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A Promoter for the Highly Spliced EBNA Family of RNAs of Epstein-Barr Virus

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A transcription start for the highly spliced EBNA group of RNAs in B95-8 cells has been identified in the short unique region of the virus genome. This promoter is used in many (but not all) human cell lines carrying Epstein-Barr virus, including a tightly latent human lymphoblastoid cell line. Another promoter for the EBNA RNAs was described previously in the internal repeat region of the virus genome. The existence of these alternative promoters may be important for differential control of EBNA gene expression.

Epstein-Barr virus (EBV) is a human herpesvirus which infects B lymphocytes and certain epithelial cells (reviewed in P. J. Farrell and B. G. Barrell, *The Herpesviruses*, vol. 5, in press). Cell lines containing EBV have been obtained either from Burkitt's lymphoma biopsies or by infection of lymphocytes with EBV. B lymphocytes infected with EBV are immortalized to perpetual growth. Depending on the source of the lymphocytes, the resulting B lymphoblastoid cell line (LCL) may produce no virus, maintaining the EBV genomes in a latent state in all the cells, or may spontaneously produce virus in a small fraction of the cells. B-cell lines derived by immortalization of cord lymphocytes are usually tightly latent, whereas lines made from adult peripheral lymphocytes are usually spontaneously productive. Particular patterns of EBV gene expression are associated with the latent or productive state (summarized in reference 12).

B cells from primates closely related to humans can also be infected with EBV. The B95-8 and FF41 cell lines are spontaneously productive marmoset LCLs containing EBV from human patients. Other cell lines used in this study are human in origin. Raji, BL29, BL37, BL72, BL74, BL18, ELI, WW1, Jijoye, and HR1 clones 5 and 16 are cell lines derived from Burkitt's lymphoma biopsies. PRBL18 and PRWW1 are LCLs made by introducing the EBV from BL18 and WW1, respectively, into peripheral lymphocytes from an English donor. IB4, X50-7, and JC-5 are tightly latent human cord LCLs.

The EBV genome is a double-stranded DNA molecule of nearly 175 kilobases (kb). Two clusters of tandemly repeated sequences, designated TR and IR, delimit the U_S and U_L regions. The sequence of the B95-8 virus genome has been determined (1). EBV apparently contains 80 to 100 genes (1), but in the latent cycle, only a few of these are expressed (19). These include the EBNA-1, -2, and -3 genes, the latent membrane protein gene, the EBER RNA genes, and a gene known variously as EBNA-4 (30), EBNA-5 (9), and leader protein (LP) (32, 38). It is presumed that some of these genes are involved in the maintenance of the EBV DNA episomes within infected cells and that some contribute to the immortalization of host cells. EBNA-1 is required for the maintenance of the EBV genome (21, 40). EBNA-2 (6, 8, 23, 26) and the latent membrane protein (37) apparently contribute to the immortalizing capacity of the virus.

The reading frames which encode EBNA-1 (11, 16), EBNA-2 (10, 17), one member of the EBNA-3 group (18), LP (38), and the latent membrane protein (15) have been established, and the structures of the mRNAs which encode those proteins are partly understood (Farrell and Barrell, in press). This paper is about the structures of the EBNA mRNAs, which seem to constitute a family of transcripts. They share long 5′ leaders, which are spliced in a very complex fashion, joined to 3′ coding regions, which are unique to each EBNA (3, 5, 32–34).

The first EBV cDNA sequence to be reported was that of the T1 cDNA clone from Raji cells (4). The sequence of clone T1 revealed that the RNA has a repetitive core including multiple copies of two short exons (W66 and W132, also known as W1 and W2, respectively) from the major internal repeat regions of EBV. This cDNA may be a partial copy of an mRNA encoding LP. Further cDNAs which appear to be partial copies of the mRNAs encoding EBNA-1 and the EBNA-3 group were isolated from B95-8 cells (3, 5). Other cDNAs isolated from latently infected human LCLs encode EBNA-1 (5, 32, 33), EBNA-2 (32), and LP (32, 34).

The cDNAs from B95-8 cells are generally very similar to those sequenced from human LCLs, with the significant difference that the B95-8 cDNAs contain 5′ exons from the U_S region of EBV, whereas the human LCL cDNAs all have 5′ ends in the IR region. These facts have led to the notion that the transcription start for the highly spliced RNAs in B95-8 cells may be different from that in the human LCLs. Since B95-8 cells are marmoset cells (human cells are presumably the natural host), this start might be an aberrant one. In this paper we accurately map the boundaries of the 5′ exon of the B95-8 cDNAs and demonstrate the transcription initiation point. We further show that this 5′ exon is present in the highly spliced EBNA family of RNAs from a wide variety of human cell lines infected with EBV, including one tightly latent human cell line.

