressed during viral latency, such as EBNA-1 (33), EBNA-2 (16), and latent membrane protein (15, 22), appear to play important roles in cell immortalization; however, the process is still poorly understood. Some in vitro evidence suggests that glucocorticoids influence this process, although the data are conflicting as to whether the effect on viral latency is positive or negative (2, 10, 23, 34). In addition, EBV immortalization of B lymphocytes is associated with increases in the concentration and absolute number of glucocorticoid receptors (GR) (35).

Glucocorticoids exert their physiological effects on development and differentiation through the GR, which acts as a ligand-dependent transcription factor (3, 12). Glucocorticoid-bound receptor complexes activate transcription by binding to specific DNA sequences, called glucocorticoid-responsive elements (GRE). The GRE consensus sequence generally consists of variations of the perfect palindrome $AG^{A}_{G}ACAnnnTGT^{T}_{C}CT$. Many of the glucocorticoid-inducible genes which have been identified are characterized by a cluster of multiple GREs at various distances upstream of the promoter region (8, 17, 24, 38). Examples of systems in which GREs enhance transcription of viral promoters include the Moloney murine sarcoma virus (24) and the mouse mammary tumor virus (38).

We hypothesized that the EBV genome might contain one or more GREs and that localization of these regulatory elements might contribute to a better understanding of the influence of glucocorticoids on EBV immortalization. By sequence searching, we identified three regions of the EBV genome which could potentially possess GRE-like properties. One region, which we call ES-1, in the *Bam*HI-C region, proved to have GRE properties and enhanced transcription from the latent-cycle *Bam*-C promoter (BC-R2), which regulates the synthesis of the highly spliced EBNA family of RNAs (4). This regulatory activity of glucocorticoids may provide an explanation for some of the observed effects of glucocorticoids on the EBV-lymphocyte interaction.

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MATERIALS AND METHODS

Plasmid constructions. All DNA constructions were done by standard procedures and verified by restriction enzyme analysis (21). Plasmid pCTGre was constructed by ligating the 36-base-pair (bp) *Xba*I fragment of pmGTCO (which contains a single GRE derived from mouse mammary tumor virus [Fig. 1C]) into the *Bam*HI site of pBLCAT2 (19) after the cohesive ends were made blunt by filling in with the Klenow fragment of DNA polymerase I. Plasmid pCTC14 was constructed in the same manner as pCTGre except that the insert was the 197-bp *Taq*I-*Ava*I fragment (Fig. 1B) of pSV2neoBamHI-C, which contains the *Bam*HI C fragment of EBV (provided by G. Miller, Yale University). Plasmid pCTC13 is identical to pCTC14 except that the insert is in the reverse orientation. Plasmid pCEC9 was constructed by ligating the *Sau*3AI fragment (1,426 bp, see Fig. 4A) of pSV2neoBamHI-C into the *Hin*dIII site of pSV0CAT (14) after the cohesive ends were made blunt by filling in with the Klenow fragment of DNA polymerase I. Plasmid pCP11 was constructed by ligating the *Sac*I-*Sau*3AI (240 bp, see Fig. 4A) fragment of pSV2neoBamHI-C into the *Hin*dIII site of pSV0CAT after the cohesive ends were made blunt with the Klenow fragment of DNA polymerase I and T4 DNA polymerase. Plasmid pCPG4 was constructed by inserting the 197-bp *Taq*I-*Ava*I fragment of pSV2neoBamHI-C into the *Nde*I site of pCP11 after the cohesive ends were made blunt by filling in with the Klenow fragment of DNA polymerase I, thus positioning the former *Ava*I site of the insert approximately 300 bp upstream of the chloramphenicol acetyltransferase (CAT) initiation codon.

