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A Promoter of Epstein-Barr Virus That Can Function during Latent Infection Can Be Transactivated by EBNA-1, a Viral Protein Required for Viral DNA Replication during Latent Infection

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A viral promoter that functions on recombinant plasmids in cells immortalized by Epstein-Barr virus was identified and characterized. It is identical to that mapped on the viral genome by Bodescot et al. (M. Bodescot, M. Perricaudet, and P. J. Farrell, J. Virol. 61:3424–3430, 1987) which functions during the latent phase of the viral life cycle in some but not all cells to encode several latent viral gene products. Experiments with these plasmids indicated that this promoter requires the enhancer within *ori*P of Epstein-Barr virus in *cis* to function efficiently. They also indicated that it requires the EBNA-1 gene in *trans* to function efficiently. The EBNA-1 gene therefore positively affects both viral DNA replication (J. L. Yates, N. Warren, and B. Sugden, Nature [London] 313:812–815, 1985) and viral transcription.

The herpesvirus Epstein-Barr virus (EBV) infects and immortalizes human B lymphocytes in culture (15). In the latently infected, immortalized cells, only 10% of the viral open reading frames (ORFs) have been found to be expressed detectably as RNAs which serve as messengers for translation (11). Some or all of the products of these viral genes are likely to be involved in the maintenance of the immortalized state. What regulates the expression of these viral genes during latent infection is not now known.

The EBV genome is usually maintained as multiple copies of a complete plasmid in latently infected cells. The only *cis*-acting element of EBV required for plasmid DNA replication is *ori*P, which is composed of two components, both of which are required for replication per se (19, 24). One of these components, a family of repeated sequences (20 members of 30 base pairs [bp] each), acts as a transcriptional enhancer for heterologous promoters (18). The EBNA-1 protein which is encoded by the BKRF-1 gene of EBV is required in *trans* for this transcriptional enhancer (18). It is also required in *trans* to support DNA replication mediated by *ori*P (13, 25). EBNA-1 is therefore a viral protein which can mediate viral DNA synthesis and potentially can affect transcription of viral DNA.

We have sought viral promoters which function during latent infection and which are transactivated by EBNA-1. cDNAs identified and characterized by Bodescot and colleagues (3, 4) indicate that some transcripts encoding EBNA-1 and EBNA-3, another viral protein expressed in latently infected cells, originate at a site near position 11305 on the viral genome. Bodescot et al. (5) have shown that this promoter functions in several EBV-transformed cell lines. We constructed a plasmid which contained this promoter embedded in the contiguous viral DNA sequences from bp 7300 to 11412 (1). This plasmid therefore included both *ori*P and the TACAAAA sequence at position 11305; it also contained a *cat* reporter gene downstream of these elements. Experiments with this plasmid and deleted variants derived from it indicated that (i) the sequences around position 11305 act as a promoter in the plasmid; (ii) this promoter depends on the family of 30-bp repeats in *cis* for its efficient expression; and (iii) it is also dependent on the EBNA-1 protein in *trans* for its efficient expression. Because some transcripts originating from this promoter in the viral genome encode EBNA-1 and EBNA-1 is required for efficient expression of this promoter, we suggest that EBNA-1 can positively affect its own synthesis.

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MATERIALS AND METHODS

Cells and electroporation. Lymphoblastoid cells were maintained as exponentially growing cultures in RPMI 1640 medium plus 10% fetal bovine serum and antibiotics. The origins of the cells used have been described previously (10, 18, 23), except for line 2783 (or GM2783), which was obtained from the mutant cell repository in Camden, N.J. Cells were electroporated with 10 μg of DNA in 0.5 ml of complete medium at densities of 0.5×10^7 to 1.5×10^7 cells per 0.5 ml under conditions described previously (12).