MATERIALS AND METHODS

IARC-BL18, BL29, BL36, BL37, BL72, BL74, ELI, and WW1 are Burkitt's lymphoma cell lines (28, 29) kindly provided by G. M. Lenoir and A. B. Rickinson. PRBL18 and PRWW1 are human LCLs made by infecting peripheral

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lymphocytes from a seronegative English donor with EBV from BL18 and WW1, respectively (28, 29). The producer status and latent antigen expression of these lines have been reviewed recently (31). Raji (25) and Jijoye (7) are also Burkitt's lymphoma cell lines. HR1 clone 5 and clone 16 are clones of the P3HR1 line (26) derived from Jijoye. JC-5, X50-7, and IB4 are tightly latent human cord LCLs (20, 30, 40). JC-5 contains the Jijoye strain of EBV. B95-8 and FF41 are marmoset cell lines immortalized with EBV (24).

Cells were grown in RPMI 1640 containing 10% heat-inactivated fetal calf serum. TPA treatment of B95-8 cells was done with 40 ng of TPA per ml for 3 days. Treatment with both PAA and TPA was the same as TPA treatment except that 125 μg of PAA per ml was added at the same time as TPA. Cytoplasmic RNA was extracted and fractionated on oligo-dT cellulose (2). All experiments used poly(A)$^+$ RNA.

Northern blotting (RNA blotting) analysis involved electrophoresis of glyoxalated nucleic acid samples on neutral agarose gels (22) followed by transfer to nitrocellulose (35) and hybridization to various probes.

Prime-cut probes were made by primed synthesis on M13 clones as described previously (2). Some M13 clones were from the DNA sequencing program (1). 406BC contains nucleotides 10,793 to 11,599 and 342BC contains nucleotides 11,294 to 11,526 of the B95-8 viral genome. HL158 contains approximately nucleotide 49,169 to exactly nucleotide 49,476, and HL130 contains approximately nucleotide 49,792 to exactly nucleotide 50,009. These clones are recombinants of M13 mp8 containing the inserts at the *Sma*I site. LEG96 contains nucleotides 148,813 to 149,079 and is a recombinant of M13 mp7 containing the insert at the *Hin*cII site. The other M13 clones were derived from the T1 cDNA (4) obtained from a cDNA library of Raji cytoplasmic poly(A)$^+$ RNA. Clones P and 20III are recombinants of M13 mp8 containing *Sma*I-*Pst*I fragments of the T1 cDNA. These fragments contain, respectively, nucleotides 296 to 1,007 and nucleotides 494 to 1,007 of the T1 cDNA. Clone 3BglI is a recombinant of M13 mp9 containing nucleotides 1 to 652 of the T1 cDNA. The insert is a *Pst*I-*Bgl*I fragment which was cloned between the *Pst*I and *Sma*I sites after digestion of the protruding *Bgl*I end.

The inserts of all the M13 clones were oriented in such a way relative to the primer that the prime-cut probes could hybridize to RNAs transcribed rightward from the viral genome.

Preparation of prime-cut probes with *Eco*RI and *Hin*dIII involves cutting at the restriction site in the M13 polylinker which is downstream of the insert so that probes called *Eco*RI or *Hin*dIII contain the whole EBV insert of the M13 clone. The probes derived from the B95-8 viral genome and from the T1 cDNA are shown in Fig. 1a and b, respectively.

S1 nuclease analysis with single-stranded, internally labeled, prime-cut probes was done as described previously (13). Each hybridization reaction was performed with 1 μg of poly(A)$^+$ RNA and 50,000 cpm of probe in 50% formamide–0.5 M NaCl–40 mM piperazine-*N*,*N'*-bis(2-ethanesulfonic acid) (PIPES) (pH 6.4)–1 mM EDTA overnight at 42°C. Digestion was done with 400 U of S1 nuclease for 30 min at 37°C in a volume of 0.2 ml.

Primer extension was done as described previously (2).

RESULTS

The analysis of open reading frames (1) and a summary of deduced mRNA structures in B95-8 cells (12) for the region of the EBV genome under study (0 to 50 kb) are shown in Fig. 1. The expanded parts of the map show the detailed exon structure and the relationship of the various single-stranded probes to the exons of EBV mRNA in the regions under study. The boundaries of the exon from nucleotides 11,333 to 11,479 shown in the first expansion were deduced from results presented below.

