VIROLOGY 181, 172–179 (1991)

The BI'LF4 *trans*-Activator of Epstein–Barr Virus Is Modulated by Type and Differentiation of the Host Cell

MANFRED MARSCHALL, FRITZ SCHWARZMANN, ULRIKE LESER, BARBARA OKER, PETER ALLIGER, HELGA MAIRHOFER, AND HANS WOLF[1]

Max von Pettenkofer-Institute, Pettenkoferstasse 9a, 8000 Munich 2, Federal Republic of Germany

Received July 31, 1990; accepted November 13, 1990

We have analyzed the activity and regulated expression of a new Epstein–Barr virus (EBV) *trans*-activator (I'ta) encoded by left reading frame 4 (BI'LF4) of the *Bam*HI I' fragment. The gene was detected in all genomes of established EBV strains and individual isolates, with the exception of B95-8, where the type-specific deletion of this open reading frame is tolerated *in vitro*. Specific *trans*-activation of two EBV promoters (early MS and I'ta promoter) could be shown in cotransfection assays. The I'ta product affected autoactivation but had no influence on heterologous target promoters. The I'ta promoter segment was shown to be costimulated in the process of host cell differentiation in the absence of other EBV gene products. Expression of the reading frame in bacteria identified a 48-kDa protein as a stable gene product. I'ta-specific antibodies were detected in sera from EBV-positive persons (nasopharyngeal carcinoma). When expressed with suitable eucaryotic vectors, a nuclear protein could be immunostained in transfected cells. Our experiments suggest a cell type-specific requirement for I'ta in the lytic cycle of EBV at a determined differentiation stage of the host cell. © 1991 Academic Press, Inc.

INTRODUCTION

The Epstein–Barr virus (EBV), a human herpesvirus causing infectious mononucleosis as a primary disease, is an ubiquitous pathogen. Lifelong persistence in B lymphocytes is under control of the immune system; however, chronic active progression of an acute infection or of neoplasias, like Burkitt's lymphoma or nasopharyngeal carcinoma, can develop in certain circumstances (Wolf *et al.*, 1987). Lymphoid cells are far from being the only site for EBV replication and a tissue-specific virus/host interaction is a central feature of EBV pathobiology (Wolf *et al.*, 1984). At present, epithelial tissue appears to be a site of viral persistence and a source of virus production. Regulation of viral replication is dependent on the differentiation of its host cells (Becker *et al.*, 1989). EBV regulation is characterized by latency and the cascade of the lytic cycle of replication, comparable to several other herpesvirus systems, such as herpes simplex virus (Roizman and Batterson, 1985) or *Herpesvirus saimiri* (Hell *et al.*, 1985).

Lytic activation is based on the induction of *trans*-acting, nuclear proteins (Chevallier-Greco *et al.*, 1986; Countryman *et al.*, 1987; Hardwick *et al.*, 1988; Liebermann *et al.*, 1986; Marschall *et al.*, 1989a) belonging to the immediate early regulatory class (Biggin *et al.*, 1987). RNA analysis by our group suggested an additional transcript, which appeared to be independent of prior synthesis of viral proteins and maps in the EBV *Bam*HI I' section (Seibl *et al.*, 1987). In this work we characterize the Epstein–Barr virus BI'LF4 *trans*-activator, designated I'ta. Gene functions including promoter activity, protein synthesis in bacteria and cell systems, *trans*-acting properties, and autoregulation are described. We established a dynamic model system using a secreted reporter product to prove the transcriptional dependence of this gene on the proceeding differentiation of the host cell. A specific differentiated stage emerges to play a key role in the lytic cycle of EBV. In epithelial cells, the I'ta gene was shown to be inducible and functional. In lymphoid cells, deletion of this lytic regulator seems to be tolerable under favorable conditions, but this deletion was shown to be rather exceptional in natural isolates.

MATERIALS AND METHODS

Plasmid constructions

The coding sequence of the open reading frame (ORF) BI'LF4 was derived from cm 302-21 cosmid DNA (Polack *et al.*, 1984) using the restriction sites of *Sal*I and *Bam*HI (1390-bp fragment). For the construction of pSVI', regulatory elements of the simian virus 40 (SV40) (early promoter/enhancer as 830-bp *Taq*I–*Stu*I fragment and splice/polyadenylation signals as 1640-bp *Nco*I–*Pst*I fragment) were excised from the original

[1] To whom reprint requests should be addressed.

