The Epstein–Barr virus ZEBRA protein activates transcription from the early lytic F promoter by binding to a promoter-proximal AP-1-like site

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The ZEBRA protein encoded by the Epstein–Barr virus (EBV) genome activates a switch from the latent to the lytic gene expression programme of the virus. ZEBRA, a member of the basic leucine zipper family of DNA-binding proteins, is a transcriptional activator capable of inducing expression from several virus lytic cycle promoters by binding to activator protein 1 (AP-1)-like sites. The Epstein–Barr virus BamHI F promoter, Fp, was for some time believed to initiate EBNA1-specific transcription in EBV-transformed latent cells. More recent data, however, show that Fp is an early lytic promoter and that the dominant EBNA1 gene promoter in latent cells is Qp, located about 200 bp downstream of Fp. In the present investigation we confirm that Fp displays the characteristics of a lytic promoter. Fp is downregulated in latently EBV-infected cells, both in the endogenous virus genome and in reporter plasmids that carry Fp regulatory sequences upstream of position −136 and down to +10 relative to the Fp transcription start site (+1), and is activated on induction of the virus lytic cycle. We show that the repression of Fp in latent stages of infection can be abolished by ZEBRA, and demonstrate that ZEBRA activates Fp through a direct interaction with an AP-1-like site at position −52/−46 in the promoter-proximal Fp region.

Introduction

Epstein–Barr virus (EBV) establishes a latent infection in human B lymphocytes, efficiently transforming the cells into lymphoblastoid cell lines (LCLs), and is implicated in the aetiology of infectious mononucleosis, Burkitt’s lymphoma (BL), Hodgkin’s disease, nasopharyngeal carcinoma (NPC) and lymphoproliferative diseases in immunocompromised individuals (Kieff & Rickinson, 2001). The virus is capable of adopting four programmes of latency (latency 0, I, II and III). In healthy individuals, latent EBV infection appears to be primarily confined to resting memory B cells (Babcock et al., 1998). The only EBV gene product that is consistently detected in these cells is the latent membrane protein LMP2A, a pattern of gene expression at present termed latency 0 (Miyashita et al., 1995, 1997; Thorley-Lawson et al., 1996). In Burkitt’s lymphoma biopsies, only Epstein–Barr virus nuclear antigen 1 (EBNA1) is expressed (latency I) (Rickinson & Kieff, 2001; Thorley-Lawson et al., 1996). In Hodgkin’s disease, NPC and T cell lymphomas, EBNA1 and variable combinations of the three members of the latent membrane protein family (LMP1, LMP2A and LMP2B) are expressed (latency II) (Rickinson & Kieff, 2001; Thorley-Lawson et al., 1996). During acute infectious mononucleosis, in lymphoproliferative syndromes in immunocompromised individuals and in LCLs, all six nuclear antigens (EBNA1–6) are expressed (latency III) (Bodescot et al., 1987). In addition, all three LMPs are expressed (Hudson et al., 1985). EBV-infected cell lines are either completely non-productive for virus particles or else contain a small subpopulation of cells that have switched spontaneously from a latent stage of infection into the lytic cycle. The mechanism of switching is not fully understood, but one of the first detectable changes in viral gene expression is activation of

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the EBV immediate-early gene BZLF1, which encodes the lytic switch transactivator ZEBRA, a member of the basic leucine zipper family of DNA-binding proteins (Flemington et al., 1991). ZEBRA, together with the protein product of the BRLF1 gene, then initiates the lytic cycle cascade (Biggin 1991). ZEBRA, together with the protein product of the BRLF1 switch transactivator ZEBRA, a member of the basic leucine zipper family, activates transcription from Fp by binding to a promoter-proximal AP-1-like site.

