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Epstein–Barr virus RNA VII: Size and direction of transcription of virus-specified cytoplasmic RNAs in a transformed cell line

(mRNA/small cytoplasmic RNA/growth transformation/recombinant DNA)

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ABSTRACT **At least three separate regions of the Epstein–Barr virus (EBV) genome encode RNA in a cell line that is growth transformed and nonpermissively infected with EBV. Six polyadenylylated cytoplasmic RNAs have been identified from these three regions. An abundant RNA 3.0–3.1 kilobases (kb) long is encoded by DNA of the internal reiteration, I_R, and DNA that maps at 25.7–30 megadaltons. A second, abundant, 2.9-kb RNA is primarily encoded by DNA at 110–03 megadaltons but probably has a 3′ end to the left of 110 megadaltons. A third, abundant, 3.7-kb RNA is largely encoded by DNA at 63–66 megadaltons and has a 5′ end to the left of 63 megadaltons. A less-abundant 1.5-kb RNA is also encoded by I_R. The least-abundant polyadenylylated RNAs identified are 2.3 and 2.0 kb. These RNAs have 3′ ends mapping at 5–7 megadaltons and 5′ ends mapping to the right of 7 megadaltons. The data suggest that there may be two additional polyadenylylated cytoplasmic RNAs, a 3-kb RNA mapping at 26.2–30 megadaltons and a minor RNA mapping at 102–110 megadaltons. An abundant 0.16-kb nonpolyadenylylated RNA is also present in the cytoplasm of IB-4 cells. This RNA precipitates from the cytoplasm in the presence of high concentrations of magnesium, indicating that it is complexed with protein or polyribosomes.**

Infection of normal B lymphocytes with Epstein–Barr virus (EBV) results in conversion of the cell to a state of perpetual growth (1, 2). The resultant cell lines frequently have normal karyotype and B-lymphocyte characteristics, including production of immunoglobulin and interferon (3). The viral genome is retained in the transformed cell through thousands of generations (4–6). Most of the viral DNA is in an episomal (7), closed circular (8), form. Some molecules are maintained in the same format as viral DNA except that the ends are covalently joined (9–11). Defective molecules formed by recombination between distant and nonhomologous regions of the viral genome are also frequently present (12). Expression of the viral genome is usually tightly restricted so that there is no detectable virus production. Nor can any of the antigens associated with virus replication such as early antigen, viral capsid antigen, or membrane antigen be detected. A virus-specified intranuclear antigen, EBNA, is invariably present, indicating that the viral genome is always active in the infected cell (13). One polypeptide component of EBNA is 48 kilodaltons (kDal) (14). A second antigen, LYDMA, has been detected on the cell surface but is yet uncharacterized (15).

To facilitate analysis of viral RNAs in nonpermissively infected, growth-transformed cells, cell lines were established by infection of neonatal lymphocytes with the B95-8 EBV isolate (9). This isolate was used because its DNA has been well characterized and cloned (11, 16, 17). The viral genome is maintained in a closed circular form in these cell lines but is otherwise similar in organization to viral DNA (9). Previous analysis of one of these cell lines, IB-4, indicated that, although antigen expression is limited, there is extensive transcription of EBV DNA (9). RNA encoded by at least 30% of EBV DNA can be detected (9). The DNA encoding these RNAs maps to many fragments (9). In contrast, polyribosomal RNA is selectively enriched for RNA encoded by 10% of EBV DNA and mapping at 5–30, 54–59, 63–66, and 110–03 MDal (for map positions, see Fig. 3) (9). At least 40% of the DNA of the 2-MDal internal reiteration (I_R) and at least 25% of the nearby fragment, *Bam*HI H (map positions 9.5–25.5 and 26.2–29.9 MDal respectively) encode polyribosomal RNA in IB-4 cells (9). The overall abundance of viral mRNA is low and constitutes approximately 0.06% of the polyadenylylated cellular RNAs (9). These results are similar to those obtained with two other nonpermissively infected cell lines, Namalwa and Raji, established by cultivation of Burkitt tumor cells (18–22). In the latter instance (22), RNA encoded by DNA at 54–59 MDal could not be detected until the cells were treated with IUdR and induced to express early antigen.