DNA constructions. Recombinant DNAs were made by standard methods. *ori*PBamCpCAT (Fig. 1) was derived from pCΔJ (24) by inserting the *Hin*dIII-to-*Bam*HI fragment from pSV2CAT containing the *cat* structural gene (8) between the *Sfi*I site (position 11412 of EBV) and the end of the *Bam*HI C fragment (position 13215 of EBV). The non-EBV sequences in the parental vector pCΔJ consist of the bacterial gene encoding resistance to ampicillin from pBR322, the ColE1 origin from pBR322, and the bacterial gene encoding resistance to hygromycin B expressed from the herpes simplex viral thymidine kinase promoter and the thymidine kinase polyadenylation signal (24). *ori*PΔBamCpCAT (Fig. 1) was made by deleting the 324-bp fragment between the *Sac*I site (position 11088 of EBV) and the *Sfi*I site (position 11412 of EBV) from *ori*PBamCpCAT. This deletion removed both the CAAT and TACAAAA sequences from this promoter. Δ*ori*PBamCpCAT (Fig. 1) was derived by deleting from *Sac*I (position 6285 of EBV) to *Sac*I (position 11088 of EBV) in pBamC (24). The *cat* structural gene was then introduced between the *Sfi*I and *Bam*HI sites as it was for *ori*PBamCpCAT. All of *ori*P (positions 7337 to 9134 of EBV) was therefore removed in Δ*ori*PBamCpCAT. DSBamCp CAT (Fig. 1) was constructed by inserting a dyad symmetry element, DS (positions 8994 to 9134 of EBV), into the *Sac*I site of Δ*ori*PBamCpCAT. FRBamCpCAT (Fig. 1) was con-