Methods

**Plasmid constructions.** All constructs made were verified by dye-exchange sequencing (Sanger et al., 1977) using the ABI PRISM Big Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems). The Fp transcription initiation site at position 62239 of strain B95-8 EBV DNA (Baer et al., 1984), Fp, was numbered as +1 (Bulfone–Paus et al., 1995). The series of chloramphenicol acetyltransferase (CAT) reporter plasmids with different 5′ deletions of Fp was constructed as follows. The NheI–BamHI (∆F1), XhoI–BamHI (∆F2), Eco52I–BamHI (∆F3), NarI–BamHI (∆F4) and MluI–BamHI (∆F5) subfragments of the BamHI F fragment of the EBV genome were cloned in the HindIII site of pSVECAT (obtained from M. Yaniv, Institute Pasteur, Paris) replacing the SV40 early control region. The plasmids were named p∆F1CAT, p∆F2CAT, p∆F3CAT, p∆F4CAT and p∆F5CAT, respectively (Fig. 1). To make an Fp CAT reporter plasmid with a specific promoter-proximal AP-1 mutation, PCR amplification was performed with p∆F5CAT as the template. The 5′ primer contained the natural NheI site at position −59 relative to the Fp transcription initiation site and the desired AP-1 mutation (TGACTAA to CAGTCGG, at positions −52 to −46). The 3′ primer was specific for a sequence in the CAT gene. The amplified fragment was cleaved with NheI and BamHI and ligated to a Sull–Nhel fragment of pAF5CAT. The resulting fragment was cloned into a Sull–BamHI digested CAT vector, yielding the pAF5mutCAT plasmid.

The expression vector for ZEBRA was kindly provided by G. Miller (Yale University School of Medicine).

**Cell lines and induction of virus lytic cycle.** The cell lines used in this study and their phenotypic characteristics are listed in Table 1. All cell lines were propagated in RPMI 1640 (Life Technologies) supplemented with 10% foetal bovine serum (Life Technologies), penicillin and streptomycin. Induction of the virus lytic cycle in Rael was performed by the addition of 5 μM (Masucci et al., 1989). Induction of the virus lytic cycle in Rael was also performed by transfection of 5 × 10^4 cells with 10 μg of ZEBRA expression plasmid by electroporation, as described below. Induction of the virus lytic cycle in Akata was performed by the addition of anti-IG antibodies (DAKO) to a concentration of 0.2% (Takada & Ono, 1989). Induction of the lytic cycle was confirmed by direct immunofluorescence using a monoclonal anti-ZEBRA antibody (M7005; DAKO).
mixture was treated with 750 U (2 days after transfection, essentially as described by Dignam by electroporation as described above. Nuclear extracts were prepared 3 DG75 cells, were transfected with 10

Electrophoretic mobility shift assay (EMSA).

RNA initiated at the Fp transcription start site 62239 is 15 nucleotides.

NaCl and 50% formamide in a total volume of 20

µg of the respective antibody was added after
dialysis steps and PMSF was replaced with Pefabloc (0.5 mM). ZEBRA expression was verified by SDS-PAGE and immunoblotting using a monoclonal anti-ZEBRA antibody (M7005; DAKO). Aliquots were frozen in liquid nitrogen and stored at —80 °C. A double-stranded, blunt-ended, synthetic oligonucleotide containing the sequence between positions 62179 and 62202 in the EBV genome, (−60)−37, was used as the probe in the EMSAs. In competition experiments, the following double-stranded consensus oligonucleotides were used: (i) AP-1 consensus: 5′ GGCTAGCC 3′; and (ii) sequence identical to the probe, except that the AP-1-like site was transversely mutated (AP-1 mut): 5′ GGCTAGGCAAGTCGGGGGTGAGGC 3′ (mutated sequence underlined). Non-specific competitor was an unrelated DNA sequence. One strand of the oligonucleotide probe was labelled with [γ-32P]ATP (6000 Ci/mmol, NEN Life Science Products) using polynucleotide kinase (Boehringer Mannheim) and annealed to the complementary strand. The labelled probe was purified by electrophoresis in an 8% polyacrylamide gel in TBE (0.1 M Tris, 0.1 M sodium borate, 2 mM EDTA, pH 8.3). The wet gel was autoradiographed and the DNA fragment was excised, electroeluted by isochromatography (Överstedt et al., 1984) and precipitated. Binding reactions were carried out in a volume of 30 µl containing 10 mM Tris–HCl, pH 7.5, 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 0.5% glycerol, 1 µg polyclonal–dC–), 5 fmol labelled probe (approximately 70000 c.p.m.) and 5 µg of crude nuclear extracts from ZEBRA-transfected DG75. In competition experiments, 3 pmol of unlabelled oligonucleotides were added into the reaction mixture. After incubation at room temperature for 30 min, the samples were loaded on a 0.6% polyacrylamide gel (acrylamide: bisacrylamide 29:1) in TGE (25 mM Tris–HCl, 0.19 M glycine, 1 mM EDTA, pH 8.3). After electrophoresis gels were dried and autoradiographed. The supershift experiments were performed as described above for the EMSAs except that 2–10 µl of the respective antibody was added after the incubation at room temperature. A second incubation was carried out

<table>
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<tr>
<th>Cell line</th>
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<th>EBNA expression†</th>
<th>Relative CAT activity (%)†</th>
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<td>pΔF2CAT</td>
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* Values are expressed as the percentage of chloramphenicol acetylation, using the results obtained with pSV2CAT as 100%, after subtraction of the CAT activity obtained with empty vector (pCAT). Results are means of two independent experiments. Numbers in parentheses indicate the standard errors of the means.