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pSV2CAT construction (Gorman *et al.,* 1982). As shown in Fig. 1B these fragments were inserted into plin vectors, derived from pUC by insertion of new synthetic polylinkers (Max von Pettenkofer-Institute, Munich). In pSCl', the TATA box of the SV40 early promoter was replaced by the human cytomegalovirus (HCMV) immediate early promoter/enhancer (*Nco*I–*Sal*I insertion of the subcloned HCMV sequence as described below) to optimize expression. For bacterial protein synthesis, the ORF BI'LF4 was positioned under the control of either the hybrid *trp*/*lac* promoter in vector pTrc99A (Amann *et al.,* 1988) (Fig. 1B) or the combined phage T7 promoter (A1)/*lac* operator in vector pUHE24-2 (Bujard *et al.,* 1987). The I'ta promoter element, an 890-bp *Alu*I–*Alu*I fragment, subcloned from H3-D1 cosmid DNA (Polack *et al.,* 1984), was inserted into reporter gene constructs using the coding sequence for either CAT or HBsAg as a reporter element. Cloning strategies for all EBV promoter regions and reporter constructions were described in detail previously (Marschall *et al.,* 1989b). For controls, the HCMV immediate early promoter/enhancer (770-bp *Hin*dIII–*Hpa*II fragment) (Boshart *et al.,* 1985) was subcloned into pUC18, cut by *Hin*dIII and *Acc*I, and subsequently inserted upstream from the reporter sequences generating pCMVHBs and pCMVCAT. In the BZLF1 expression vector pCMVZ (including two spliced exons at the 3'-end) (Manet *et al.,* 1989), this HCMV element was used to promote transcription of the genomic *Bam*HI Z sequence (1065-bp *Nae*I–*Nco*I fragment) (Skare *et al.,* 1980).

Cell culture, transfections, and induced differentiation

HeLa, COS-1, and mouse teratocarcinoma F9 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, 100 units/ml of penicillin, and 250 μg/ml of streptomycin. Eight hours before transfection, the cells were seeded at a density of 10^6/100-mm petri dish. The cells were transfected by the $CaHPO_4$ method (McCutchan *et al.,* 1968) with 10 to 40 μg of DNA, using vector DNA to achieve equimolar concentrations in separate tests. The $CaHPO_4$–DNA precipitate was removed 4 to 8 hr after addition and the cells were grown in fresh medium. TPA (tumor promoting agent, phorbol-12-myristate-13-acetate) was added to a final concentration of 20 n*M*. Cell differentiation of the F9 line was induced by treatment with 1 μ*M* retinoic acid for at least 4 days. Spontaneously growing B-cell lines were cultured from EBV-positive peripheral blood lymphocytes. Two weeks after isolation of the lymphocytes by centrifugation in Ficoll–metrizoate gradients (density 1.077), cyclosporin A was added at a concentration of 1 μg/ml to suppress T-cell growth, and the medium was exchanged once a week. Within 4 to 6 weeks spontaneous growth of EBV-positive B cell populations was observed.

HBsAg reporter system and CAT assay

The HBsAg reporter system is based on the secretion of HBsAg (missing pre-S sequences) into the culture supernatant and its quantification correlating to the tested promoter activity (Marschall *et al.,* 1989b). HBsAg production was assayed in supernatant aliquots by the commercially available, quantitative radioimmunoassay for HBsAg (Abbott). Since cell lysis was not required, transfection assays could be continuously monitored over a period of time. For CAT assays, the cells were washed in phosphate-buffered saline (PBS) 2 days post-transfection and lysed by sonication in 100 μl of 0.25 *M* Tris–HCl, pH 8.0, on ice. Cell debris was removed by centrifugation for 5 min at 500 *g*. The protein concentrations were equilibrated after measurement of the optical densities at 260 and 280 nm. The protein extract (50 μl) was mixed with a reaction mixture containing 100 μl of 0.25 *M* Tris–HCl (pH 8.0), 20 μl of 4 m*M* acetyl coenzyme A (Pharmacia-LKB), and 10 μl of [^{14}C]chloramphenicol (0.25 μCi, Amersham) and incubated for 1 hr at 37°C. Chloramphenicol and its acetylated products were extracted with 400 μl of ethyl acetate and evaporated in a speed-vacuum centrifuge. Dried samples were resuspended in 10 μl of ethyl acetate and spotted on silica gel thin-layer chromatography plates (Merck 5737) in chloroform:methanol (95:5) solvent. The plates were exposed on hyperfilm-βmax (Amersham) for 12 to 24 hr.