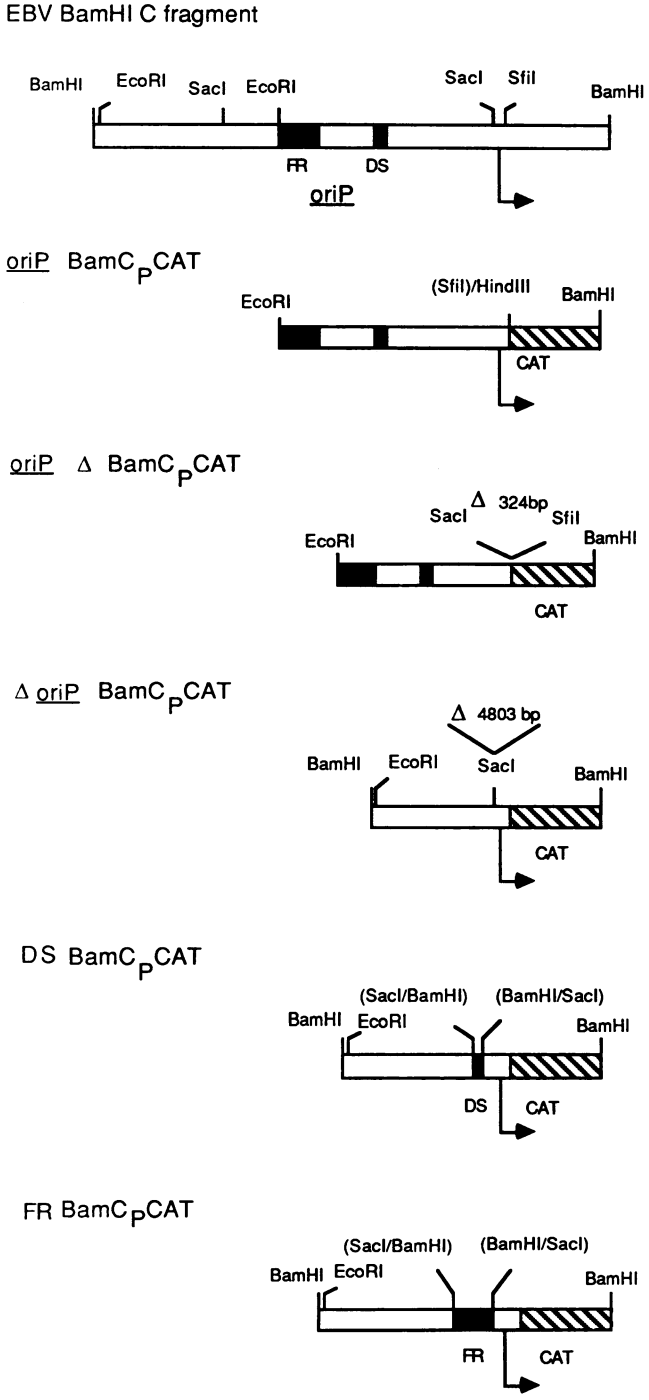

FIG. 1. Schematic diagrams of the DNA in the EBV *Bam*HI C fragment and *cat* expression inserts constructed from it. At the top of the figure is shown the *Bam*HI C fragment (nucleotides 3994 to 13215 of EBV) (1). The open boxes represent EBV DNA, and the solid boxes represent the two components of *ori*P, the putative plasmid origin of replication of EBV. The black box denoted FR represents the family of 30-bp repeats and that denoted DS represents the dyad symmetry element (see reference 19 for a dissection of *ori*P). The arrow just upstream of the *Sfi*I site represents the site of the promoter identified by Bodescot et al. (5) and studied here. The *cat* structural gene (shown as a hatched box) derived from SV2CAT by digestion with *Hin*dIII and *Bam*HI was cloned between the *Sfi*I and *Bam*HI sites as shown for *ori*PBamCpCAT. The five *cat* expression inserts were introduced into pkan2 (24) between the *Eco*RI site at position 1 and the unique *Bam*HI site or into the unique *Bam*HI site. The pkan2 sequences are not depicted in this figure.

structed similarly by inserting the family of 30-bp repeats, FR (positions 7337 to 8190 of EBV), into the *Sac*I site of Δ*ori*PBamCpCAT. CMVpEBNA-1 was made by first introducing the immediate-early promoter-enhancer of cytomegalovirus derived from pwt760CAT (21) on a 760-bp *Bam*HI-to-*Hin*dIII fragment into pHEBo (22) between the *Cla*I and *Hin*dIII sites. The structural gene for EBNA-1 (BKRF-1 ORF) was then introduced on a *Hin*dIII fragment (positions 107945 to 110493 of EBV) at the *Hin*dIII site downstream of the cytomegalovirus promoter-enhancer. *ori*PBamCpEBNA-1 was made by substituting the structural gene for EBNA-1 for the *cat* gene in *ori*PBamCpCAT. A *Hin*dIII-to-*Pvu*II fragment (positions 107945 to 110176 of EBV) containing the BKRF-1 ORF was substituted for the *cat* structural gene between the *Sfi*I and *Bam*HI sites of *ori*PBamCpCAT.

CAT assays. Chloramphenicol acetyltransferase (CAT) assays were performed as described previously (8, 18) 72 h after the cells were electroporated, except that each set of assays included a reconstruction assay of 1×10^3 to 100×10^{-3} U of purified enzyme. The measurements of activity of the cell extracts were then converted to units of activity by comparing the results of the unknowns with those of the standards. The units of the purified enzyme used were those measured by the supplier (Pharmacia, Inc., Piscataway, N.J.). One unit of activity results in the acetylation of 1 nmol of substrate in 1 min at 30°C.

Primer extension. Whole-cell RNAs were purified by the method of Chirgwin et al. (6). The primer used to analyze RNA by reverse transcription was a 32-base oligonucleotide complementary to CAT RNA beginning with the first base upstream of the AUG in pSV2CAT. The primer was labeled with [^{32}P]ATP and T4 polynucleotide kinase and used as described previously (14). The extended products were resolved electrophoretically on an 8% polyacrylamide–8 M urea gel and detected by autoradiography.

Western blotting. Western blotting (immunoblotting) was performed as described previously (2). An affinity-purified antibody raised against a purified *cro*–EBNA-1–β-galactosidase immunogen was used to detect EBNA-1.