Cell lines. The following cell lines were used in this study: HeLa, a human epidermoid carcinoma line, originally designated the S3 subline and grown in our laboratory for many years; HepG2, a human hepatoma line recently obtained from M. Karin (University of California, San Diego); D98/

A) AG[A/G]ACAnnnTGT[T/C]CT

B) XbaI XbaI
TCTAGAAAATGTTCTGATCTGAGCCTCGAG

C) ES-1

10240- TTTCGACTGTCATTTACAATAAAATGAAACCTTTTATTCTTGATTGCCTCTTGTGTTCTT

10300- GCCGCCCAGGTACCTTCCTGTGTTCTCCCCACGGGAAAAAGAATAGCTTCTGCAGAAGGC

10360- CATTGACGCAAGTTTTGCCCGTGGGGATTACCCGACCCAGCCACTTACAGCACATTTTGT

10420- TCTAGGTCCATCTTAGGAGC

ES-2

54020- TTTCCTCCCACCCCTGTCCTGGCTGTGGCAAATGCGACCCTCATAGAGTTGTGTTTCAGG

54080- TCTGTGTCCTGTTTGCGGT

ES-3

82190- AACGACCGGAGTCCTGTCTCTTTGTGTTCTTGGGGGACTTGAGTTAGCTGTCTTTCCTCT

82250- TATTACATTGGGCTAACGGGAGGAAATGAACCCAGGGGTGGCAGTGGATGGGGTCATTTA

82310- TGGGCAAAACTCACAGGACATGTTTGGGGAGTTAGCATTGGCGTCGGGAAACACAGCTCT

82370- GGCAGTTATAACCGCACCAGCTAACAGGACATGTTTGGGG

FIG. 1. EBV sequences selected as potential GREs. (A) Perfect palindrome GRE consensus sequence. (B) Single GRE sequence from mouse mammary tumor virus subcloned into pBLCAT2 for the construction of pCTGre (Fig. 2A). (C) Three EBV sequences selected for construction of CAT reporter plasmids. Underlined regions represent the very highly conserved hexanucleotide sequence common to most GREs. Numbers correspond to the EBV nucleotide sequence coordinates.

HR1, an adherent human line constructed as a hybrid between the Detroit-98 epidermal cell line and the Burkitt's lymphoma line P3HR1; Jijoye and Raji, human cell lines derived from Burkitt's lymphoma biopsy specimens; FF41, a spontaneously productive marmoset lymphoblastoid cell line containing EBV from human patients; X50-7, a lymphoblastoid cell line made by in vitro immortalization of human B lymphocytes by EBV. These last five lines were all recently obtained from G. Miller (Yale University), in whose laboratory the X50-7 line originated. The HeLa and D98/HR1 lines were grown in minimum essential medium with 5% fetal calf serum. In addition, D98/HR1 cells were maintained in 1× MAGGT (6×10^{-7} M methotrexate, 5×10^{-5} M adenosine, 5×10^{-5} M guanosine, 1×10^{-4} M glycine, 1.6×10^{-5} M thymidine) (27). The HepG2 line was grown in Dulbecco modified Eagle medium with 10% fetal calf serum. Jijoye, Raji, FF41, and X50-7 cells were grown in RPMI 1640 medium with 10% fetal calf serum. Penicillin and streptomycin were added to all media.

Transient transfections. D98/HR1, HeLa, and HepG2 cells were transfected at 30% confluency in 10-cm dishes with a total of 18 pmol of DNA by the DEAE-dextran technique as described previously (1). The DEAE-dextran solution was removed after 30 min and the cells were treated with medium containing 0.1 mM chloroquine diphosphate. After a 12-h recovery period in normal medium, the cells were incubated in serum-free medium for 24 h with or without 1 μM dexamethasone before harvesting. Jijoye, Raji, FF41, and X50-7 cells were transfected at a cell density of 3×10^5 cells per ml with a total of 18 pmol of DNA by the DEAE-dextran technique. The DEAE-dextran solution was removed after 30 min, and the cells were incubated in normal medium for 5 h. The cells were then incubated in normal medium with or without 1 μM dexamethasone and harvested after 36 h. When indicated, Raji and FF41 cells were cotransfected with 3 pmol of the p6RGR, a plasmid which encodes the GR.