Western blot and immunofluorescence

For Western blots, bacterial protein extracts were prepared from exponentially growing transformed *Escherichia coli* clones. Protein synthesis was induced by addition of 2 m*M* isopropyl-β-D-thiogalactopyranoside for at least 2 hr. Lysates were prepared in 2% sodium dodecyl sulfate (SDS) by sonication. Standard immunoblotting and modified staining procedures were described previously (Marschall *et al.,* 1989a). For indirect immunofluorescence, transfected cells (2 days post-transfection) were fixed in ice-cold acetone for 5 min. Human antisera and fluorescein isothiocyanate-linked goat anti-human antibodies (Medac) were applied 1:50 for 1 hr at 37°C. After the cells were washed with PBS, the slides were evaluated at a microscope magnification of 250.

Southern blot

For Southern blots, 10 μg of total cell DNA was digested with *Sst*I restriction enzyme and separated in 1.5% (w/v) agarose gels. The DNA transfer onto nylon membranes was achieved by suction through Whatman filters in 20× SSC buffer (3 *M* NaCl, 0.3 *M* Na citrate) for 8 hr. Crosslinking was done by a 5-min exposure to ultraviolet light. Hybridization of the blots was performed as described in detail by Boehringer Mannheim, "DNA Labeling and Detection Kit, Nonradioactive." As BI'LF4-specific probe, a 1.0-kb *Sst*I fragment was isolated from the *Sst*I digest of pSVI'. The nonradioactive labeling with digoxigenin (Dig-dUTP) was again done following the instruction mentioned above.

RESULTS

Genome localization and subcloning

The ORF BI'LF4 is located in the *Bam*HI I' region of the Raji genome, starting with the ATG codon at position 14115 (paul.sin-sequence, kindly offered by Paul Farrell) (Baer *et al.,* 1984; Farrell *et al.,* 1987; Hatfull *et al.,* 1988). Sequence analysis identified conserved promoter motifs, e.g., TATA box, a cryptic CAAT signal (position −75), and a perfect AP-1 binding site (position −450) (Angel *et al.,* 1987). As the *Bam*HI I' fragment is partly deleted in the B95-8 standard genome (Fig. 1A), all subclones were constructed using M-ABA strain DNA (Polack *et al.,* 1984).

In the eucaryotic expression vectors pSVI' and pSCI', the ORF BI'LF4 was expressed under the control of heterologous promoters and terminated by the simian virus 40 polyadenylation signal. Efficient RNA transport was achieved by the insertion of the SV40 splice acceptor and donor. In pSCI', the coding sequence was controlled by a strong hybrid promoter (SV40 early enhancer plus HCMV immediate early promoter/enhancer). Two vectors with different promoter/operator systems were used for the I'ta protein synthesis in *E. coli,* pTrcI' (Fig. 1B) and pUHI' (see Materials and Methods). The I'ta promoter was analyzed using the reporter plasmids pI'CAT and pI'HBs.

trans-Activity and autoregulation

trans-Regulatory functions of the I'ta product were tested in cotransfection assays. Reporter plasmids with selected promoters were analyzed for increased activity as a consequence of I'ta expression (Fig. 2A). No effect was seen on heterologous targets, such as the SV40 early promoter/enhancer (pSVCAT) and the HCMV immediate early promoter/enhancer (pCMVCAT). Using a reporter construction with the SV40 early promoter, lacking the 72-bp enhancer repeats (p21CAT not shown), the basal level of expression was reduced to where no responsiveness to I'ta was detected. Several EBV promoters of different regulatory classes, e.g., immediate early (pZCAT, pRCAT), early (pACAT), and latent (pNCAT), were likewise not activated. A specific *trans*-activation, however, was shown for two test constructions of the EBV early MS promoter (pS1CAT: 127-bp basic promoter, pSCAT: 1.4-kb upstream element plus basic promoter) and for the I'ta promoter itself (pI'CAT). The strong influence on the proximal part of the MS promoter was studied in detail under concentration-dependent test conditions using the HBsAg (hepatitis B virus surface antigen) reporter system (Fig. 3A). Autoactivation of the I'ta promoter by its own gene product could be intensified with the efficient expression vector pSCI' (Fig. 2B).