RESULTS

Identification of promoter active in latent infection within *Bam*HI C fragment of EBV. The plasmid *ori*PBamCpCAT contains the contiguous EBV DNA sequences from nucleotides 7300 to 11412. The structural gene for CAT is jointed at position 11412, which lies within exon 1 of the cDNAs characterized by Bodescot and Perricaudet (4). This plasmid was introduced into a variety of EBV-positive cell lines, including clones immortalized in vitro and lymphoid lines established from Burkitt's lymphomas. In each of these lines, a significant level of CAT activity was expressed from the plasmid (Table 1). To identify tentatively the promoter yielding CAT expression, we introduced a deletion mutant, *ori*PΔBamCpCAT, which removes only the EBV DNA sequences between 11089 and 11412, into the same set of EBV-positive cell lines. The level of CAT activity induced in the EBV-positive cell lines by *ori*PΔBamCpCAT was at background (Table 1). A *cis*-acting element required for expression of CAT activity therefore lies within the 323 bp

TABLE 1. A 324-bp deletion tentatively identifies a promoter with the *Bam*HI C fragment

Electroporated plasmid	CAT activity (normalized) measured in 10^7 EBV-positive recipient cells[a]:			
	721	11/17-5	2783	GG68
SV2CAT	1	1	1	1
A10CAT	0.4	0.1	0.002	0.03
*ori*PBamCpCAT	2	0.7	0.2	5
*ori*PΔBamCpCAT	0.2	0.1	0.002	0.006

[a] Each electroporation of each cell line was repeated twice. The CAT activities were averaged, and then the values were normalized to those found for SV2CAT. The plasmid A10CAT lacks the 72-bp enhancer of simian virus 40 and serves as a negative control. The average units for SV2CAT per 10^7 cells were: for 721, 3×10^{-3} U; for 11/17-5, 6×10^{-3} U; for 2783, 290×10^{-3} U; for GG68, 120×10^{-3} U.

deleted in *ori*PΔBAMCpCAT. To determine whether these deleted sequences contain a promoter, we identified the 5′ end of the CAT RNA by primer extension (14). The plasmid *ori*PBamCpCAT, which encodes resistance to G418, was introduced stably into three EBV-positive lymphoblastoid cell lines by selecting the cell lines for resistance to G418. Total RNAs were analyzed from pools of these resistant cells, which now maintained the input DNA as a plasmid and expressed CAT activity constitutively. The 5′ end of the CAT RNA mapped to position 11336±3 nucleotides (Fig. 2). This finding defines the promoter for the CAT RNA expressed from *ori*PBamCpCAT constitutively and shows that it is, within experimental error, identical to that found to function on the complete viral genome (5). In addition, the levels of signals found in the experiment with primer extension correlated with the different levels of CAT activity measured in the three pools of resistant cells. This correlation indicates that the expression of RNA from this promoter is paralleled by the CAT enzyme it encodes, the activity of which is measured in our experiments.

The work of Bodescot et al. (5) demonstrated that expression of RNA from this promoter at 11,336±3 nucleotides in the viral genome was not enhanced when cells were treated with 12-*O*-tetradecanoylphorbol-13-acetate (TPA). TPA induces EBV-positive cells to support the expression of the early and late genes of EBV (26). EBV promoters whose function is not affected by exposure of a host cell to TPA are termed latent promoters. To test whether the promoter in *ori*PBamCpCAT was regulated as a latent promoter, we introduced this plasmid into four EBV-positive cell lines, Raji, 721, 2783, and GG68. The cells were divided into two aliquots, and one aliquot was treated with TPA. In one of the recipient cell lines, GG68, 15 to 30% of the cells express late gene products 72 h after exposure to TPA (10). After 72 h, CAT activity was measured and found not to vary whether or not the cells had been treated with TPA (data not shown). This promoter within the context of the plasmid is thus also regulated as a latent promoter.