† EBNA is abbreviated as E.

**Table 1. Relative activity of Fp reporter constructs in phenotypically distinct EBV-positive and -negative B cell lines and in two EBV-negative non-B cell lines**

Transactivation of Fp by ZEBRA

### Transient transfections and CAT assay

All cell lines were transfected by electroporation (Potter et al., 1984) with a Gene Pulser System (Bio-Rad). Transfections were performed with 5 × 10^6 cells and 10 µg of the reporter plasmids. Cells were harvested 2 days after transfection. To study ZEBRA transactivation of Fp, 5 × 10^6 DG75 cells were co-transfected with 10 µg of the pAF5CAT reporter plasmid and either 10 µg of expression vector for ZEBRA (37 pmol) or 8.5 µg of parent empty vector (37 pmol). Cells were harvested 3 days after transfection. Cell extracts were prepared and analysed for CAT activity as previously described (Ricksten et al., 1988).

### RNA analysis

Cytoplasmic RNA was prepared by a standard procedure (Sambrook et al., 1989). Transcription initiated at Fp in the endogenous EBV genome was determined by S1 mapping using a single-labelled probe (150 fmol) was annealed for 12–16 h at 37 °C with 100 µg of cytoplasmic RNA in 40 mM PIPES, pH 4, 1 mM EDTA, 0.4 mM NaCl and 50% formamide in a total volume of 20 µl. The hybridization mixture was treated with 750 U/mL of S1 nuclease for 45 min at 37 °C. Protected fragments were fractionated by electrophoresis in a denaturing 12% polyacrylamide gel. The expected size of a fragment protected by RNA initiated at the Fp transcription start site 62239 is 15 nucleotides.

### Electrophoretic mobility shift assay (EMSA)

For preparation of ZEBRA-containing nuclear extracts, 40 aliquots, each consisting of 10^7 DG75 cells, were transfected with 10 µg of ZEBRAP/ Fp expression plasmid by electroporation as described above. Nuclear extracts were prepared 3 days after transfection, essentially as described by Dignam et al. (1983), except that antipain (5 µg/ml), leupeptin (5 µg/ml) and aprotinin (2 µg/ml) were added to the buffer in the final homogenization and

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Results

Activity of Fp reporter plasmids in cells of different phenotypes

The activity of reporter plasmids carrying Fp and different parts of its 5’ flanking sequences (Fig. 1) was determined in a panel of B lymphoid cell lines of different phenotypes and in one EBV-negative T cell line and one EBV-negative epithelial cell line. The results obtained with the CAT reporter plasmids are summarized in Table 1. The pAF3CAT, pAF4CAT and pAF5CAT plasmids, which contain intermediate or large fragments of Fp, induced expression of CAT only at very low or background levels in all examined cell lines. However, when sequences upstream of −136 (pAF1CAT and pAF2CAT) were deleted, Fp induced significant CAT expression in latency I B cells as well as in the EBV-negative cell lines, indicating the presence of a negative regulatory cis-element in the deleted upstream sequences. Invariably, the activity of the short Fp fragments was high in latency I B cells and EBV-negative cells and below detection level in latency III B cells. The very low activity of pAF1CAT and pAF2CAT in latency III cells suggests that they may be regulated by transcription factors expressed in a cell-phenotype-dependent manner. Nevertheless, the main finding was the low or undetectable Fp activity in reporter plasmids carrying intermediate or large Fp fragments (pAF3CAT, pAF4CAT and pAF5CAT), confirming that Fp is inactive in EBV-positive cell lines in latent stages of infection and in EBV-negative cell lines.