Cell differentiation and promoter stimulation

The mouse teratocarcinoma line F9 was used as a cell system for inducible differentiation by retinoic acid (Umesono *et al.,* 1988). Coactivation of different EBV promoters by differentiation-dependent cellular regulators was studied in the absence of viral proteins. Most of the EBV target promoters did not respond to changes in the differentiation stage and growth behavior, while the I'ta promoter and the distal segment of the MS promoter were clearly activated in retinoic acid-treated cells (Fig. 3B). Induction of other constructs with very low basal activity was likewise not detected in independent assays, even after attempts to enrich the reporter protein particles by ultracentrifugation.

Two further effects of influencing the activity of the I'ta promoter were tested. Since the regulatory upstream sequence contains an AP-1 consensus binding site, high promoter activity was assumed to be induced by TPA incubation (Lee *et al.,* 1987) or by expression of Zta (Farrell *et al.,* 1989; Urier *et al.,* 1989). Our findings indicate that both pathways of activation stimulate the I'ta promoter to a certain extent (Fig. 2B), but to a level much lower than that observed for autostimulation. We propose therefore that mainly cellular differentiation triggers the synthesis of the I'ta protein, which subsequently accelerates its own expression.

Expression and nuclear localization

Using procaryotic in-frame vectors, the ORF BI'LF4 was expressed as an authentic viral protein without heterologous carboxy-terminal fusion (Fig. 4A). Dependent on reliable promoter/operator systems, the polypeptide was stably expressed in *E. coli* in amounts sufficient for further characterization. We determined a molecular weight of 48 kDa, which was in line with the