Family of 30-bp repeats in *cis* and EBNA-1 in *trans* increase expression from latent promoter in *Bam*HI C fragment of EBV. A series of plasmids was constructed to test whether the family of 30-bp repeats affected the expression of the latent promoter in *Bam*HI-C. In one plasmid, Δ*ori*PBam CpCAT, all of *ori*P including the family of 30-bp repeats and the dyad symmetry was deleted. The dyad symmetry element (DS) was inserted back into ΔoriPBamCpCAT to form DSBamCpCAT, and the family of 30-bp repeats (FR) was also inserted into the deletion to form FRBamCpCAT. Each of these plasmids was introduced into the EBV-positive Raji cells in three independent experiments and tested for its CAT expression (Table 2). These experiments indicated that deleting *ori*P reduced CAT expression from the plasmid to close to background levels; reintroduction of the dyad symmetry element into the deleted plasmid did not increase its expression detectably, whereas reintroduction of the family of 30-bp repeats increased its CAT expression to 25% of the wild-type level (Table 2). This increase indicates that the family of 30-bp repeats can function in *cis* to increase expression from the latent promoter in *Bam*HI-C. The family of 30-bp repeats has also been found to enhance the herpes simplex viral *tk* promoter and the simian virus 40 early

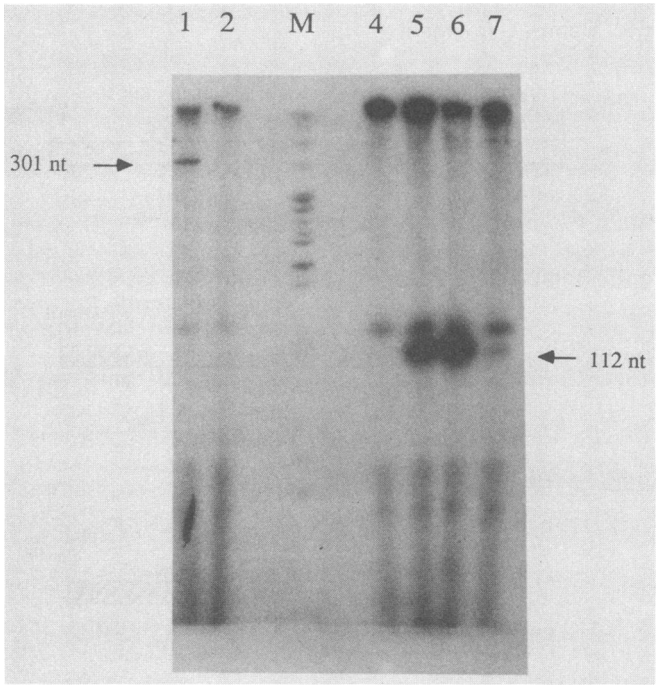

FIG. 2. Autoradiogram from an analysis of CAT RNAs by primer extension. Total cellular RNAs were hybridized to 3 ng of a ^{32}P-end-labeled primer, the primer was extended with avian myeloblastosis virus reverse transcriptase, the template RNA was destroyed with alkali, and the extended products were separated by electrophoresis on an 8% acrylamide–8 M urea gel. The products were detected by autoradiography at −70°C for 14 h. Lane 1 contains the products from 10 μg of cellular RNA isolated from CV-1 cells transfected with an RSVpCAT vector and serves as a positive control yielding an extension product of 301 nucleotides (nt). Lane 2 contains the products from 10 μg of CV-1 cellular RNA alone. Lane M contains end-labeled fragments of pBR322 DNA derived by digestion with *Msp*II which serve as markers and which were readily identified on 36-h exposure (not shown). Lanes 4, 5, 6, and 7 contain the products from 25 μg of cellular RNA from BL60 cells, which are EBV-positive B lymphoblasts, from 721 cells carrying *ori*PBamCpCAT, from Raji cells carrying *ori*PBamCpCAT, and from GG68 cells carrying *ori*PBamCpCAT, respectively. These latter three cell lines are EBV positive and G418^r. The CAT activity assayed in extracts of 10^6 cells carrying *ori*PBamCpCAT was, in increasing order, GG68 < 721 < Raji. This order differs from that found for CAT activity measured 72 h after electroporation of these three cell lines with *ori*PBamCpCAT (Table 2). The level of CAT activity 72 h after electroporation reflects in part the efficiency of electroporation, while the CAT activity measured in the G418^r cells reflects the steady-state efficiency of the *Bam*HI-C latent promoter. The band identified at 112 nucleotides is derived from the extension of the primer and maps the 5′ end of the CAT RNA in *ori*PBamCpCAT to position 11336 ± 3 on the EBV genome.