The purpose of the experiments described here was to determine the size and direction of transcription of the mRNAs, in IB-4 cells, encoded by the DNA mapping at 5–30, 63–66, and 110–03 MDal. In the course of these studies, we discovered a small cytoplasmic nonpolyadenylylated RNA that is also encoded by EBV DNA.

MATERIALS AND METHODS

Analysis of the Size of Cytoplasmic RNAs. IB-4 cells were cloned in soft agarose (9, 23). IB-4 and Loucks (an EBV-negative, B-lymphocyte cell line derived from a lymphoma) cells were harvested in logarithmic phase (9). Cytoplasm was prepared by differential centrifugation of cells lysed at 2°C in 0.01 M NaCl/0.02 M Tris·HCl, pH 7.4/0.25% Triton X-100/1.25% sucrose/0.01 M vanadyl ribonucleoside complex (24). RNA was purified (25). Polyadenylylated and nonpolyadenylylated RNAs were separated by two cycles of oligo(dT)-cellulose column chromatography (19).

The size of RNAs was determined from their electrophoretic mobility in agarose gels under denaturing conditions. RNA (5 μg) was denatured for 5 min at 60°C in 20 μl of 50% (vol/vol) recrystallized formamide and 2.2 M formaldehyde in electrophoresis buffer [0.02 M morpholinopropanesulfonic acid (Sigma), pH 7.0/5 mM sodium acetate/1 mM EDTA] and applied to 0.8% or 1.2% agarose gels containing 2.2 M formaldehyde in electrophoresis buffer with ethidium bromide (1 μg/ml). After electrophoresis at 50 V for 10–12 hr, the gel was soaked in 20× NaCl/Cit (NaCl/Cit is 0.15 M NaCl/0.015 M Na citrate). The RNA was transferred onto nitrocellulose paper (Millipore) by

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Abbreviations: EBV, Epstein–Barr virus; EBNA, E–B nuclear antigen; Dal, dalton(s); I_R, internal reiteration; NaCl/Cit, 0.15 M NaCl/0.015 M Na citrate; oligo(dN), oligodeoxynucleotide.

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using 10× NaCl/Cit (26), and the positions of marker RNAs were determined under UV illumination. ^{32}P-Labeled (11, 16) DNAs were hybridized to the RNA blots in 50% formamide/10% dextran sulfate/5× NaCl/Cit/0.02 M sodium phosphate, pH 6.5, containing 100 μg of denatured salmon sperm DNA per ml, 0.1% $NaDodSO_4$, and 0.02% Ficoll, polyvinylpyrrolidone, and bovine serum albumin for 15 hr at 50°C (27, 28). ^{32}P-Labeled denatured recombinant DNA (5–10 × 10^6 cpm) consisting of plasmid or phage DNA and a specific EBV DNA fragment (11) were used in most experiments. For some experiments, EBV DNA fragments mapping at 5–7 (*Eco*RI J), 7–11, 11–25 (*Bam*HI V), 102–106, and 106–110 MDal were separated from the vector by restriction enzyme digestion and agarose gel electrophoresis prior to labeling (9).

Preparation and Hybridization of cDNAs. Polyribosomal RNA was prepared from IB-4 cell cytoplasm by magnesium precipitation (29, 30), and "intact" polyadenylylated RNA was selected by two cycles of oligo(dT)-cellulose column chromatography (19). Oligo(dT)-primed cDNAs of different lengths and randomly primed cDNAs were synthesized by using avian myeloblastosis virus reverse transcriptase (R. Gallo, National Institutes of Health). Long oligo(dT)-primed cDNA (average size, 300 bases) was made by using intact polyadenylylated RNA as template. The longest oligo(dT)-primed cDNA (average size, 600 bases) was prepared by using intact polyadenylylated RNA as a template in the presence of 4 mM sodium pyrophosphate (31). Templates for the short oligo(dT)-primed cDNA (average size, 125 bases) and for random oligodeoxynucleotide (dN)-primed cDNA were made by treating polyadenylylated RNA for 10 min with iodoacetate-treated DNase I (RNase-free, Worthington) (20). This DNase had sufficient RNase activity that marker RNAs could no longer be distinguished in agarose gels. The polyadenylylated RNA was rechromatographed on an oligo (dT)-cellulose column to separate the poly(A)-containing 3′ end for short oligo(dT)-primed cDNA synthesis from the rest of the RNA which was used as a template for randomly [oligo(dN)] primed cDNA synthesis (32). Oligo(dT)-primed cDNAs were size-fractionated on preparative alkaline sucrose velocity gradients (33). Aliquots of each fraction were sized on alkaline agarose gels relative to *Hin*fI restriction fragments of pBR322 DNA (34, 35). Labeled cDNAs were hybridized to nitrocellulose blots of agarose gels of cloned EBV restriction fragments at 72°C for 36 hr in 6× NaCl/Cit containing 1 mg of denatured salmon sperm DNA per ml, 1 mM EDTA, 0.5% $NaDodSO_4$, and 0.2% Ficoll, polyvinylpyrrolidone, and bovine serum albumin. Blots were washed in decreasing concentrations of NaCl/Cit in 0.1% $NaDodSO_4$ at 72°C.