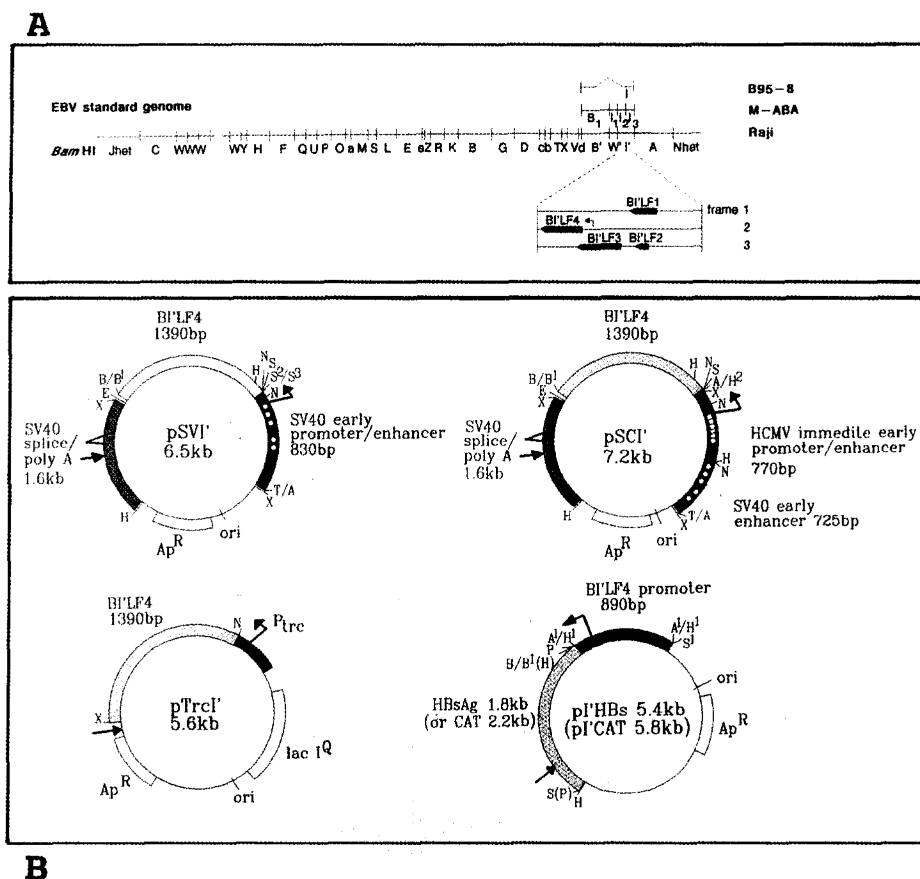

FIG. 1. Genome mapping of the I'ta gene (A) and construction of expression and reporter plasmids (B). The structure of three EBV genome types is shown in the linearized form, subdivided into *Bam*HI fragments. The ORFs of the Raji strain *Bam*HI I' fragment are presented in an enlarged drawing (boldface arrows). Promoter elements are indicated by bent, horizontal arrows. In the eucaryotic expression vectors pSVI' and pSCI', the enhancer repeats are drawn as open circles (SV40 21 and 72 bp) or rhombs (HCMV 17, 18, 19, and 21 bp). Polyadenylation signals and splice acceptors/donors are marked by vertical arrows and angles, respectively. Procaryotic elements of pTrcI' are abbreviated as ori (origin of replication), Ap^R (ampicillin resistance), lac I^Q (repressor allele), and P_{trc} (inducible hybrid promoter). The test constructions for the I'ta promoter, pI'HBs and pI'CAT, contain the coding sequences for the hepatitis B virus surface antigen (HBsAg) or chloramphenicol acetyltransferase (CAT) as reporters. Restriction enzymes: A, *Acc*I; A^1, *Alu*I; B, *Bam*HI; B^1, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; H^1, *Hin*cII; H^2, *Hpa*II; N, *Nco*I; P, *Pst*I; S, *Sal*I; S^1, *Sst*I; S^2, *Sma*I; S^3, *Stu*I; T, *Taq*I; X, *Xba*I.

sequence prediction. High titers of I'ta-specific antibodies in human sera, derived from patients with nasopharyngeal carcinoma, indicate the relevance of the gene product during natural infection.

Expression of I'ta in transfected eucaryotic cells was analyzed by immunofluorescence. A small percentage (2–5%) of transfected cells showed a bright, mostly nuclear fluorescence when incubated with human antisera. Most of the positive cells showed a nuclear staining (Fig. 4B). Mock-transfected cells and EBV-negative sera (preabsorbed with a mix of *E. coli* and BJA-B cell extracts, respectively) were used in control experiments and did not give positive signals.

Presence in individual EBV isolates

We tested the presence of the ORF BI'LF4 on DNA level by Southern blot analysis (Fig. 5). Several established EBV-positive and -negative Burkitt lymphoma lines were compared to spontaneously growing B cells of seven EBV-positive persons. The BI'LF4-specific 1.0-kb *Sst*I fragment, originally subcloned from the M-ABA type, was found in all individual isolates in its unaltered molecular size. The Raji and the P3HR-1 strain were used as positive controls. Both *Sst*I recognition sequences were conserved in all EBV types tested. As expected, no BI'LF4 signal was detected in DNA from EBV-negative cells and in the mutant B95-8 strain.

DISCUSSION

The most important interpretations of our observations are the following: (i) the EBV genome encodes a *trans*-acting factor in the ORF BI'LF4; (ii) this factor has strong and specific regulatory effects but can be dis-