The B95-8 cDNAs T2 to T6 (5), which appear to include the B95-8 versions of EBNA-1 and EBNA-3 mRNAs, are partial clones but nevertheless extend 5' on the RNA as far as the U_S region of EBV. All the EBNA cDNAs sequenced so far from B95-8 and human LCLs contain the W1 and W2 exons. We first sought to establish whether all the B95-8 RNAs which contained W1 and W2 sequences started in the U_S region or whether certain members of the family started in the IR region, as has been reported in human LCLs. Probes from the Raji cDNA T1 (4) were convenient for this analysis, since several copies of W1 and W2 are present in this clone. When either of the overlapping probes 3BglI and 20III was used, a complex pattern of RNAs was detected in B95-8 and Raji cells (Fig. 2).

The sizes of RNAs detected in B95-8 cells ranged from 4.8 to 0.35 kb (Fig. 2a and b). Several RNAs were also seen in Raji cells, but the average size was smaller than that in B95-8 cells, ranging from 4.0 to 1.4 kb (Fig. 2a and b). The major bands in B95-8 cells were both broad bands around 3.4 and 4.0 kb in length and around 2.3 kb in length. In Raji cells the major broad band was around 2.8 to 3.3 kb in length. All of these broad bands appeared to be composed of multiple individual bands. When RNA from the tightly latent cell line IB4 was probed (Fig. 2f), a simpler pattern of RNAs, with the predominant band at 2.5 kb and other weak bands, was seen.

The M13 clone 406BC yielded a probe which covered the 5' ends of the B95-8 cDNAs (Fig. 1). 406BC produced the same pattern of hybridization with B95-8 RNA as did probes 3BglI and 20III (Fig. 2a, b, and c), although there is no sequence relationship between 406BC and T1 cDNA. This result implied that all of the RNAs containing exons W1 and W2 are represented among the RNAs originating in the U_S region. Since several cDNA clones (T2 to T6) were found to end within the region covered by 406BC, it seemed likely that the 5' end might be present within the area covered by this clone. It was noteworthy that 406BC did not hybridize to Raji cell RNA (Fig. 2c), indicating that the B95-8 U_S exons are absent in Raji cells.

On these gels, RNA and DNA do not migrate identically, but it is more reliable and convenient to use DNA markers. By running a separate gel (data not shown) with standard RNAs and DNAs, we established a correction curve, and all RNA sizes quoted are the corrected RNA values.

Preliminary Northern blotting experiments had indicated that probes 406BC-*Ava*II and 406BC-*Bgl*I both hybridized to the RNAs, so they were used as probes in S1 nuclease mapping experiments to define the exon boundaries. Fragments of both probes were protected from S1 nuclease digestion by B95-8 RNA (Fig. 3). The protected fragments were sized at about 148 base pairs (bp) for 406BC-*Ava*II (Fig. 3a, lane 3) and about 71 bp for 406BC-*Bgl*I (Fig. 3a, lane 7). Since the two probes only differed at one end and since 406BC-*Bgl*I was the shorter probe, the reduction in length of the protected fragment with 406BC-*Bgl*I meant that the *Bgl*I site lay within the exon hybridizing to the probes. 406BC-*Bgl*I protected a fragment of 71 bp, so the 3' end of the exon must have been 71 bp from the *Bgl*I site, i.e., at nucleotide 11,479 on the standard map. The longer probe, 406BC-