Enzyme assays. Cells were washed twice in phosphate-buffered saline, scraped from the dish in the case of D98/HR1 cells, centrifuged at 1,000 × *g*, and suspended in 150 μl of 250 mM Tris chloride (pH 7.8). Cells were lysed by three freeze-thaw cycles and then incubated at 60°C for 10 min to inactivate endogenous acetylases. The cellular debris was removed by centrifugation at 7,000 × *g* for 5 min at 4°C. Equal amounts of protein from the crude extracts were incubated at 37°C for varying time courses (1 or 2 h) in 150 μl of an assay mixture containing 250 mM Tris chloride (pH 7.8), 4 mM acetyl coenzyme A, and 0.125 μCi of [^{14}C]chloramphenicol. Reaction mixtures were extracted with ethyl acetate, spotted onto thin-layer chromatography plates, and developed in a mixture of chloroform-methanol (19:1). The acetylated and nonacetylated forms of [^{14}C]chloramphenicol were excised from the plate and quantitated by liquid scintillation counting.

Northern (RNA) blot analysis. Jijoye and X50-7 cells were seeded approximately 24 h preinduction in RPMI 1640 medium with 10% fetal calf serum. At a concentration of 3×10^5 cells per ml, cells were treated with dexamethasone for various times. The cells were harvested, and total RNA was extracted as described previously (6). Total RNA was electrophoresed on a 1% agarose–6% formaldehyde gel and

transferred to a nylon membrane (Gene Screen Plus; Dupont, NEN Research Products, Boston, Mass.). RNA blots were hybridized with a ^{32}P-labeled probe produced by random primer synthesis (13). Hybridization was done at 42°C for 15 h in 50% deionized formamide–1 M NaCl–1% sodium dodecyl sulfate–10% dextran sulfate. Membranes were washed twice in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at room temperature for 5 min, twice in 2× SSC–1% sodium dodecyl sulfate at 60°C for 30 min, and twice in 0.1× SSC at room temperature for 30 min.

Mobility shift assay. DNA probe was end labeled with [α-^{32}P]dCTP and Klenow fragment of DNA polymerase I to a specific activity of 10^7 cpm/μg. In a 15-μl reaction volume, 0.5 ng of labeled DNA probe and 3 ng of purified T7X556, a truncated protein containing the DNA-binding domain of the GR, were incubated under the following conditions: 100 μM Tris chloride (pH 7.8), 50 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, and 300 μg of bovine serum albumin per ml for 15 min at 20°C. For the competition assay, various amounts of unlabeled specific and nonspecific probe were added to each reaction 15 min before the addition of labeled probe. Specific probe was the 197-bp ES-1 DNA (Fig. 1), and nonspecific probe was the 275-bp *Hin*cII-*Bam*HI fragment of pBR322. A 4% acrylamide gel (acrylamide to bisacrylamide ratio of 80:1) was preelectrophoresed for 1 h at 20 mA in low-ionic-strength buffer (5). Electrophoresis was done at 30 mA for 3 h while recirculating the buffer. The gel was then vacuum dried and autoradiographed.

Microdensitometry. Microdensitometry readings of the autoradiographs from Northern analyses and mobility shift assays were measured on a Joyce-Loebl Mark III recording microdensitometer. The areas under the curves were determined by planimetry.

RESULTS

Selecting potential GREs. Initially, we devised a strategy using various criteria for selecting specific EBV sequences which might possess GRE-like activity. Although GRE consensus sequences are variations of the palindrome $AG^A_GACAnnnTGT^T_CCT$, the most highly conserved motif is the hexanucleotide TGT^T_CCT. Since the majority of glucocorticoid enhancers consist of a cluster of two or more consensus sequences, we selected three regions from the EBV genome which contained a cluster of hexanucleotide motifs located adjacent to promoters proved to be functional (Fig. 1).

EBV sequence 1 (ES-1) is located in the *Bam*HI C region approximately 850 bp upstream of the latent-cycle *Bam*-C promoter, BC-R2. EBV sequence 2 (ES-2) is located in the *Bam*HI H region, approximately 1,230 bp upstream of the BH-L1 promoter. EBV sequence 3 (ES-3) is located in the *Bam*HI M region, approximately 1,280 bp downstream of the BM-R2 promoter.