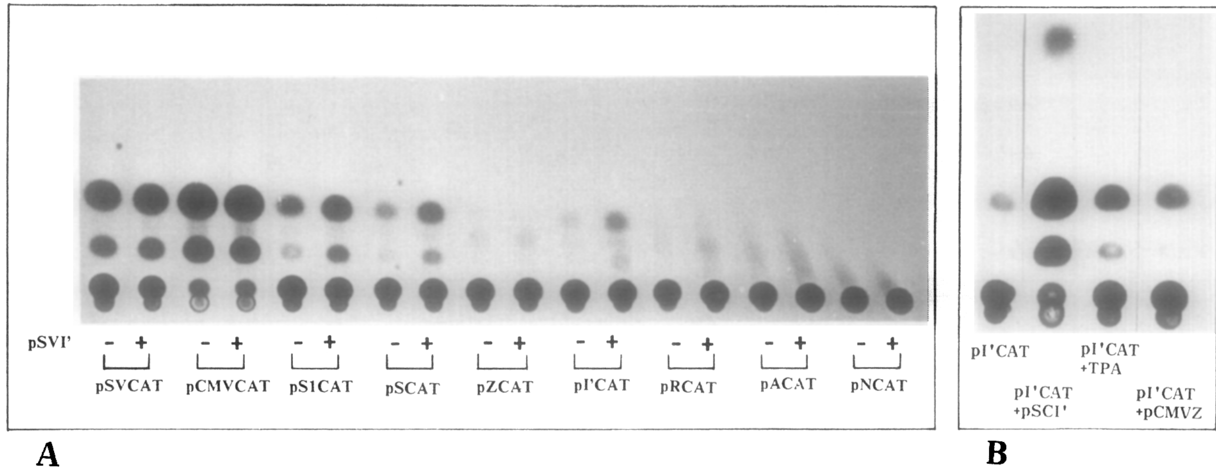

FIG. 2. CAT assays, showing the *trans*-activation by the I'ta product (A) and the regulation of its promoter (B). HeLa cells were transfected with 20 μg of total DNA. The reporter plasmids contained the following promoters: SV40 early (pSVCAT), HCMV immediate early (pCMVCAT), EBV BMLF1/BSLF2 (pS1CAT, 127-bp basic promoter; and pSCAT, 1.4-kb promoter/upstream sequence), BZLF1 (pZCAT), BI'LF4 (pI'CAT), BRLF1 (pRCAT), BALF2 (pACAT), and BNLF1 (pNCAT). Promoter activation was assayed using the expression vectors pSVI', pSCI', and pCMVZ or by TPA induction.

pensed *in vitro* at least in lymphoid cells, where a moderate lytic cycle of EBV in marmoset-derived B95-8 cells is observed; (iii) its promoter is regulated by cellular factors and by its own gene product; (iv) the I'ta activity is not restricted to lymphoid cells and is influenced by the differentiation stage of the host cell; (v) the gene product is a 48-kDa protein which is transported into the nucleus; and (vi) the gene is present in individual EBV isolates, indicating its general importance in natural infection.

We characterized a 48-kDa EBV gene product which is mapping in the *Bam*HI I' region of EBV but is deleted in the B95-8 cell-derived EBV genome. Recent transcript analyses proved the affiliation of I'ta to the immediate early regulatory class. Studies in freshly infected or superinfected B-cell lines, in the presence of translation inhibitors, delineated that I'ta is an immediate early gene because its expression does not depend on prior protein synthesis (U. Leser, 1990, Ph. D. thesis, University of Munich, FRG; U. Leser, manuscript in preparation). Parallel to the two other immediate early factors of EBV, BZLF1 (EB1; Chevallier-Greco *et al.*, 1986) (ZEBRA; Countryman *et al.*, 1987) (Zta; Cox *et al.*, 1990) and BRLF1 (R; Hardwick *et al.*, 1988) (Rta; Cox *et al.*, 1990), I'ta activates the early antigen level by turning on the BMLF1/BSLF2 gene. The promoter of this universal, post-transcriptional MS regulator (Buisson *et al.*, 1989; Kenney *et al.*, 1989a; Kenney *et al.*, 1989b) was characterized as a target for different pathways following the lytic switch (Marschall *et al.*, in press).

Although the regulatory properties of the I'ta product are proven, its role in the lytic cascade can obviously be substituted. This situation is in line with the regulation of herpes simplex virus, where the powerful ICP0 *trans*-activator is not essential for the viral cycle (Sandri-Goldin *et al.*, 1987). In context with the central role of nonlymphoid, in particular differentiating epithelial cells in the life cycle of EBV (Becker *et al.*, 1989; Wolf *et al.*, 1984), the responsiveness of the I'ta promoter to differentiation-dependent host factors may play a key role in the biology of EBV. Although mouse cells, as used in our model system, are unlikely to support either latent or lytic EBV replication, our results clearly show the responsiveness of distinct EBV promoter elements to cellular factors with transient activity. Morphological change in the differentiating cells was correlated to the induction of the I'ta promoter independent from EBV.

The fact that the gene is deleted in the viral genome of the B95-8 line, extensively passaged in nonnatural marmoset lymphocytes, suggests selective pressure against efficient virus replication for the benefit of the host cells. *In vivo* the selective pressure apparently is reversed in favor of the most active viruses, as this deletion was never found in recently established cell lines and only once in a cell culture with an extensive history of viral propagation (Shope *et al.*, 1973).