RESULTS

Size of Cytoplasmic RNAs. The size of cytoplasmic RNAs was determined from electrophoretic mobility in denaturing agarose gels relative to lymphocyte ribosomal RNA, *Escherichia coli* ribosomal RNA, brome mosaic virus RNAs, and *E. coli* tRNA (36, 37). To facilitate detection of EBV mRNAs, the polyadenylylated RNAs were separated from the bulk of the cytoplasmic ribosomal RNA by chromatography on oligo(dT)-cellulose columns. The position of EBV RNAs in the gels was determined by blotting the RNAs onto nitrocellulose filters and hybridizing the filters to labeled EBV recombinant DNAs. To detect EBV-specific cytoplasmic RNAs that were not polyadenylylated, blots of the RNA that did not bind to oligo(dT)-cellulose columns were included in each hybridization. Blots of cytoplasmic RNAs of a non-EBV-infected B lymphoblastoid cell line (Loucks) were also included in each hybridization to ensure that the recombinant DNAs did not hybridize to a cellular RNA.

Attempts to detect EBV RNAs with labeled viral DNA were unsuccessful, presumably as a consequence of the greater kinetic complexity of whole genomic DNA. *Bam*HI V (11–25 MDal), X (25–26.2 MDal), and H (26.2–30 MDal) probes iden-