To determine whether these EBV sequences enhanced transcription in a glucocorticoid-dependent manner, we constructed CAT reporter plasmids with these various sequences inserted directly upstream of the herpes simplex virus thymidine kinase (HSV-TK) promoter. Figure 2A depicts a set of constructs for ES-1 and the results of CAT activity from transiently transfected D98/HR1 cells with these constructs. CAT activity was highly inducible by dexamethasone in pCTC14 (47-fold) and pCTC13 (30-fold). This demonstrated that ES-1 possesses orientation-independent, GRE-like activity. As expected, pCTGre, which contains a single GRE derived from mouse mammary tumor virus, was also inducible by dexamethasone (18-fold); however, absolute levels of induced CAT activity were higher in pCTC14 and pCTC13.

Similar experiments in transient transfections of HepG2 cells and HeLa cells also demonstrated dexamethasone-responsive activity by ES-1 (data not shown). ES-2 and ES-3 did not demonstrate induction with dexamethasone in these CAT assay experiments in any of the above cell lines (data not shown).

GRE activity of ES-1 with latent *Bam*-C promoter. Having identified a GRE in EBV, ES-1, which was active with the heterologous HSV-TK promoter, we attempted to determine whether this activity existed in the context of the homologous EBV promoter, the *Bam*-C promoter (BC-R2), 850 bp downstream. We constructed CAT reporter plasmids containing ES-1 and various components of downstream sequence which contained the *Bam*-C promoter (Fig. 3). The level of CAT activity after transient transfection of these constructs in Jijoye cells and X50-7 cells is also depicted. After dexamethasone treatment, a threefold enhancement of CAT activity in Jijoye cells and fourfold enhancement in X50-7 cells occurred with ES-1 in natural context 850 bp upstream of the *Bam*-C promoter (pCEC9).

The specific effect of glucocorticoid responsiveness was delineated further by inserting ES-1 directly upstream of the promoter region. Deletion of intervening sequences between ES-1 and the promoter regions resulted in an even greater degree (ninefold) of CAT activity induced in Jijoye cells (pCPG4). In X50-7 cells, the degree of dexamethasone induction increased to 14-fold with pCPG4; however, the absolute level of CAT activity in induced and uninduced cultures was substantially decreased compared with that with pCEC9. This phenomenon possibly suggests that a generalized enhancer or other transcriptional regulatory domain present in pCEC9 has been deleted in pCPG4.

Transient transfections of pCEC9 were done in HepG2 and D98/HR1 cells. No CAT activity could be detected, suggesting that the *Bam*-C promoter is not functional in these cell lines. Transient transfections of pCEC9 were done also in Raji cells and FF41 cells. Glucocorticoid induction of CAT activity occurred in FF41 cells, but only if cotransfected with p6RGR, a plasmid encoding the GR. In Raji cells, glucocorticoid induction of CAT activity was not observed, even when cotransfected with p6RGR (data not shown).

Glucocorticoid induction of transcription from latent *Bam*-C promoter. From the CAT assay data, we predicted that transcription of native mRNAs from the *Bam*-C promoter could be induced by dexamethasone. The specific coordinates of the first exon transcribed from the *Bam*-C promoter have been well characterized, and deduced structures of mRNAs transcribed from this promoter are presented in Fig. 4A. Therefore, we performed Northern analyses on total RNA and probed with a *Dde*I fragment of EBV corresponding to exon 1 to evaluate the level of specific mRNA expression (Fig. 4A and B). We treated cultures of Jijoye cells at the mid-log growth phase with dexamethasone at various times and extracted total RNA. With exon 1 as the probe, Northern analysis demonstrated induction of specific mRNA beginning at 6 h (Fig. 4B, lane 4). Microdensitometry readings of the autoradiograph from this Northern blot revealed a 10-fold induction of specific mRNA 15 h after dexamethasone treatment (Fig. 4B, lane 5). These results correlate with the CAT assay data and probably represent a more physiological determination of glucocorticoid-induced enhancement of transcription from the *Bam*-C promoter.