TABLE 2. Family of 30-bp repeats enhances expression from the latent *Bam*HI C promoter

Electroporated plasmid	CAT activity (normalized) in 10^7 EBV-positive Raji cells[a]
SV2CAT	1
A10CAT	0.02
*ori*PBamCpCAT	1.3
Δ*ori*PBamCpCAT	0.06
DS*ori*PBamCpCAT	0.06
FR*ori*PBamCpCAT	0.3

[a] Raji cells were electroporated with each of the listed plasmids on three separate occasions. CAT activities were measured from extracts of 10^7 of these cells, averaged, and normalized to those of SV2CAT. The average value for SV2CAT was 60×10^{-3} U.

promoter in the presence of EBNA-1 in a variety of cells (18, 19). It is likely that the family of 30-bp repeats does not restore 100% of the CAT activity of *ori*PBamCpCAT in Raji cells because the latter plasmid replicates in Raji cells while FRBamCpCAT does not. The presence of the dyad symmetry element is absolutely required for transient replication of *ori*P vectors (19). Replication of a plasmid in the recipient cells would be expected to increase both its average copy number in the population of cells and its stability.

The plasmid *ori*PBamCpCAT was introduced into two EBV-negative lymphoid cell lines established from Burkitt's lymphomas and into a strain of human fibroblasts. Little or no CAT activity was expressed from this plasmid in these EBV-negative cell lines (Table 3). A plasmid encoding the EBNA-1 protein was introduced along with *ori*BamCpCAT into the same EBV-negative recipients, which then expressed significant levels of CAT activity (Table 3). That this CAT activity was expressed from the latent promoter within the *Bam*HI C fragment is demonstrated by the deletion variant, *ori*PΔBamCpCAT, which failed to express CAT activity in the parallel experiment (Table 3). These observations indicate that the BKRF-1 gene which encodes EBNA-1 is required in *trans* for the latent promoter within the *Bam*HI C fragment of EBV to be expressed efficiently. They also indicate that this promoter functions not only in human B-lymphoid cell lines but also in a human fibroblast strain.

The above findings indicate that the EBNA-1 protein can positively regulate a latent promoter within the *Bam*HI C fragment of EBV. The work of Bodescot and colleagues (3, 4) indicates that some transcripts initiating from this promoter encode EBNA-1. We asked whether or not a construction with the BKRF-1 ORF downstream of this promoter would express EBNA-1 in the absence of another source of EBNA-1. A recombinant DNA with EBNA-1 expressed from the latent *Bam*HI-C promoter (*ori*PBamCpEBNA-1) was first introduced into Raji cells, and G418^r cells were selected and analyzed for the expression of EBNA-1. The EBNA-1 protein expressed by Raji cells migrates more rapidly than that encoded by *ori*PBamCpEBNA-1, so that it could be readily established that the introduced construction did express EBNA-1 in Raji cells, which themselves provide EBNA-1 in *trans* (Fig. 3). No EBNA-1 could be detected in EBV-negative cells into which *ori*PBamCpEBNA-1 was introduced (data not shown). An assay for EBNA-1 which is more sensitive than its detection by Western blotting is to screen for its transactivation of the enhancer within *ori*P. However, when *ori*PBamCpEBNA-1 was coelectroporated into EBV-negative cells (Wilson) along with *ori*PBamCpCAT, no CAT activity was detected (Table 4). At the level of detection of our assays, *ori*PBamCpEBNA-1 requires EBNA-1 in *trans* to express EBNA-1 in *cis*.