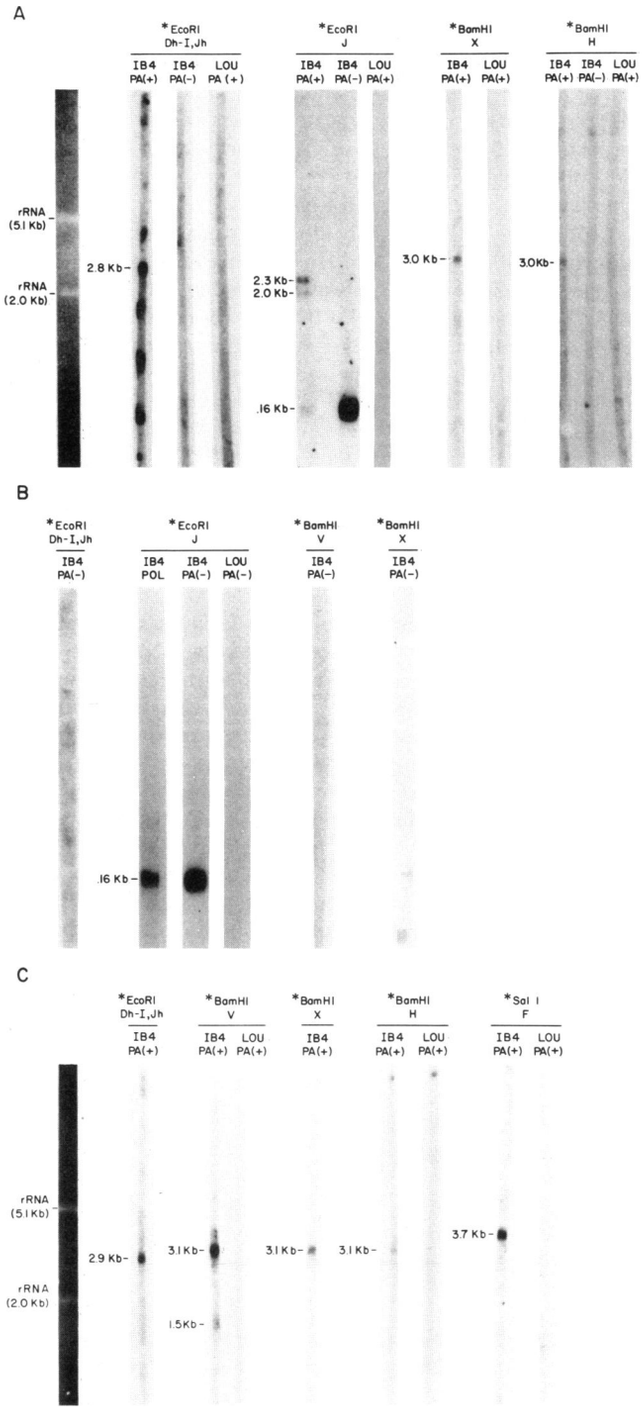

FIG. 1. Size of EBV-specified RNAs in the cytoplasm of IB-4 cells. ^{32}P-Labeled recombinant DNA probes (indicated by *) were hybridized to blots of denaturing agarose gels of IB-4 (IB4) or Loucks (LOU) polyadenylylated [PA(+)] or nonpolyadenylylated [PA(−)] cytoplasmic RNA or IB-4 polyribosomal (POL) RNA. Loucks cells are an EBV-negative lymphoblastoid cell line, and hybridization of probes to blots of RNA prepared from them served as a negative control. Sizes of RNAs were determined by comparison of electrophoretic mobility to that of marker RNAs. The blots shown under the *Eco*RI J probe in *A* were made from another similar gel in which the 2.3- and 2.0-kb polyadenylylated RNAs were more clearly identified. Left-hand lane in *A* and *C* shows ethidium bromide fluorescence with IB-4 RNA.

tified polyadenylylated RNA(s) 3.0–3.1 kb long (Fig. 1). The 3.0- to 3.1-kb RNA(s) identified by these three probes were not resolved as more than one species in any of the gels. *Bam*HI V also identified a less-abundant polyadenylylated 1.5-kb RNA. Polyadenylylated RNAs larger than 8 and 10 kb became evident on long exposures of blots hybridized to labeled *Bam*HI H (data not shown) and are presumed to reflect leakage of nuclear RNAs. *Sal* I F (62–67 MDal) and *Eco*RI Dhet–I, Jhet (110–03 MDal) identified polyadenylylated RNAs of 3.7 and 2.9 kb, respectively. None of these probes hybridized to any RNA on blots of nonpolyadenylylated IB-4 RNA or on blots of Loucks cytoplasmic polyadenylylated RNAs.

*Eco*RI J (5–7 MDal) readily identified an abundant nonpolyadenylylated 0.16-kb RNA in IB-4 cytoplasm (Fig. 1). A much smaller amount of this RNA was detected in polyadenylylated RNA. The amount of 0.16-kb RNA in IB-4 polyadenylylated RNA was compatible with the contamination of polyadenylylated RNA with other nonpolyadenylylated RNAs, including ribosomal RNAs. This small RNA is also present in polyribosome and ribonucleoprotein complexes prepared by magnesium precipitation (Fig. 1*B*). On long exposure of the blot of IB-4 polyadenylylated RNA hybridized to labeled *Eco*RI J, 2.3- and 2.0-kb RNAs in lower abundance were also detected. *Eco*RI J did not hybridize to any polyadenylylated or nonpolyadenylylated Loucks cytoplasmic RNA. No additional RNAs were detected with labeled *Bam*HI C (5–11 MDal).

Direction of Transcription of RNAs Relative to EBV DNA. To determine the direction of synthesis of RNAs, the DNA encoding the 3′-terminal 125, 300, and 600 nucleotides and the DNA encoding the bulk of the viral polyadenylylated polyribosomal RNAs were mapped. For these experiments, cDNA was synthesized from polyadenylylated polyribosomal RNA templates with oligo(dT) as a primer specific for the 3′ end or with oligo(dN) as a random primer. The cDNAs were hybridized to Southern blots of restriction enzyme fragments of cloned EBV DNAs (Fig. 2). The shortest oligo(dT)-primed cDNA (125 bases) hybridized to fewer fragments than did the other cDNAs and therefore presumably identifies fragments encoding 3′ ends of RNAs. The longer oligo(dT)-primed cDNA (300 and 600 nucleotides) hybridized to all the DNA fragments to which oligo(dN)-primed cDNA hybridized. As expected, these cDNAs hybridized more strongly than the oligo(dN)-primed cDNA to the DNA fragments identified as encoding 3′ ends of RNAs. The results of hybridization of the 125- and 300-nucleotide oligo(dT)-primed and oligo(dN)-primed cDNAs are shown in Fig. 2. The results of the 600-nucleotide oligo(dT)-primed cDNA hybridizations (data not shown) were intermediate between those obtained with the 300-nucleotide oligo(dT)-primed cDNA and oligo(dN)-primed cDNA.