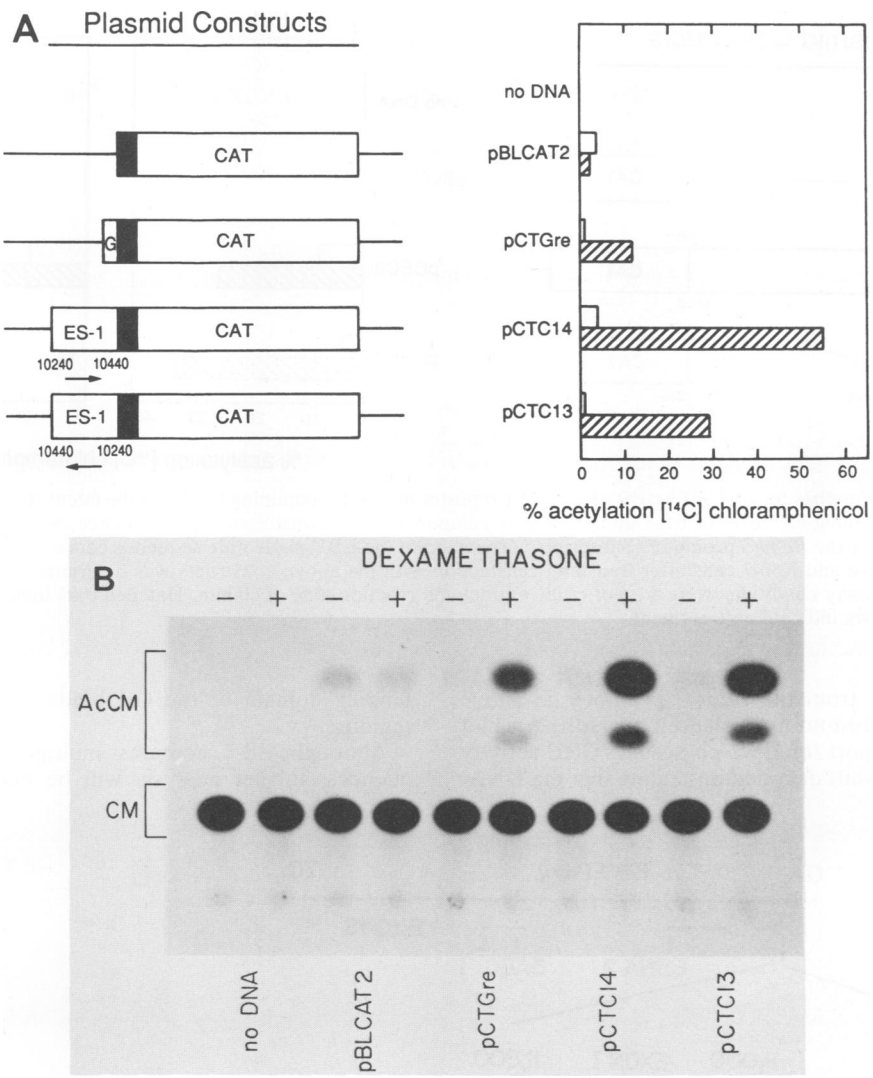

FIG. 2. Effect of dexamethasone on CAT reporter plasmids containing ES-1 and the HSV-TK promoter. (A) CAT plasmid constructs for testing ES-1 and corresponding CAT activity from crude extracts of transiently transfected D98/HR1 cells, determined as percent acetylation of [^{14}C]chloramphenicol. Numbers correspond to the EBV nucleotide sequence coordinates. The solid area indicates the HSV-TK promoter region. G refers to the single GRE from pmGTCO, originally derived from mouse mammary tumor virus. Hatched bars indicate treatment with 1 μM dexamethasone; open bars indicate no treatment. Equal amounts of crude extract were used for each assay within an individual experiment. The results presented are the mean of three separate transfections. (B) Representative example of thin-layer chromatography of a CAT assay described above. CM, chloramphenicol; AcCM, acetylated chloramphenicol.

Similar experiments were done in X50-7 cells with polyadenylated RNA, but induction of mRNA with dexamethasone could not be detected (data not shown).