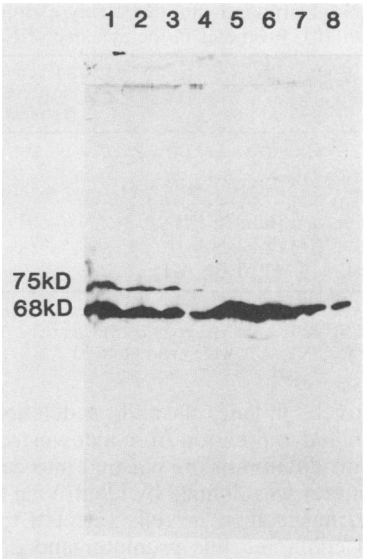

FIG. 3. Western blot detecting the EBNA-1 protein in Raji cells carrying *ori*PBamCpEBNA-1 and in Raji cells alone. Extracts of 8×10^6, 4×10^6, 2×10^6, and 1×10^6 Raji cells plus *ori*PBamCpEBNA-1 (lanes 1 to 4) and of 8×10^6, 4×10^6, 2×10^6, and 1×10^6 Raji cells (lanes 5 to 8) were separated by electrophoresis in a sodium dodecyl sulfate-containing 8% polyacrylamide gel. Proteins were transferred electrophoretically to nitrocellulose and detected with an immunoaffinity-purified anti-EBNA-1 antibody. Prestained marker proteins were included in the gel. The faster-migrating band represents the EBNA-1 protein (approximately 68 kilodaltons [kD]) endogenous to Raji cells which is known to migrate more rapidly than the EBNA-1 protein (approximately 75 kilodaltons) in B958 cells (9). The slower-migrating band represents the EBNA-1 protein encoded by *ori*P BamCpEBNA-1. The DNA encoding EBNA-1 in this vector is derived from the B958 strain of EBV.

TABLE 3. EBNA-1 transactivates the latent *Bam*HI C promoter in EBV-negative cells

Electroporated plasmid	CAT activity (normalized) in recipient cells[a]:		
	Ramos	Wilson	Human fibroblasts
SV2CAT	1	1	1
A10CAT	0.04	0.006	ND[b]
*ori*PBamCpCAT	0.04	0.02	0.04
*ori*PBamCpCAT + CMVpEBNA-1	0.3	0.4	1.3
*ori*PΔBamCpCAT + CMVpEBNA-1	0.04	0.004	ND

[a] Ramos and Wilson cells were electroporated twice and human fibroblasts once with each of the listed plasmids, and 72 h later, extracts of 10^7 Ramos cells, 10^7 Wilson cells, and 10^6 human fibroblasts were prepared and assayed for CAT activity. The values for Ramos and Wilson cells were each averaged. The values for each cell line were normalized to that for SV2CAT. The value for SV2CAT for 10^7 Ramos cells was 12×10^{-3} U, for 10^7 Wilson cells it was 80×10^{-3} U, and for 10^6 human fibroblasts it was 9×10^{-3} U.

[b] ND, Not determined.

DISCUSSION

We showed that a promoter whose TATAA consensus sequence (TACAAAA) lies at position 11305 within the *Bam*HI C fragment of EBV (1) functions on recombinant vectors in cells latently infected by EBV. This promoter was

TABLE 4. EBNA-1 protein is not detectably expressed from the latent *Bam*HI C promoter in the absence of EBNA-1

Electroporated plasmid	CAT activity (normalized) in 10^7 EBV-negative recipient Wilson cells[a]
SV2CAT	1
A10CAT	0.02
*ori*PBamCpCAT	0.04
*ori*PBamCpCAT + *ori*PBamCp EBNA-1	0.02
*ori*PBamCpCAT + CMVpEBNA-1	0.3
*ori*PΔBamCpCAT + CMVpEBNA-1	0.02

[a] Wilson cells were electroporated with each of the listed plasmids, and 72 h later, extracts of 10^7 cells were assayed for CAT activity. The values were normalized to that for SV2CAT, which was 80×10^{-3} U.