Randomly primed cDNA hybridized to the 61.6–63 and 63–66 MDal components of *Sal* I F; oligo(dT)-primed cDNAs hybridized only to the 63–66 MDal component of *Sal* I F (Figs. 2 and 3). These data indicate that the RNA encoded by *Sal* I F is transcribed from left to right.

Randomly primed cDNA hybridized to the DNA on both sides of the *Bam*HI site at 110 MDal in the *Eco*RI Dhet–I, Jhet fragment, but more strongly to the DNA to the right of this site (110–03 MDal) (Figs. 2 and 3). Progressively shorter oligo(dT)-primed cDNAs hybridized increasingly to DNA to the left of this site (106–110 MDal). However, even the shortest oligo(dT)-primed cDNA hybridized to the DNA mapping at 110–03 MDal as well as to DNA at 106–110 MDal. Therefore, either the 3′ end maps within 106–110 MDal and very close to the restriction site at 110 MDal or the 3′ end of one RNA is within the 110–03 MDal fragment and there is an additional RNA that has a 3′ end mapping within 106–110 MDal. Longer oligo(dT)- and oligo(dN)-primed cDNAs hybridized to a limited extent to DNA at 106–110 MDal (Figs. 2 and 3) and also to DNA mapping even farther left, at 102–106 MDal (data not shown), suggesting that there is an additional RNA encoded by this region but that this second RNA is probably a minor species. Attempts to detect this putative minor RNA on RNA blots by using labeled 102–106 or 106–110 MDal DNA have not been successful. Because of the extensive hybridization of short oligo(dT)-primed cDNA to DNA at 106–110 MDal, it is likely that this identified the 3′ end of an abundant RNA. The direction of transcription of the major RNA mapping largely within 110–03 MDal is therefore likely to be from right to left.

The analysis of the direction of transcription of RNAs encoded by DNA at 5–30 MDal is even more complex. The DNA fragments from this region were divided into segments with map coordinates 4.5–5, 5–7, 7–11, I_R (*Bam*HI V, 11–25), 25–25.7, 25.7–26.2, 26.2–26.6, 26.6–26.9, 26.9–29, and 29–30 MDal (Figs. 2 and 3). I_R was further divided into components