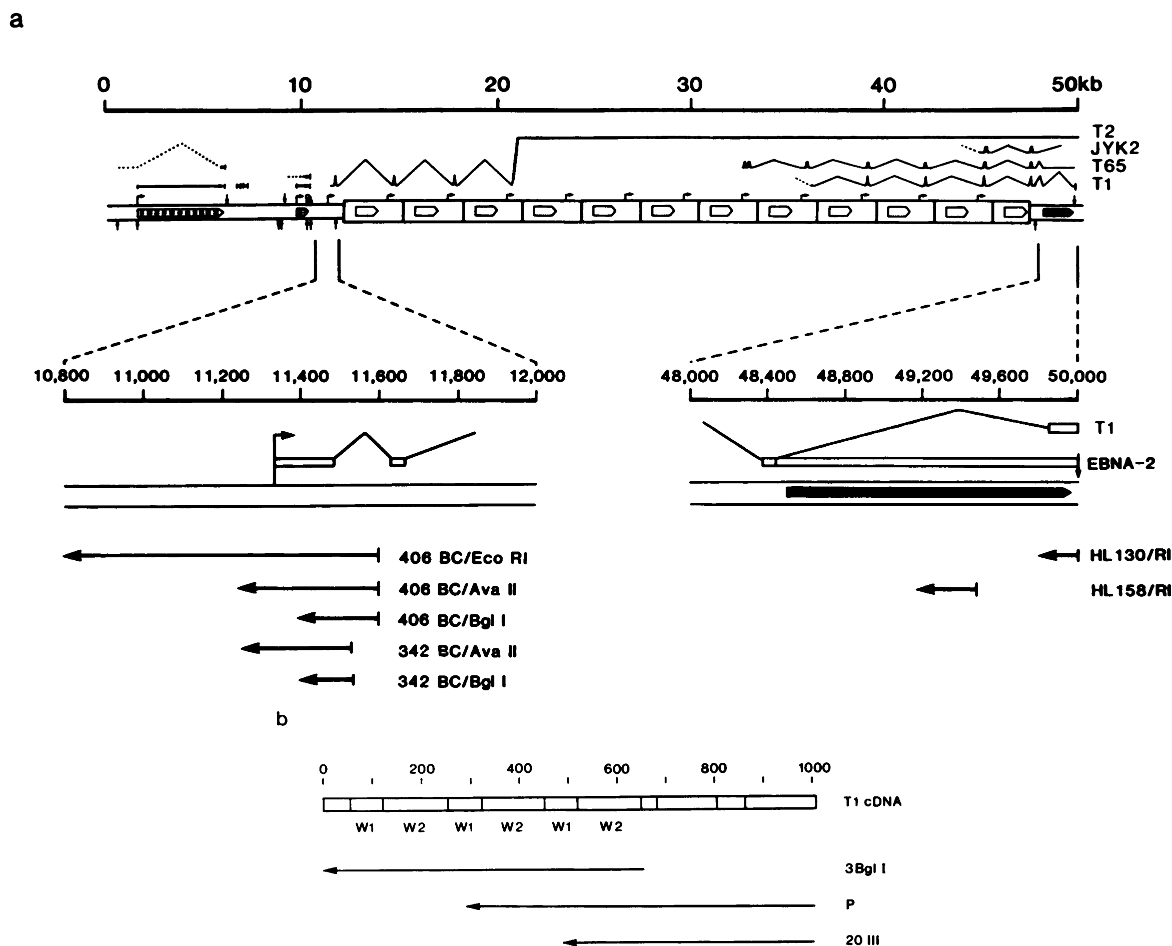

FIG. 1. (a) Region of the B95-8 genome under study with the deduced RNA structures, open reading frames, and poly(A) sites represented by the notations established previously (12). In the upper part of the drawing, below the scale in kilobases, the reading frames are shown as horizontal pointed boxes. Reading frames corresponding to latent-cycle genes have black shading, late productive-cycle genes have horizontal dashed shading, and unknown reading frames have no shading (12). RNAs are shown as horizontal arrows above and below the reading frames, and poly(A) sites (AAUAAA) are shown as small vertical arrows. The structures of several cDNAs that have been sequenced are shown: T2 (3), JYK2 (33), T65 (32), and T1 (4). Below this part of the drawing the relevant parts of the EBV genome are shown on an expanded scale, and the relationship of the mapping probes (406BC-*Eco*RI, etc.) used in this study to the RNA structures is shown. (b) Exon structure of the T1 cDNA (4) shown in relation to the probes 20III, 3BglI, and P used in this study. The vertical lines within the T1 cDNA box represent the exon boundaries. The W1 and W2 exons are indicated (the first exon is a partial copy of W2). Scale is in base pairs.

*Ava*II, protected the whole exon, producing a fragment of 148 bp. Since we have defined the 3′ end of the exon as being at nucleotide 11,479, this means that the 5′ end is at nucleotide 11,333. We show below that this is the true 5′ end of the RNA. Consistent results were obtained with probe 342BC-*Ava*II (Fig. 3b).

As predicted from the failure to detect this exon on Northern blots with 406BC in Raji cells, none of the S1 nuclease mapping experiments gave any evidence for this exon in Raji cell RNA (Fig. 3a, lanes 4 and 8, and Fig. 3b, lane 6).