Specific binding of GR to ES-1 sequence. Based on the CAT assay data, ES-1 functioned as a GRE in vivo. We hypothesized that if the enhancer function of ES-1 was acting through the GR, then this fragment should bind specifically to the GR protein. To determine whether specific binding was occurring, we performed mobility shift assays with ES-1 and the DNA-binding domain of the GR (Fig. 5). In lane 2, a shifted band migrated more slowly than the protein-free probe in lane 1 and probably represents a DNA-protein complex. To determine whether there was sequence specificity to this binding, we added either nonspecific or specific (ES-1) DNA to the binding reaction. In this competition assay (Fig. 5), approximately ninefold more nonspecific than specific probe was required to compete with the labeled ES-1 probe to equivalent levels. Microdensitometry of the autoradiographs allowed quantitation of the fraction of radioactive ES-1 sequence bound. This experiment demonstrated that ES-1 contains a nucleotide sequence(s) to which the DNA-binding domain of the GR binds specifically.

DISCUSSION

Our results demonstrated that the EBV genome contains a GRE, which we call ES-1, located between coordinates 10,240 and 10,440, approximately 850 bp upstream of the *Bam*-C promoter region. ES-1 enhanced *cat* gene expression under the control of the HSV-TK promoter in dexamethasone-treated D98/HR1 cells by as much as 47-fold. In dexamethasone-treated Jijoye and X50-7 cells, ES-1 enhanced *cat* gene expression under the control of the *Bam*-C promoter by three- to fourfold. In addition, dexamethasone

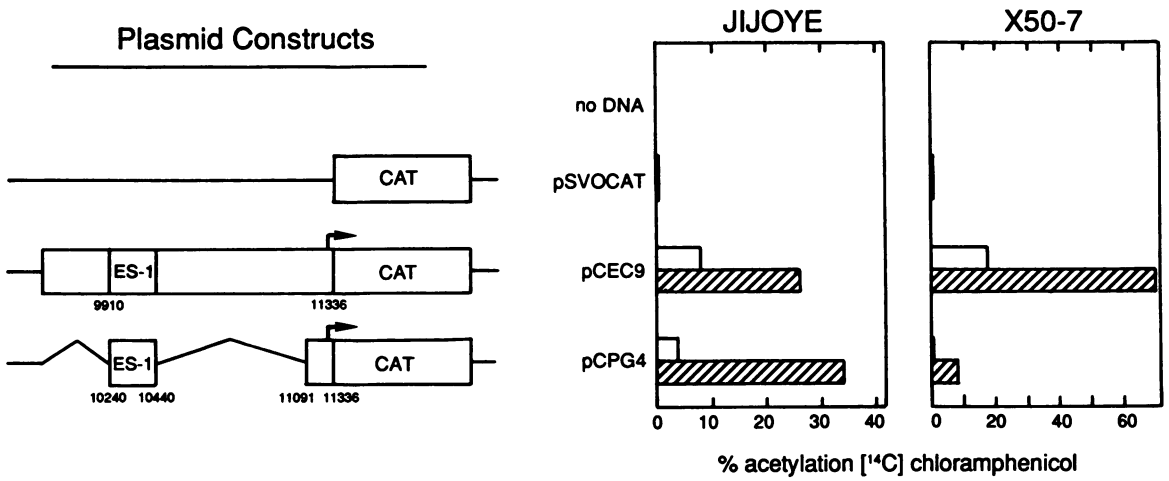

FIG. 3. Effect of dexamethasone on CAT activity from CAT reporter plasmids containing ES-1 and the latent *Bam*-C promoter. Left, CAT plasmid constructs containing elements of ES-1 and/or various components of downstream EBV sequence. ↱ represents the transcription initiation site identified for the *Bam*-C promoter. Subscripts correspond to the EBV nucleotide sequence coordinates. Right, CAT activity of crude extracts from Jijoye and X50-7 cells after transient transfection with the above constructs was determined by percent acetylation of [^{14}C]chloramphenicol. Assay conditions were 8 μg of crude extract and reaction time of 60 min. Hatched bars indicate treatment with 1 μM dexamethasone; open bars indicate no treatment.

induced transcription from the *Bam*-C promoter in Jijoye cells by as much as 10-fold as measured by Northern blot analysis. Further support for ES-1 possessing GRE activity comes from mobility shift data demonstrating that the DNA-binding domain of the GR binds specifically to the ES-1 region.