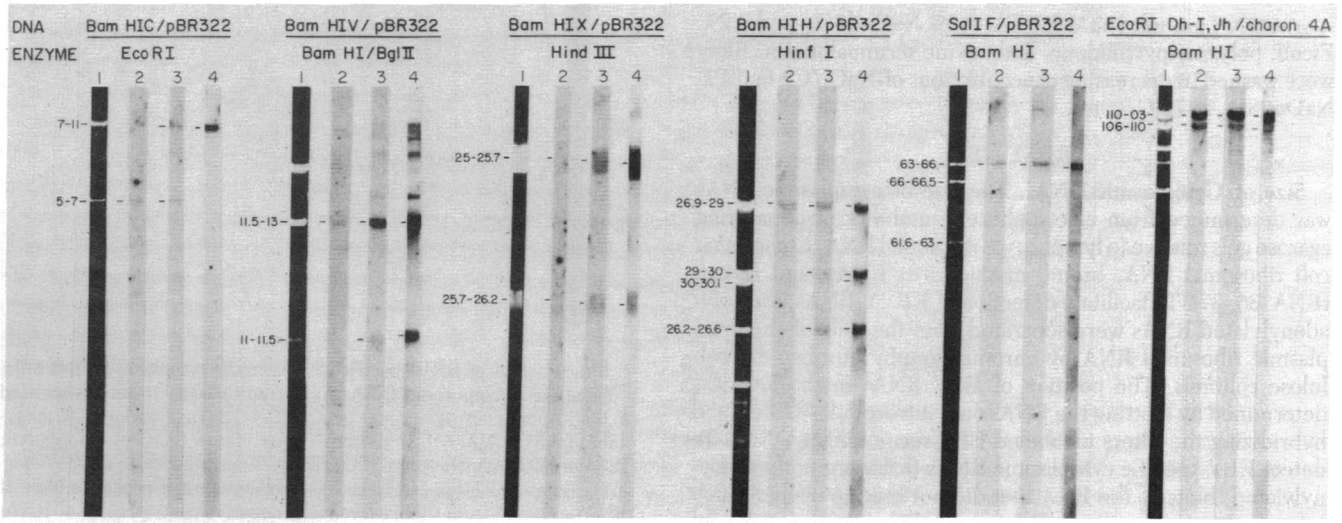

FIG. 2. Hybridization of cDNAs to blots of gels of EBV recombinant DNAs. Lane 1 is the UV-illuminated ethidium bromide-stained gel. Map coordinants (in MDal) of the fragments are given relative to the map in Fig. 3. Hybridization of 3′-end specific 125-nucleotide and 300-nucleotide oligo(dT)-primed cDNAs in lanes 2 and 3, respectively. Hybridization of oligo(dN)-primed cDNA made from RNA after removal of the 3′-polyadenylylated ends is shown in lane 4. The lines to the left of lanes 2–4 indicate where hybridization was observed in the original autoradiograms.

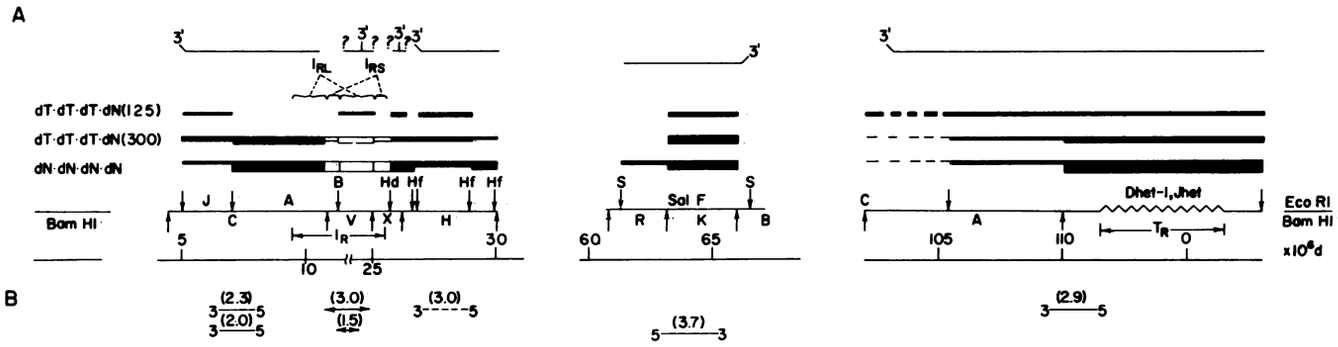

FIG. 3. Summary and interpretation of the data shown in Figs. 1 and 2. (*A*) Restriction enzyme map of three regions of the EBV genome which encode cytoplasmic RNA in IB-4 cells, with map coordinants below (as MDal). Unlabeled arrows above the line indicate *Eco*RI restriction sites; arrows below the line indicate *Bam*HI restriction sites. Other restriction sites identified are: *Bgl* II, B; *Hin*dIII, Hd; *Hin*fI, Hf; and *Sal* I, S. I_R, internal repetition; T_R, terminal repetition. For simplicity, only one copy of *Bam*HI V is shown in I_R. Above the map, the results of hybridization of cDNAs are indicated by a bar directly over the fragment to which the cDNAs hybridized. The height of each bar indicates the relative intensity of hybridization. At the top of *A*, the location of 3′ ends of RNA based on hybridization of the shortest oligo(dT)-primed cDNA is shown. The lines are drawn the full width of the fragment to which the cDNA hybridized. I_{RL} and I_{RS} are the large and small fragments, respectively, produced by cleavage of I_R with *Bam*HI and *Bgl* II. I_R begins in *Bam*HI C and ends in *Bam*HI X. Most of I_{RL} is contained in *Bam*HI C from 9.5 to 11 MDal and most of I_{RS} is in *Bam*HI X between 25 and 25.5 MDal. It cannot be determined from these data whether the RNAs are encoded by I_{RL} and I_{RS} which are in proximity to unique sequences in *Bam*HI C and *Bam*HI X, respectively, or by repetitions of I_R. This ambiguity is indicated by open bars in the diagram. In the region of the genome from 11 to 27 MDal, there is such extensive transcription that the direction of transcription of mRNAs for which 3′ ends have been mapped cannot be determined. This is indicated by 3′ ends flanked by question marks. (*B*) Tentative map of EBV mRNAs in IB-4 cells, showing size (in kb) and orientation.