The experiments with the 342BC probes were extended to examine RNA from B95-8 cells chemically induced into the productive cycle. Productive-cycle RNAs were dramatically induced by TPA treatment in B95-8 cells; see, for example, the late productive-cycle RNA detected at around position 149,000 on the genome with probe LEG96 (Fig. 2g and h). The S1 nuclease mapping experiments with 342BC showed little or no effect of TPA on the steady-state level of this exon, showing that it is not part of a productive-cycle RNA.

The S1 nuclease mapping experiments defined an upstream exon of the highly spliced RNAs but did not reveal whether this exon represented the true 5′ ends of the RNAs. Accordingly, probe 342BC-*Bgl*I was hybridized to B95-8 cell RNA and extended with reverse transcriptase in a primer extension analysis. Primer extension did occur, as shown by the band at approximately 241 nucleotides in Fig. 3c, lanes 2 to 4. This result indicates that the 5′ end of the RNA is about 76 bases 5′ of the *Bgl*I site, i.e., at about nucleotide 11,333, the same result as that defined by S1 nuclease mapping. These results show that about nucleotide 11,333 is the 5′ end of at least a significant fraction and possibly all of the highly spliced RNAs in B95-8 cells. The prime-cut probe used in this primer extension experiment produced a considerable amount of self-priming, resulting in the large band present at about 250 nucleotides. The specific primer extension product, present only when B95-8 RNA was present, was visible at 241 nucleotides.

Having defined a 5′ end, we now sought to relate some of the cDNAs to the pattern of RNAs observed in Northern

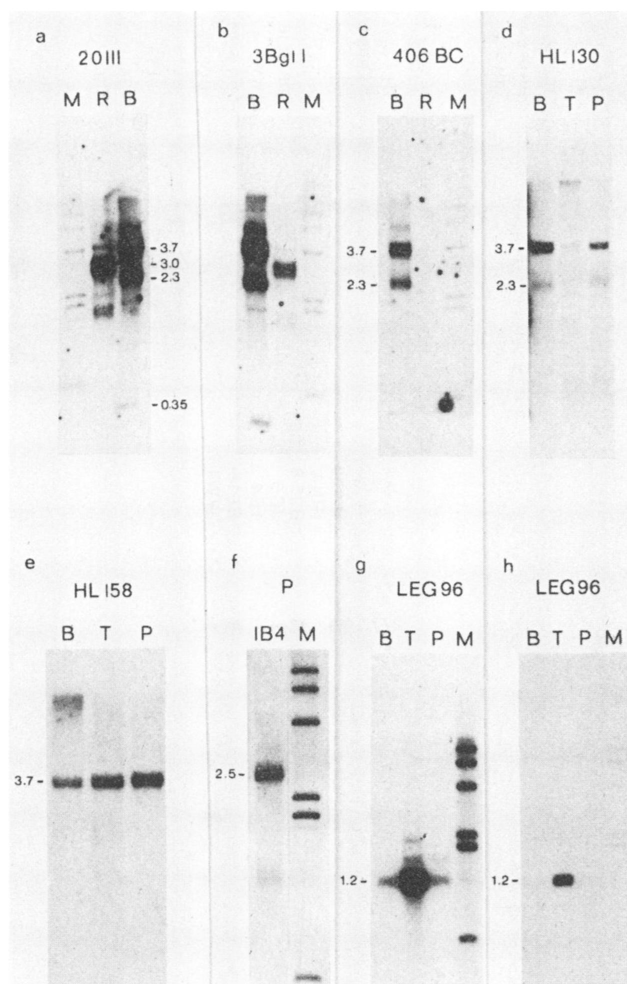

FIG. 2. Northern blots of B95-8, Raji, and IB4 RNAs with the various probes shown in Fig. 1. The RNA electrophoresed in each lane is indicated at the top of the lane: R, RNA from Raji cells; B, RNA from untreated B95-8 cells; T, RNA from B95-8 cells treated with TPA; P, RNA from B95-8 cells treated with TPA and PAA; and IB4, RNA from IB4 cells. The probes with which the filters were hybridized were as follows: (a) 20III-*Eco*RI; (b) 3BglI-*Hin*dIII; (c) 406BC-*Eco*RI; (d) HL130-*Eco*RI; (e) HL158-*Eco*RI; (f) P-*Eco*RI; (g and h) LEG96-*Eco*RI. Panel h is a lighter exposure of panel g. The radioactive lambda *Hin*dIII markers are shown in lanes M. The LEG96-*Eco*RI probe contains EBV nucleotides 148,758 to 149,080 in the *Hin*cII site of M13 mp7 and detects an abundant late productive-cycle RNA. Numbers represent kilobases.

blots. We used Northern blotting with short probes around the EBNA-2 region to understand which RNA detected with the 20III and 3BglI probes might correspond to EBNA-2 RNA.