Fp activity in endogenous virus genomes

To answer the question of whether Fp activity could be detected in the endogenous virus genome and upregulated on induction of the virus lytic cycle, S1 protection analysis was performed. An end-labelled oligonucleotide containing the Fp transcription initiation site at position 62239 was used as the probe (Fig. 2). Fp-initiated transcripts were only detected in the B95-8 cell line, which contains a proportion of spontaneously lytic subpopulations, and in cell lines in which the virus lytic cycle had been induced (Rael ZEBRA, Rael 5azaC, Mutu gr I 5azaC and Akata Ig). Induction of the lytic cycle was confirmed by direct immunofluorescence using a monoclonal anti-ZEBRA antibody. Percentages of ZEBRA-positive cells are shown, and undigested probe is indicated by an arrowhead. Protected fragments representing multiple Fp transcription initiation between positions 62232 and 62239 are indicated by a bracket. Fp-initiated transcripts were detected only in cells induced to enter the lytic cycle and in the B95-8 cell line, which contains spontaneously lytic subpopulations of cells, in this experiment 1% of the total number of cells.

ZEBRA transactivates Fp by binding to a promoter-proximal AP-1-like site

The original observation that Fp was activated on induction of the virus lytic cycle led Lear et al. (1992) to the hypothesis that Fp may be activated through a ZEBRA-induced cellular or viral intermediate. However, our TRANSFAC program scans (Wingender et al., 2000) revealed that the Fp regulatory region in pAF5CAT contained four AP-1-like sites (Fig. 1). Since ZEBRA is known to transactivate promoters through binding to AP-1-like sites, we hypothesized that ZEBRA could activate Fp directly by this mechanism. We co-transfected EBV-negative DG75 cells with pAF5CAT or pAF5mutCAT, in which the AP-1-like site in the −52/-46 Fp region was mutated, together with a ZEBRA expression plasmid (Fig. 3). The results showed that Fp was transactivated by ZEBRA and...
Transactivation of Fp by ZEBRA

Fig. 4. Identification of transcription factors that interact with the AP-1-like motif in the −60/−37 Fp region. A 32P-labelled double-stranded synthetic oligonucleotide corresponding to the −60/−37 Fp region was incubated with nuclear extracts from ZEBRA-transfected DG75 cells. The reaction mixtures were analysed by EMSA. Lane 1, binding pattern obtained with the nuclear extract; lanes 2–5, patterns obtained with a 600-fold excess of the unlabelled competitors indicated below the autoradiogram; lanes 6–8, patterns obtained after incubation with the antibodies indicated below the autoradiogram. The solid arrow indicates the ZEBRA-specific complex. The dashed arrow indicates a specific complex that is slightly reduced by incubation with anti-ZEBRA and anti-Sp1 antibodies. The arrowhead indicates the position of the anti-ZEBRA antibody-shifted complex.

that the transactivation was abolished by the promoter-proximal AP-1 mutation in spite of the presence of additional upstream AP-1-like sites in the pAF5mutCAT plasmid. The binding of ZEBRA to the AP-1-like site at the −52/−46 Fp position was investigated by EMSA and antibody supershift analysis using a probe corresponding to the −60/−37 Fp region and nuclear extracts from ZEBRA-transfected DG75 cells (Fig. 4). Two bands were evident in the extracts. Both bands could be removed by an excess of unlabelled probe and the AP-1 consensus oligonucleotide, but not by an excess of an unrelated sequence or by an oligonucleotide that was identical to the probe except that the AP-1-like site was transversely mutated. The bands were therefore considered specific, representing protein(s) bound to the AP-1-like site. Antibody supershift analysis showed that anti-ZEBRA antibodies supershifted the slowest-moving band. An antibody against the AP-1 component c-Jun did not interact with the complexes. The identity of the fastest-moving band is uncertain, although the competition experiments suggest that the complex consists of protein(s) bound to the AP-1-like site. The intensity of the band was reduced by approximately 30% in two independent supershift experiments with antibodies against ZEBRA and Sp1, but no supershifted bands were detected with the anti-Sp1 antibody. However, the major findings in the experiments presented in Figs 3 and 4 are that ZEBRA interacts with the AP-1-like site in the −52/−46 Fp region and that this interaction is essential for the ability of ZEBRA to transactivate Fp.