Although ES-1 contains multiple GRE consensus sequences, further analysis will be necessary to determine

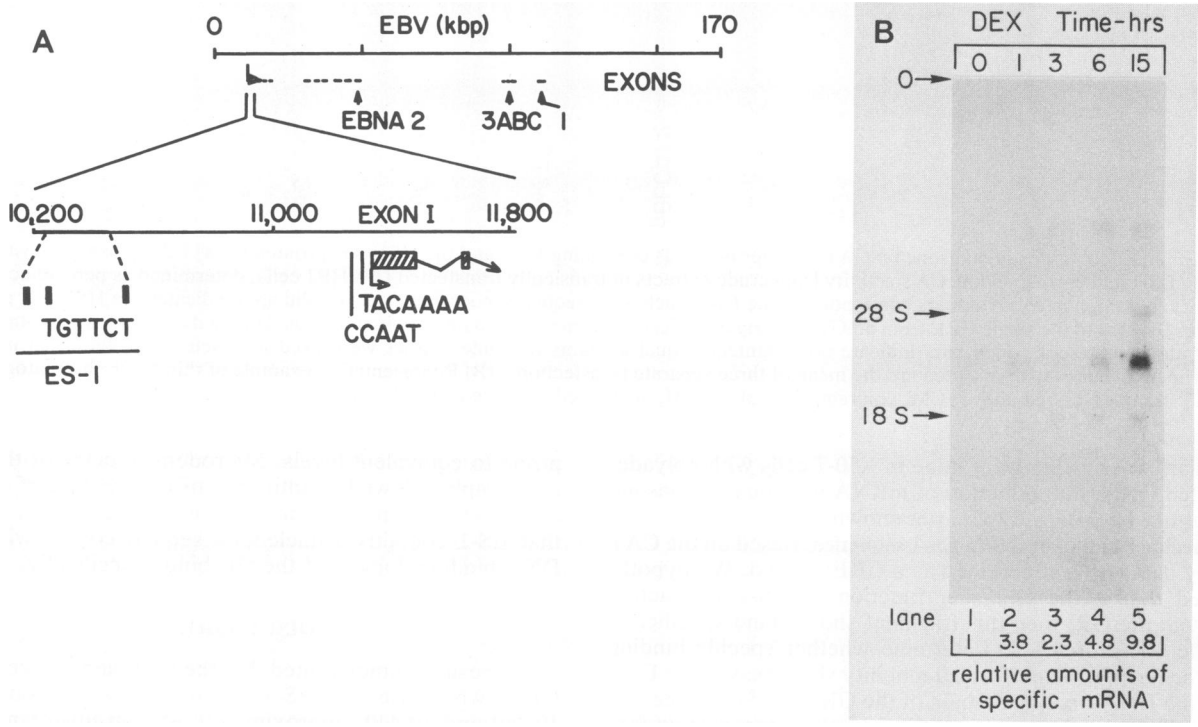

FIG. 4. Effect of dexamethasone on transcription of EBV-specific mRNA. (A) Transcription map for the *Bam*-C promoter. The locations of the exons for EBNA-1, EBNA-2, and EBNA-3A, -B, -C are shown on the complete EBV map. The expanded map shows the location of the three TGTTCT motifs of ES-1, the CAAT and TATA motifs, the RNA start site, and the first two exons. kbp, Kilobase pairs. (B) Northern blot analysis of total RNA (10 μg per lane) from Jijoye cells induced with 1 μM dexamethasone (DEX) for the indicated times. The blot was probed with the 193-bp *Dde*I fragment (EBV coordinates 11293 to 11486) corresponding to exon 1 of the highly spliced transcripts from the *Bam*-C promoter. The relative amount of specific mRNA per lane was determined by microdensitometry of the autoradiograph and standardized to the intensity of the actin mRNA hybridization on the same blots.