The finding that complex viruses have alternate, partly redundant genes sheds new light on cell type-specific regulation. Activation of the I'ta product might explain the high efficiency of lytic EBV expression in epithelial cells (seen in the tonsils during infectious mononucleosis, in the parotid gland of all seropositives, and in the hairy leukoplakia lesions of AIDS patients). Viruses will frequently not replicate in unre-

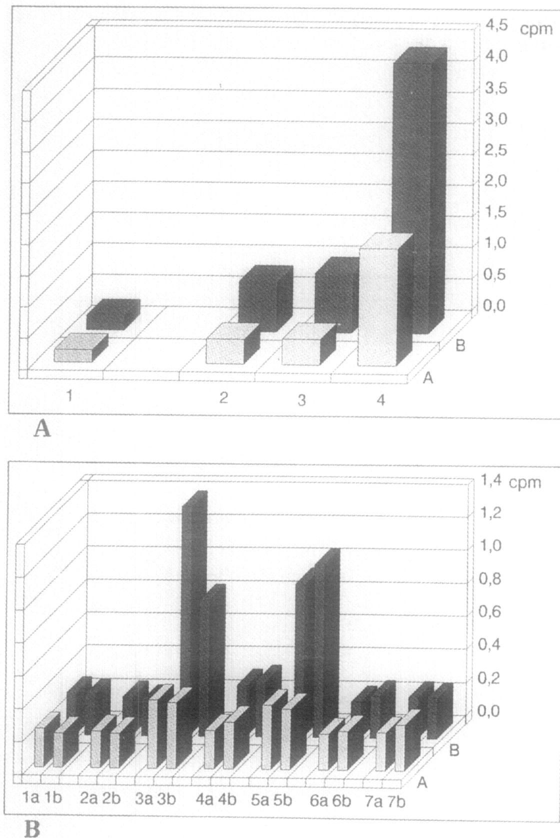

FIG. 3. Radioimmunoassay data of the HBsAg reporter system showing specific *trans*-activation in transfected HeLa cells (A) or differentiation dependence in teratocarcinoma F9 cells (B). *trans*-Activation of the EBV MS promoter (pS1HBs) by I'ta was measured by the cotransfection of different concentrations of pSVI' (1, 0 μg; 2, 10 μg; 3, 20 μg; 4, 30 μg). The values in row A of panel A refer to supernatant samples of the first 2 days and in row B of the following 2 days post-transfection. 10 ml of the culture supernatant was assayed in each case. Panel B shows HBsAg reporter expression in F9 cells, without retinoic acid treatment (row A) or with retinoic acid treatment (row B). Each left value (a) reflects the promoter activity of the first 2 days and each right value (b) of the following 2 days post-transfection. The target promoters used are described in Fig. 2 (1, pZHBs; 2, pRHBs; 3, pSHBs; 4, pS1HBs; 5, pI'HBs; 6, pAHBs; 7, pNHBs).

placeable, undifferentiated cells but will replicate in differentiated epithelium, often localized on outer surfaces. Supply of such cells is limited and self-renewing. Similar concepts can be seen for papilloma viruses.

An interesting feature of the I'ta gene is the positive feedback effect on its own promoter. This is in contrast to reports on immediate early genes of herpes simplex virus which seem to limit their gene expression by down-regulation of their own promoters (O'Hare *et al.*, 1987). Such an autorepression has not been found so far within the EBV system. However, alternative ways of positive regulation were detected (moderate responsiveness to TPA, Zta; consensus AP-1 binding site), although their relevance in the lytic cycle still awaits further experimental data.

The nucleotide sequence of the I'ta gene was compared with those of well-characterized viral or cellular regulatory genes, such as the coding regions for EBV Zta and Rta, for human c-*fos* and c-*jun*, for human immunodeficiency virus 1 tat, human adenovirus 4 E1A, human cytomegalovirus immediate early 1, herpes simplex virus 1 ICP 0, pseudorabies virus 180-kDa protein, and hepatitis B virus X gene, using GCG software