mapped tentatively in one plasmid; a deletion which removed it eliminated expression from a downstream *cat* gene shortly after introduction of the plasmid into cells (Tables 1 to 4). The promoter was defined by identifying the 5′ end of a transcript arising from it in cells selected to maintain a plasmid which contained this promoter and a downstream *cat* gene (Fig. 2). The results of the experiments presented in Table 2 indicated that deleting *ori*P and DNA sequences between *ori*P and a site close to this latent promoter in *Bam*HI-C reduces expression from this promoter. The addition of the family of 30-bp repeats (FR) to this deletion restored 25% of the expression from this promoter (Table 2). These findings are consistent with the notion that the family of 30-bp repeats serves as a transcriptional enhancer for the latent promoter within *Bam*HI-C but do not prove it. The deletion and rearrangement of DNA sequences in the vector FRBamCpCAT could also affect expression from this promoter. The results of the experiments presented in Table 3 support the notion that the family of 30-bp repeats, while in the wild-type context within the BamHI C DNA fragment, do enhance the downstream latent promoter. They show that the EBNA-1 protein is required in *trans* for detectable expression of CAT activity from the vector *ori*PBamCpCAT. The EBNA-1 protein binds specifically only to the family of 30-bp repeats and to the dyad sequence element within this vector (17), and of these two elements, only the family of 30-bp repeats acts detectably as a transcriptional enhancer for other promoters (18).

Bodescot and colleagues (3–5) have shown that this latent *Bam*HI-C promoter functions in a variety of EBV-transformed cells and can yield transcripts which encode EBNA-1. These findings together with those presented above indicate that EBNA-1 can positively affect its own synthesis, at least when it is expressed from this promoter within the *Bam*HI C fragment of EBV. This interpretation is also supported by the observation that EBNA-1 is not expressed detectably from this promoter in the absence of exogenous EBNA-1 (Fig. 3; Table 4).

This interpretation raises two questions. First, how is EBNA-1 expressed when EBV infects a resting B lymphocyte? It appears unlikely that the *Bam*HI-C promoter will function in the absence of EBNA-1 to express EBNA-1 (Table 4), so that either the EBV virion will bring EBNA-1 into the cell to activate the *Bam*HI-C promoter or a different promoter will be used in the lymphocyte to express EBNA-1. It is now practical to address this question by measuring the amount of EBNA-1 in virus particles. If EBV virions are found not to contain EBNA-1, then the latent promoter found within the *Bam*HI W fragment is another candidate for initiating the synthesis of EBNA-1 in newly infected lymphocytes (20). Other as yet unidentified viral promoters could also support expression of EBNA-1 in newly infected cells.

Second, what limits the expression of EBNA-1? EBNA-1 is required for the replication of its own gene and is likely to affect its own synthesis at the level of transcription. These two ideas might suggest that the EBV replicon is ripe for an unregulated, explosive amplification. But both EBV DNA replication and the expression of EBNA-1 are stable in recently established cell lines (unpublished observations). It seems likely that there are as yet unidentified modes of regulating the expression of EBNA-1 which act to limit its synthesis either transcriptionally or posttranscriptionally.

The interpretation that EBNA-1 can positively affect its own synthesis provides an intricate example in which the product of one gene affects both transcription and DNA replication in eucaryotic cells. It has become clear that *cis*-acting sites which can affect transcription can form parts of eucaryotic origins of replication (7). It now also appears that single proteins can participate in both the replication and the transcription of DNA. For example, the octamer transcription factor-1 (OTF-1) which is required for efficient transcription in vitro from a histone promoter is also one of the nuclear factors (NF-III) which binds specifically to the origin of DNA replication of adenovirus. It is required for efficient adenoviral DNA replication in vitro (16). As with OTF-1, EBNA-1 binds site specifically to an origin of replication (*ori*P of EBV) and to a transcriptional enhancer (the family of 30-bp repeats of EBV) (17). What is intricate about the dual role of EBNA-1 in transcription and DNA replication is that its effect on transcription within the EBV genome apparently can affect its own synthesis.

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