Probe HL130 hybridized to B95-8 cell RNA detected most but not all of the RNAs detected by probes 20III and 3BglI (Fig. 2d). The largest RNA was not detected by HL130. This RNA may be the EBNA-1 RNA in view of the structure of some of the cDNA clones of EBNA-1 and the identical sizes of this RNA and the EBNA-1 RNA detected (data not shown) with probes in the EBNA-1-coding region. RNAs in both the 3.4- to 4.0-kb and 2.3-kb groups were detected by HL130. In contrast, probe HL158 detected only an RNA in the larger (3.4- to 4.0-kb) group. Probe HL158 lies within the EBNA-2 reading frame and is completely spliced out of the T1 cDNA clone. The observation that HL158 detected only an RNA in the larger group suggests that in B95-8 cells these include the EBNA-2 RNA. In view of its length, the T1 cDNA presumably corresponds to one of the shorter (2.3-kb) group of RNAs. On the basis of the length of the cDNA (3) which probably corresponds to EBNA-3 RNA, it would also appear that the EBNA-3 RNA belongs to the larger group.

In the HL130 and HL158 experiments, RNAs from control and TPA-treated B95-8 cells were used, and similar levels of the RNAs were detected in both induced and uninduced cells. There was occasionally variation in the levels of the RNAs detected (Fig. 2), but overall, TPA treatment made

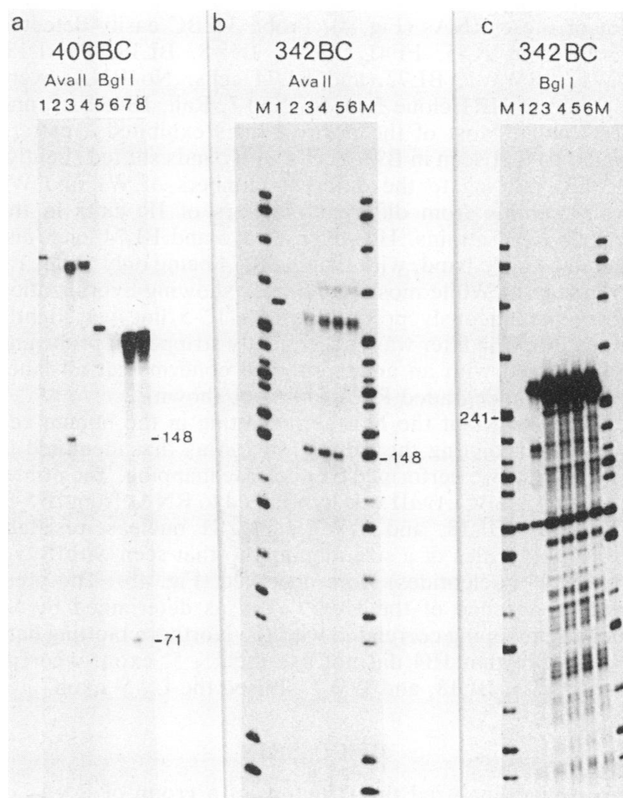

FIG. 3. Identification of the 5′ exon (nucleotides 11,333 to 11,479) of the highly spliced RNAs by S1 nuclease and primer extension analyses. (a) S1 nuclease analysis with probe 406BC-*Ava*II (lanes 1 to 4) or probe 406BC-*Bgl*I (lanes 5 to 8). Lanes 1 and 5 contain untreated probes. Other lanes contain S1 nuclease digestion products after hybridization of probes to tRNA as a negative control (lanes 2 and 6), RNA from untreated B95-8 cells (lanes 3 and 7), and RNA from untreated Raji cells (lanes 4 and 8). Sizes are interpolated from coelectrophoresed pBR322-*Msp*I size markers. (b) S1 nuclease analysis with probe 342BC-*Ava*II. Lane 1 contains untreated probe. Lanes 2 to 6 contain S1 nuclease digestion products after hybridization of probes to tRNA as a negative control (lane 2), RNA from untreated B95-8 cells (lane 3), RNA from TPA-treated B95-8 cells (lane 4), RNA from B95-8 cells treated with TPA and PAA (lane 5), and RNA from untreated Raji cells (lane 6). Markers (lanes M) are pBR322 DNAs digested with *Msp*I. (c) Primer extension analysis with probe 342BC-*Bgl*I. Lane 1 contains untreated probe. Lanes 2 to 6 contain primer extension products from hybridization of probes to tRNA as a negative control (lane 2), RNA from untreated B95-8 cells (lane 3), RNA from TPA-treated B95-8 cells (lane 4), RNA from B95-8 cells treated with TPA and PAA (lane 5), and RNA from untreated Raji cells (lane 6). Markers (lanes M) are pBR322 DNAs digested with *Msp*I. Numbers represent base pairs.