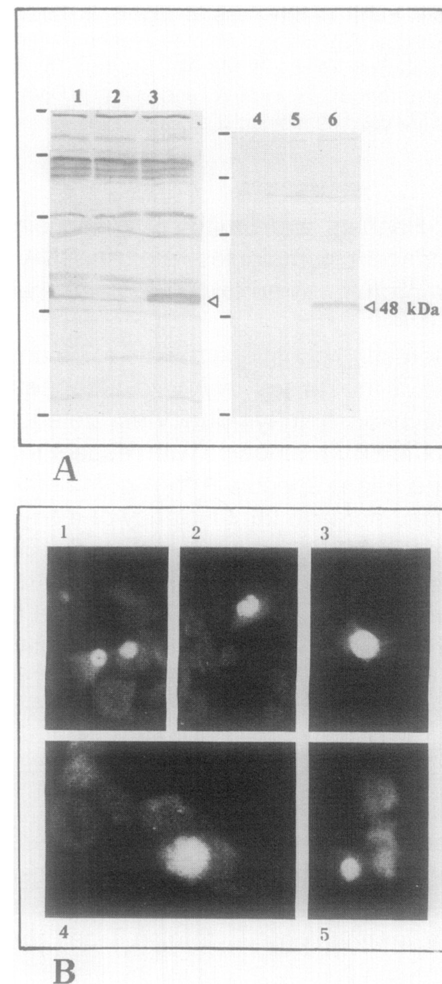

FIG. 4. Synthesis of I'ta protein in *E. coli* (A) and HeLa or COS-1 cells (B). Western blots with different bacterial extracts (1, pTrc99A; 2, pTrcI'; 3, pTrcI' induced; 4, pUHE24-2; 5, pUHI'; 6, pUHI' induced) were incubated with a human serum pool (nasopharyngeal carcinoma patients) and stained in alkaline phosphatase procedures. The molecular size was determined by comparison with a protein standard (Sigma; 116, 97.4, 66, 45, and 29 kDa). Immunofluorescence staining of transfected cells (1 to 3, pSVI'; 4 to 5, pSCI') with a human serum pool shows the nuclear localization of the I'ta protein in adherent HeLa (1 to 4) and trypsinized COS-1 cells (5).

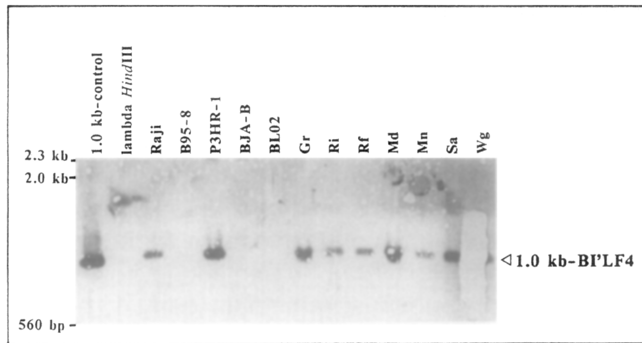

FIG. 5. Southern blot, demonstrating the presence of ORF BI'LF4 in different EBV strains. A digoxigenin-labeled BI'LF4-specific probe was used to detect a 1.0-kb *Sst*I fragment in total DNAs of EBV-positive (Raji, B95-8, P3HR-1), EBV-negative (BJA-B, BL02) B-cell lines, and seven individual EBV isolates from spontaneously growing peripheral B lymphocytes (Gr, Ri, Rf, Md, Mn, Sa, Wg). The 1.0-kb *Sst*I control, isolated from BI'LF4 in pSVI', and phage λ DNA, digested with *Hin*dIII, were used as molecular size markers.

(Devereux, Haeberli and Smithies; Wisconsin). However, no stringent homologies, e.g., in DNA binding regions or protein complexing domains, were detected.

Some interesting questions remain to be answered. For example, is the I'ta activity *in vivo* dependent on the cell type? Obviously in lymphocytes, deletion of I'ta moderates the EBV phenotype with respect to the lytic activity. One might speculate that complementation with I'ta might increase the efficiency of the lytic gene expression in B95-8 cells, comparable to the EBV regulation in the high producer strain P3HR-1. In the P3HR-1 strain, an elevated amount of Zta, derived from the WZhet DNA (Rooney *et al.,* 1988), constitutively promotes the lytic cycle. Alternatively I'ta could be a function required primarily in nonlymphoid target cells as mentioned above.

In addition to its role in the lytic infection, one would have to consider possible other functions, e.g., in cell transformation via interaction with cell proliferation, as has been shown for the tax gene of the human T lymphotropic virus-I (Leung *et al.,* 1988). It may also play a role in EBV immunology. Cytotoxic T-cell responses against immediate early proteins, as studied in detail for other herpesvirus systems (Reddehase *et al.,* 1989), have been involved in control of EBV infection of humans as well (Jilg *et al.,* 1989).

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (Wo 227/4 and FA 138). The authors are grateful to Paul Farrell (Ludwig Institute for Cancer Research, London, UK) for making the paul.sin sequence available and to Rolf Kemler (Max Planck-Institut, Freiburg, FRG) for providing F9 cells. Many thanks are due to Alain Sergeant's group (Ecole Normale Superieure de Lyon, France) for their collaboration and discussions and to Margarete Brand for reading the manuscript.

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