I_{RL} and I_{RS}. The I_R begins in *Bam*HI C, and most of I_{RL} is in *Bam*HI C. The I_R ends in *Bam*HI X and most of I_{RS} is in *Bam*HI X. The oligo(dT)-primed cDNA (125 bases) hybridizes to four fragments mapping at 5–7, I_{RL}, 25.7–26.2, and 26.9–29 MDal but not to DNA mapping between these fragments, indicating that each of these four fragments contains DNA that encodes part of the 3′-terminal 125 nucleotides of RNA.

With regard to the most leftward (2.3 and 2.0 kb) RNAs that are identified by labeled 5–7 MDal DNA on the RNA blots (Fig. 1), shorter oligo(dT)-primed cDNA hybridized more intensively to the 5–7 MDal component and much less to the 7–11 MDal component of *Bam*HI C. Longer oligo(dT)-primed cDNAs and oligo(dN)-primed cDNAs hybridized extensively to DNA at 7–11 MDal and much less to DNA mapping at 5–7 MDal, indicating that the 2.3- and 2.0-kb RNAs are probably transcribed from right to left (Figs. 2 and 3). Hybridization of cDNAs to the I_{RL} component of *Bam*HI C are unlikely to affect this interpretation because all of the cDNAs hybridize to a similar extent to the I_{RL} component of *Bam*HI V (Fig. 2). Also, contamination of the polyadenylylated RNA with the 0.16-kb nonpolyadenylylated RNA would not interfere with this analysis because labeled 7–11 MDal DNA does not hybridize to the 0.16-kb RNA on RNA blots.

With regard to the most rightward part of this region, only longer oligo(dT)-primed cDNAs or oligo(dN)-primed cDNAs hybridize extensively to the 29–30 MDal component of *Bam*HI H, indicating that RNA from this region is also transcribed from right to left (Figs. 2 and 3).

With regard to the remainder of the 5–30 MDal data, however, little can be deduced. Longer oligo(dT)-primed cDNA and oligo(dN)-primed cDNAs hybridized extensively to all of the fragments between 7 and 30 MDal (Figs. 2 and 3). Because of the interspersion of 3′-end encoding fragments between fragments that also encode mRNA, it is not possible to determine the direction of transcription of the other RNAs with 3′ ends near I_{RL} and 25.7–26.2 MDal from these data.