little difference in the levels of the EBNA family of RNAs, in contrast to the dramatic induction reproducibly observed for productive-cycle RNAs (Fig. 2g and h).

Our results so far have indicated that the B95-8 EBNA family of highly spliced RNAs starts primarily at nucleotide 11,333. Since neither Raji nor IB4 cells displayed the U_S exons, it was important to show that the U_S start was not unique to B95-8 cells, in which the virus is in marmoset cells rather than human cells. RNA was prepared from a large number of human cell lines carrying EBV. These included several Burkitt's lymphoma cell lines, tightly latent human lymphoblastoid cell lines, and spontaneously productive human LCLs. Probe 342BC corresponds closely to the 5′ exon mapped in the U_S region and was used in a Northern blot of these RNAs (Fig. 4). Probe 342BC easily detected RNAs from JC-5, FF41, Jijoye, B95-8, BL18, PRBL18, WW1, PRWW1, BL72, and BL74 cells. No RNAs were detected in HR1 clone 5 or 16, X50-7, Raji, IB4, ELI, and BL37 cells. Most of the positive lines exhibited a pattern similar to that seen in B95-8 cells with bands shifted slightly, perhaps relating to the different numbers of W1 and W2 exons coming from different numbers of IR units in the various EBV strains. However, Jijoye and BL74 each displayed a single band, with that in BL74 being only about 1.0 kb in length. While most of the lines showing hybridization were spontaneously productive, the JC-5 line was tightly latent (30). The filter was subsequently stripped of probe and rehybridized with an actin probe to confirm that all lanes contained undegraded RNA (data not shown).

To confirm that the Northern blotting in the human cell lines was detecting the same U_S exon as that identified in B95-8 RNA, we performed S1 nuclease mapping. The prime-cut probe 342BC-*Ava*II was hybridized to RNAs from B95-8, JC-5, IB4, BL18, and WW1 cells. S1 nuclease-resistant hybrid molecules of a size identical to that seen with B95-8 RNA (148 nucleotides) were observed (Fig. 4b). The presence or absence of the U_S 5′ exon as determined by S1 nuclease mapping correlated with the Northern blotting data (Fig. 4a) in that IB4 did not use the U_S 5′ exon, whereas B95-8, JC-5, BL18, and WW1 all used the U_S 5′ exon.