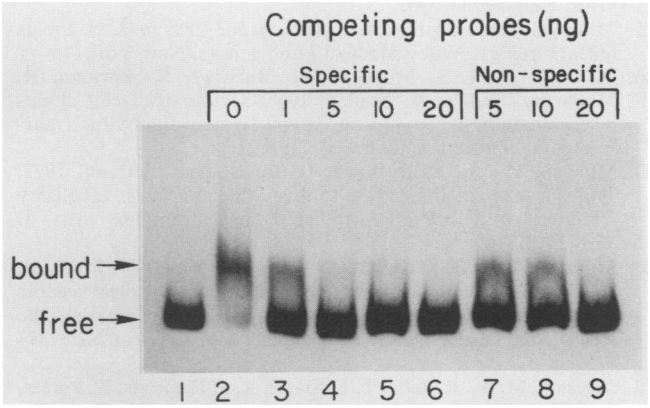

FIG. 5. Binding of GR to ES-1 sequence. Binding was detected by the shift in mobility of the DNA fragment incubated in the presence of GR protein. α-^{32}P-labeled ES-1 DNA (0.5 ng) was added to each reaction. Except for lane 1 (no protein control), 3 ng of purified T7X556 protein (DNA-binding domain peptide of the GR) was added to each reaction. The nonspecific probe is the 275-bp *Hin*cII-*Bam*HI fragment of pBR322, and the specific probe is the ES-1 DNA fragment.

whether only one or multiple consensus sequences actually possess significant GRE activity. Furthermore, our studies do not preclude the possibility of other functional GREs within the EBV genome. The lack of demonstrable glucocorticoid induction by ES-2 and ES-3 in our experiments does not eliminate the possibility of GRE function of these sequences in vivo. The construction of these respective reporter plasmids may have deleted promoter or enhancer elements that are necessary for GRE activity. Also, our inclusion criteria for screening potential GREs may have been too stringent (for example, regions containing single GRE motifs were not selected).

Other *cis*- or *trans*-acting factors also may be critical for glucocorticoid-induced enhancement of gene expression by ES-1. This possibility is suggested by the fact that in transient transfections of pCEC9 into Raji cells, glucocorticoid induction of *cat* gene expression does not occur. Although other transcription factors besides the GR are not absolutely required for glucocorticoid induction, a synergistic increase of promoter activity by GREs in combination with other transcription factor-binding sites has been demonstrated in vitro (30). EBNA-1 can act as a transcription factor for the latent-cycle *Bam*-C promoter (32); however, in the present experiments, this effect was probably not important because EBNA-1 activity appears to require *oriP* in *cis*.

In addition, it has been shown that specificity of some hormone receptor elements is not highly stringent and that these elements can mediate induction by several hormones, including progestins, androgens, and glucocorticoids (9, 37). It is possible that ES-1 modulates transcription through other hormone receptors; however, additional experiments will be required to determine this.

Most of the efforts toward understanding EBV immortalization have concentrated on the roles of latently expressed EBV proteins. Among the EBNA family of proteins, EBNA-1 is required in *trans* to mediate replication of viral plasmids in EBV-immortalized cells (20, 39), and EBNA-2 may be involved in stimulating proliferation of these cells (31). The function of ENBAs 3 to 6 in latency is unknown. The latent-cycle *Bam*-C promoter (4) may be essential to the immortalization process since transcripts of at least two latently expressed proteins, EBNA-1 and EBNA-3, are transcribed from this promoter. Since, as we have shown, glucocorticoids enhance transcription from the *Bam*-C promoter, one could speculate that glucocorticoids, likewise, enhance expression of one or several species of EBNA proteins. If this proves to be true, then glucocorticoids may be intimately involved in the regulation of EBV immortalization.

One possible scenario for physiological EBNA expression based on this novel GRE could be that glucocorticoids induce increased expression of EBNA-1, resulting in tighter viral latency. This hypothesis, however, is contradictory to evidence that cyclosporin A-glucocorticoid-induced immunosuppression results in an increased incidence of EBV reactivation and EBV-associated lymphoproliferative disorders (7, 29). Another possibility could be that glucocorticoids enhance transcription from the *Bam*-C promoter in such a way that a different pattern of EBNA proteins is expressed, contributing to a disruption of viral latency. An altered pattern of EBNA expression may affect the cytotoxic-T-cell response to EBV-transformed cells, thus impairing immunosurveillance (18, 26, 28, 36). A glucocorticoid-induced alteration of EBNA expression and glucocorticoid-induced immunosuppression may be important factors in the etiology of EBV-induced lymphoproliferative lesions. Further studies to examine the effects of glucocorticoids on EBNA protein expression and viral replication will be required to determine whether these relationships are plausible.

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