DISCUSSION

Previous mapping data with polyribosomal RNA from IB-4 cells which indicate that these RNAs are encoded by DNA that maps at 7–30, 63–66, and 110–03 MDal (9) are confirmed by these more precise cDNA mapping experiments (Figs. 2 and 3) and by the demonstration on RNA blots of six viral specified cytoplasmic polyadenylylated RNAs from these three regions. There may be two other mRNAs. DNA mapping at 102–110 MDal may encode a minor RNA. Another additional mRNA may be encoded by the region 7–30 MDal. The evidence in support of another RNA from this region is as follows. First, DNAs from I_R (9.5–25.5 MDal), *Bam*HI X (25–26.2 MDal), and *Bam*HI H (26.2–30 MDal) all hybridize extensively to a 3.0- to 3.1-kb polyadenylylated RNA. A 1.5-kb polyadenylylated RNA is detected by labeled I_R but not by *Bam*HI X or H. Previous data indicate that at least 40% of I_R (2.5 kb) and 25% of *Bam*HI H (3 kb) encode polyribosomal RNA (9). The region of *Bam*HI X from 25.7 to 26.2 MDal which does not contain I_R also encodes polyribosomal RNA. Thus, the 4.5 kb of polyadenylylated RNA identified from this region on RNA blots is at least 1 kb and possibly as much as 1.8 kb (assuming that the region 25.7–26.2 MDal fully encodes mRNA) short of estimates of the complexity of RNA from this region. Second, short oligo(dT)-primed cDNA maps the polyadenylylated end of mRNAs to three sites within this region; to I_{RL}, to 25.7–26.2 MDal, and to 26.9–29 MDal (Fig. 3). Longer oligo(dT)-primed cDNA and oligo(dN)-primed cDNA fill the gaps between the three shorter oligo(dT)-primed cDNA mapping sites and extend to the right of the most rightward site. These data therefore also suggest that there are at least three RNAs from this region. Only two RNA sizes have been identified. The simplest model suggested by these data (Fig. 3) is that the 1.5-kb RNA is almost entirely encoded by I_R; the 3.0- to 3.1-kb RNA has a 3′ end in I_R but is largely encoded by *Bam*HI X and H; there is another RNA encoded by *Bam*HI H which has a 3′ end at 26.9–29 MDal and a 5′ end mapping near 29–30 MDal. The intensity of hybridization of oligo(dN)-primed cDNA to the fragment mapping at 29–30 MDal suggests that this RNA is not a minor species (Fig. 2). Because only the 3.0- to 3.1-kb RNA is detected by hybridization of the *Bam*HI H probe to RNA blots (Fig. 1), the putative additional RNA may also be of this size.

From the amount of unique sequence DNA needed to detect

each of the mRNAs on identical RNA blots in several independent experiments (such as Fig. 1), and from the extent of hybridization of the cDNA probes to separated viral DNA fragments (such as Fig. 2), we estimate that the 3.0- to 3.1-kb, 2.9-kb, and 3.7-kb RNAs are more abundant (in that order); the 1.5-kb RNA less abundant; and the 2.3- and 2.0-kb RNAs are least abundant. The most easily detected and presumably most abundant viral specific polypeptide in nonpermissively infected cells is the 48-kDal component of EBNA (12). All of these cytoplasmic polyadenylylated RNAs (possibly with the exception of the 1.5-kb RNA) are sufficiently long to encode the 48-kDal polypeptide. However, EBNA is likely to be encoded by an abundant RNA such as the 3.0- to 3.1-, 2.9- or 3.7-kb RNA.

Whether there are only six mRNAs encoded by these three regions of EBV DNA in nonpermissively infected growth transformed cells or, as the data suggest, there are two additional RNAs, it is apparent that there is considerably more extensive expression of the EBV genome in these cells than is indicated by the immunologic studies previously published. The RNA mapping data confirm that several regions of the EBV genome encode mRNA in these cells. Together, these data indicate that the maintenance of growth transformation is a complex phenomenon as was suggested by earlier studies of viral RNA (9, 18–21) and by the sensitivity of virus induction of growth transformation to UV irradiation of the virus (38).

The finding of an abundant virus-specified nonpolyadenylylated 0.16-kb RNA in the cytoplasm of IB-4 cells was a surprising outcome of this study. The 0.16-kb nonpolyadenylylated RNA is more abundant in the cytoplasm of Raji cells than in IB-4 cells (22) and accounts for the observation that iodinated cytoplasmic RNA from Raji cells hybridizes almost exclusively to the *Eco*RI J fragment of EBV DNA (39). The size of this RNA is similar to that of small cellular and adenovirus VA RNAs (40–42). Most of these small RNAs are predominantly nuclear and are transcribed by RNA polymerase 3. The function of these RNAs is uncertain. Several of the cellular RNAs and VA RNAs have homology to splice sites and are presumed to facilitate splicing of RNA within the nucleus (43). At least one small RNA, U6 RNA, does not have homology to splice sites in heterogeneous nuclear RNA but localizes to perichromatin granules (40). The abundance of the 0.16-kb EBV-specified RNA in the cytoplasm of IB-4 and Raji cells suggests that it may play an important role in maintaining nonpermissive infection or growth transformation. A characteristic of nonpermissively infected cells is the presence of RNA in the nucleus encoded by many regions of EBV DNA (9, 18–22). The 0.16-kb nonpolyadenylylated RNA could play a role in selective processing or selective transport of RNAs from the nucleus to the cytoplasm. Alternatively, the 0.16-kb RNA could play a more direct role in cellular growth transformation by altering cellular gene regulation.

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