Discussion

ZEBRA plays a fundamental role in disrupting latency and initiating the EBV lytic cascade. Transcriptional activation of the ZEBRA-encoding BZLF1 gene is the primary underlying mechanism by which activators of lytic virus replication exert their effect. ZEBRA shares homology with the DNA-binding domain of the cellular transcription factor c-fos (Farrell et al., 1989), which is a component of AP-1 (Hunter & Karin, 1992), and functions as a transactivator that recognizes consensus AP-1 sites, as well as AP-1-like sequences (ZEBRA responsive elements; ZRE), in several cellular and viral lytic genes involved in the progression of the lytic cycle (Speck et al., 1997; Taylor et al., 1991). In the present study we tested the hypothesis that ZEBRA activates the lytic F promoter by direct binding to the Fp regulatory region. We have confirmed that Fp displays the characteristics of a lytic promoter and have shown that ZEBRA transactivates Fp by binding to a promoter-proximal AP-1-like site.

Previous studies of regulatory elements in Fp should be carefully interpreted, since they were undertaken during a period when Fp was considered an EBNA1-specific promoter in latency I and II cells. More recent data have demonstrated that EBNA1-specific initiation of transcription in restricted latency stages arises from the downstream Q promoter (Schaefer et al., 1995b; Tsai et al., 1995; Zetterberg et al., 1999). Fp was reported to be regulated by sequences in BamHI Q (Nonkwelo et al., 1995; Sung et al., 1994). However, the measured promoter activity in these studies most likely arose from Qp. Bulfone-Paus et al. (1995) investigated a short Fp fragment (146 bp, resembling our pAF2CAT construct) that spanned the Fp TATA box without BamHI Q sequences. In concordance with our data, their results indicated a remarkably
high activity from this short promoter fragment in EBV-positive and -negative B lymphoid cells. They identified three positive cis-acting elements: one Sp1 site and two tandem LR1 sites (Fig. 1). The activity of the short Fp fragments in our experiments (p∆FICAT and p∆F2CAT) was high in latency I B cells and EBV-negative cells and below detection level in latency III B cells, suggesting that the promoter fragments may be regulated by viral or cellular transcription factors expressed in a cell-phenotype-dependent manner. Since there are no such differences between latency I and III B cells with regard to Sp1 and LR1, we suggest that viral proteins or other cellular factors may contribute to the differential regulation. For example, latency I cells may contain additional activating factors not identified in this study, or express higher levels of activating factors, whereas latency III cells may contain a repressor(s). The significance of the differential regulation is, however, unclear since the full-length Fp, which presumably is biologically more relevant, behaves in the same manner irrespective of the original latency type, i.e. is repressed in latent cells and upregulated on induction of the virus lytic cycle. Interestingly, there was a significant activity of the short Fp fragments in the epithelial HeLa cell line, which should not contain the B cell-specific transcription factor LR1 (Bulfone-Paus et al., 1995). Since Sp1 usually functions together with other transcription factors (Ryu et al., 1999), it will most likely collaborate with other factors in this cell type (Bulfone-Paus et al., 1995). The promoter-proximal AP-1-like site in the −52/−46 Fp region overlaps one of the LR1 sites (Fig. 1). However, Bulfone-Paus et al. (1995) ruled out a functional role for AP-1 by binding experiments and by comparing reporter gene expression in cells cultured with and without phorbol 12-myristate 13-acetate. Taken together, it seems that Sp1 and LR1, presumably together with additional factors, activate the short Fp fragments in latency I cells and EBV-negative cells and that this activity is downregulated by a repressor element(s) upstream of −136. It should be noted that two other research groups have employed p∆F2CAT-like reporter plasmids for studying Fp activity and measured a low promoter activity (Schaefer et al., 1995a; Sung et al., 1994). In both studies the Fp transcription start site was assigned to nucleotide 62229 in the EBV-genome, 10 bp upstream of the transcription start site defined by Bulfone-Paus et al. (1995). In our study we detected multiple initiations between positions 62232 and 62239. The consequence of this is that the p∆F2CAT-like construct used by Sung et al. (1994) did not include the major Fp transcription start sites, explaining the low promoter activity in their experiments. The construct used by Schaefer et al. (1995a) was identical to p∆F2CAT, except that it contained the luciferase reporter gene instead of CAT. Thus, there is no obvious explanation for our differing results regarding this specific question. Nevertheless, the main finding of the transient transfection experiments performed in our study was that reporter plasmids carrying larger fragments of Fp (p∆F3CAT, p∆F4CAT and p∆F5CAT) were inactive in all examined cell lines, corroborating that Fp is silent in cells that are not induced to undergo the virus lytic cycle.