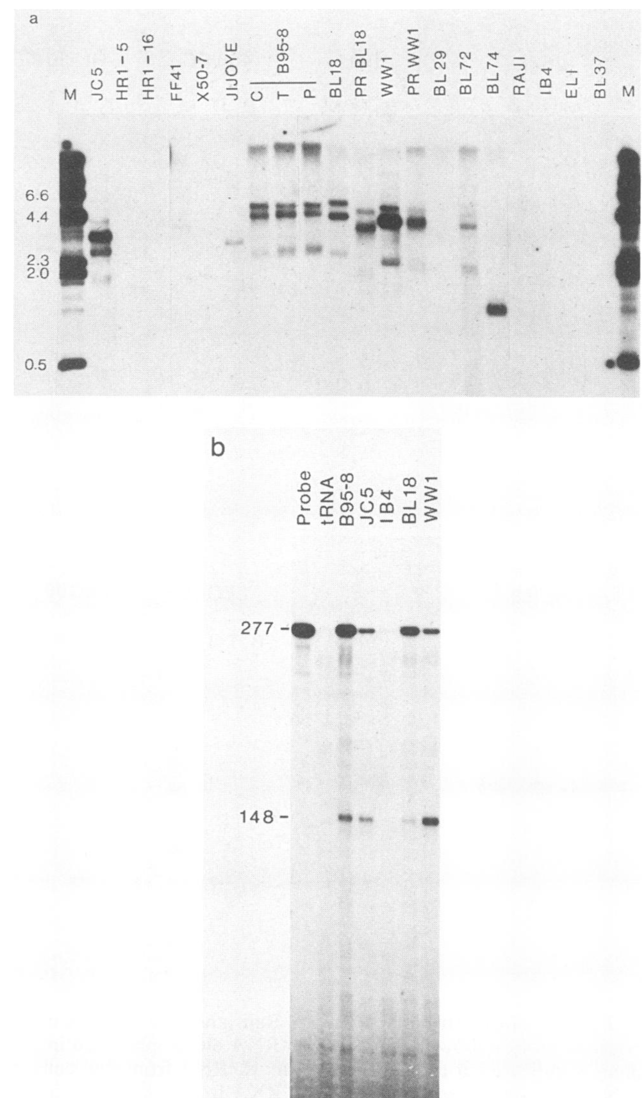

FIG. 4. (a) Northern blotting analysis with the 5′ exon probe. Cytoplasmic poly(A)$^+$ RNA (1 μg) from various cell lines was glyoxalated, electrophoresed in a 1% agarose gel, and blotted to nitrocellulose. The filter was hybridized with the prime-cut probe 342BC-*Ava*II. HR1-5 and HR1-16 are clone 5 and clone 16 of P3HR1 cells, respectively, and B95-8 cells were untreated (C), treated with TPA (T), or treated with TPA and PAA (P). Lanes M contain size markers (end-labeled *Hin*dIII digest of phage λ DNA). Numbers represent kilobases. (b) S1 nuclease analysis of the 5′ exon with probe 342BC-*Ava*II. Lanes contain untreated probe or S1 nuclease digestion products after hybridization to tRNA as a negative control, RNA from untreated B95-8 cells, JC-5 RNA, IB4 RNA, BL18 RNA, and WW1 RNA. Sizes were deduced from an *Msp*I digest of pBR322 DNA coelectrophoresed on the same gel. Numbers represent base pairs.

DISCUSSION

We have analyzed the structure of a group of RNAs of EBV. They are related in having highly spliced common 5′ leaders which are joined to unique 3′ exons. These RNAs probably encode the latent-cycle proteins EBNA-1, -2, and -3 and LP. Using Northern blotting, S1 nuclease mapping, and primer extension analysis, we have identified the transcription start of some or all of these mRNAs in B95-8 cells in the U_S region of the EBV genome at about nucleotide 11,333. A TATA box-like sequence (TACAAAA) is present about 30 bp upstream of the transcription start, and a CCAAT sequence is apparent at −70. The activity of this promoter may be influenced by the enhancer located in the *oriP* region of the virus genome, about 3.3 kb away (21, 27). Northern blotting and S1 nuclease analysis showed that this exon is used in a wide variety of EBV strains in human cells, including JC-5, BL18, and WW1. The JC-5 line is latently infected with EBV, but not all latently infected cells use the U_S start, IB4 and X50-7 being notable exceptions.

Our results are consistent with the analysis of B95-8 cDNA clones (3, 5). The cDNA clones show that a second, very short exon distal to the one detected by S1 nuclease mapping but still in the U_S region intervenes between the 5′ exon and the W1 and W2 exons (3, 5).

cDNA sequencing work (32) and in vitro transcription (36) have identified a promoter in the IR region of the virus with a transcription start at about nucleotide 14,384. This promoter apparently gives rise to a very short exon (W_0) which then splices into the W1 and W2 repeats. It is not strictly known whether this promoter is active in EBV-infected cells because the transcription start has only been demonstrated in vitro. Nevertheless, it seems likely that both the promoter in the U_S region and the potential promoter in the IR region

can give rise to mRNAs which contain the W1 and W2 exons in common in their highly spliced structures. One of the cDNAs sequenced by Sample et al. (32) can encode (38) both LP and EBNA-2 and contains the W_0 exon at its 5′ end.

We presently cannot tell whether the W_0 exon is used in B95-8 cells. Our extensive S1 nuclease mapping and Northern blotting experiments (unpublished data) around this region would probably not have detected such a short exon. The existence of the alternative promoters for the EBNA group of RNAs may imply some regulatory function for the two starts. The complexity of the virus-cell interaction that EBV displays at the level of gene expression has recently been illustrated in studies on the restricted expression of latent-cycle antigens in Burkitt's lymphoma cells (31). We suspect that differential use of the two promoters may be important for EBV in different cell types or different stages of B-cell differentiation, when perhaps the transcription factors available to the virus change.

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