Since Fp was silent in strictly latent cells, both in endogenous genomes and in reporter plasmids carrying large fragments of the Fp regulatory region, we asked if Fp activity could be upregulated by induction of the virus lytic cycle. We have shown that endogenous Fp activity was induced by treatment of EBV-infected cells with known activators of lytic virus replication, confirming previous investigations (Lear et al., 1992; Schaefer et al., 1995a; Zetterberg et al., 1999). Moreover, the silent p∆F5CAT was activated in DG75 cells in the absence of other virus proteins when co-transfected with a ZEBRA expression plasmid. The induction depended on the promoter-proximal AP-1-like site, since p∆F5mutCAT was only expressed at background levels in spite of the presence of ZEBRA. Notably, the AP-1 mutation introduced in the p∆F5mutCAT plasmid changed three bp in the promoter-proximal LR1 site (11 bp). However, the substitutions did not significantly reduce the basal activity of Fp in DG75 cells when introduced in p∆F2CAT (data not shown). This finding also supports the conclusion drawn by Bulfone-Paus et al. (1995) that AP-1 is not involved in the regulation of Fp. Moreover, the substitutions did not alter the nucleotides essential for LR1 binding (Dempsey et al., 1998). EMSA and supershift experiments identified ZEBRA as a component of one of the protein–DNA complexes detected with an oligonucleotide that spanned the −60/−37 Fp region and nuclear extracts from ZEBRA-transfected DG75 cells. We identified one additional band representing protein binding to the AP-1-like site. The intensity of the band was reproducibly slightly reduced by incubation with antibodies against ZEBRA and Sp1. There is no Sp1 binding site in the −37 Fp region. We could not detect the band with nuclear extracts from untransfected DG75 (data not shown). Either the reduced intensity is an unspecific phenomenon, or it might be a result of an interaction between ZEBRA and Sp1. Such an interaction has not yet been described in the literature. In conclusion, we have identified one additional ZEBRA-responsive element in the EBV genome. There are three more AP-1-like sites in the upstream regulatory region of Fp. Conceivably, these are also involved in ZEBRA-induced transactivation of Fp and initiation of the EBV lytic cascade.

A major question remains: what is the 3′ end exon of the Fp-initiated transcripts? In a previous investigation of relative levels of EBNA1 gene transcripts in latent and lytic stages of infection, we demonstrated that only 1–2% of Fp-initiated transcripts in cells induced to virus lytic cycle splice from the U exon to the EBNA1-encoding K exon (FpQ/U/K splicing pattern) (Zetterberg et al., 1999). The large majority of the Fp-initiated transcripts displayed an FpQ (52–75% of Fp-initiated transcripts) or FpQ/U (24–47%) splicing pattern. Furthermore, we identified a BamHI K-containing lytic cycle-specific transcript, which extends upstream of the BamHI f/K cleavage site. It is possible that there is a relationship between the Fp-
initiated transcripts with presently unidentified 3' end exon(s) and the lytic cycle-specific BamHI K-containing mRNA, which may give rise to EBNA1. In a study of the transregulatory effects of ZEBRA on different classes of EBV promoters, Kenet et al. (1989) showed that ZEBRA down regulates Cp and Wp. In spite of the downregulation of latent promoters, EBNA1 is not repressed on induction of the virus lytic cycle (Rowe et al., 1992; Weigel et al., 1985). These data suggest that EBNA1 gene transcription in the lytic cycle may be driven by a lytic cycle-specific transcription unit, possibly initiated at Fp, which could compensate for the repression of the latent EBNA1 gene promoters. EBNA1 is known to bind to oriP sequences and enhance nuclear import of oriP-containing plasmids in transient transfection experiments, most probably through its strong nuclear localization signal (Langle-Rouault et al., 1998). It is tempting to speculate that EBNA1 may serve an important function in the virus lytic cycle through binding to oriP in the genomes of newly packaged virions and facilitate the nuclear import of virus genomes on infection of resting B cells.

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Epstein–Barr virus
Bam 63
EBV promoter and induces the virus productive cycle. spliced BZLF1 gene of Epstein–Barr virus (EBV) transactivates an early Sciences chain-terminating inhibitors. Proceedings of the National Academy of Sciences antigen 1 transcriptional initiation site. expression in Burkitt lymphoma is due to a different Epstein–Barr nuclear promoters used in latently infected lymphocytes. Burkitt lymphoma arises from a third promoter, distinct from the promoters used in latently infected lymphocytes. Proceedings of the National Academy of Sciences, USA 88, 6550–6554.


