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Mapping of Epstein–Barr Virus Proteins on the Genome by Translation of Hybrid-Selected RNA from Induced P3HR1 Cells and Induced Raji Cells

RUDOLF SEIBL AND HANS WOLF[1]

Max von Pettenkofer Institute, Pettenkoferstr. 9a, 8000 Munich 2, Federal Republic of Germany

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RNA was isolated from induced P3HR1 cells which synthesize Epstein-Barr virus (EBV) particles and therefore a full set of early and late antigens and from induced Raji cells which synthesize only early EBV proteins and hybridized to cloned EBV-DNA fragments spanning the entire genome. Bound mRNA was eluted and translated *in vitro* with rabbit reticulocyte lysate. The translation products were analyzed on SDS-polyacrylamide gels either directly or after immunoprecipitation with human sera. Most proteins could be mapped to short defined regions of the EBV genome using short restriction fragments and overlapping sheared fragments and there is evidence of splicing for some mRNA species. The synthesis of five early proteins can be seen only with hybrid-selected RNA from induced Raji cells. These mRNAs seem to be enriched in the cells restricted to early antigen synthesis.

INTRODUCTION

No efficient lytic system is available for the production of Epstein-Barr virus (EBV), thus the major sources of viral RNA, viral proteins, and virus particles are lymphoblastoid cell lines. Although all cell lines used contain viral genomes in every single cell, some lines cannot produce virus. For example Raji, which is originally derived from a Burkitt's lymphoma, synthesizes only the EBV nuclear antigen (EBNA). Others, e.g., B95-8 (*in vitro* transformed marmoset lymphocytes) or P3HR1 (also derived from a Burkitt's lymphoma) spontaneously produce early antigen (EA), virus capsid antigen (VCA), and membrane antigen (MA), but only in a small almost constant fraction of cells. After induction with chemicals, such as butyric acid (Luka *et al.*, 1981) or phorbol esters (TPA) (zur Hausen *et al.*, 1978), the percentage of cells synthesizing EBV antigens increases in producer cell lines. Raji cells only synthesize EA after induction (Lenoir, 1979).

Virus produced by P3HR1 cells, but not that derived from B95-8 cells, can be used to superinfect Raji cells which then enter into a lytic cycle of virus replication. On the other hand, P3HR1 virus has lost its ability to transform lymphocytes. This may correlate with a deletion in the left part of the EBV genome, since the transforming capacity can be rescued after replacement of the lost DNA by recombination (Skare *et al.*, 1981; Stoerker *et al.*, 1983). The consequences on molecular level of a 13-kb deletion in the right part of the B95-8 DNA are still obscure.

Transcribed regions on the EBV genome in noninduced and iododeoxyuridine-induced Raji cells (King *et al.*, 1981) and EBV proteins in Raji cells induced by butyric acid and TPA (Mueller-Lantzsch *et al.*, 1979; Kawanishi *et al.*, 1981b; Bayliss *et al.*, 1983; Kallin and Klein, 1983) have been identified. EBV proteins in the productive cycle have been studied in induced P3HR1 cells (Mueller-Lantzsch *et al.*, 1979; Kallin *et al.*, 1979; Kawanishi *et al.*, 1981a; Edson and Thorley-Lawson, 1981) and extensively in superinfected Raji cells (Bayliss and Wolf, 1981, 1982). In B95-8 cells (Hummel and Kieff, 1982a) and in a cellular subclone of P3HR1 cells (Weigel and Miller, 1983), EBV mRNAs have been mapped to specific restriction fragments

[1] Author to whom requests for reprints should be addressed.

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of the EBV DNA. By hybrid-selected translation with RNA from B95-8 cells, the coding regions for many EBV proteins have been mapped on the genome (Hummel and Kieff, 1982b).

We have mapped EBV proteins of induced P3HR1 cells and early EBV proteins of induced Raji cells by translating hybrid-selected mRNA using cloned EBV-DNA fragments spanning the entire genome. Many proteins could be mapped more precisely and differences between the EBV strains could be documented. A repeat region in the EBV *Bam*H1 K fragment which is transcribed from EBV DNA (Heller *et al.*, 1982a) encodes part of EBNA (Hennessy and Kieff, 1983) and hybridizes to cellular DNA (Heller *et al.*, 1982b). This fragment selected a translatable mRNA from EBV-negative cells which would indicate that the homologous region within the cellular DNA is also transcribed.

MATERIALS AND METHODS

Tissue culture and RNA preparation. P3HR1, Raji, and BJAB cells were grown in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal calf serum. P3HR1 and Raji cells were induced with 40 ng/ml TPA and 3 m*M* butyric acid. For *in vivo* labeling of proteins, the cells were labeled 3 days after induction in methionine-free MEM with 50 μCi [^{35}S]methionine (NEN) per milliliter for 4 hr. After washing, the cells were lysed in immunoprecipitation buffer.

RNA was prepared by lysing the cells 2 days after induction with 4 *M* guanidine isothiocyanate (Fluka), 0.5 *M* 2-mercaptoethanol, and 50 m*M* sodium acetate, pH 5.5 (Chirgwin *et al.*, 1979). The lysate was centrifuged for 1 hr at 20,000 rpm in a SW-41 rotor (50,000 *g*) and the supernatant layered on top of 2 ml CsCl (density 1.8 g/cm^3) in 10 m*M* triethanolamine and 1 m*M* EDTA, pH 7.4. After centrifugation for 17 hr at 150,000 *g* and 15° the RNA pellet was dissolved in water, extracted with chloroform/4% isoamyl alcohol, and precipitated with ethanol.

EBV DNA. The following cloned EBV-DNA fragments were isolated using standard procedures: Overlapping, sheared fragments from the B95-8 strain of EBV cloned in Charon 4A (Buell *et al.*, 1981). *Bam*H1 restriction fragments from the B95-8 strain cloned in pBR 322 (Skare and Strominger, 1980). Subclones from Charon 4A EB 90-99 (M 3-1-1, M 3-5-6) cloned in pUC8 (Motz and Wolf, unpublished results). Restriction fragments from EBV-strain M-ABA containing the region which is deleted in B95-8 (H3-D1, H3-K2, H3-D2) and the terminal fragment (966-20) from circularized M-ABA DNA (Polack *et al.*, 1984).

Hybrid selection. DNA (8 μg) was sonicated 3 × 60 sec to give DNA fragments of approximately 1000 bp, incubated for 10 min at 100° and spotted on a nitrocellulose filter (about 5 × 5 mm, Schleicher & Schüll BA85). The filter was dried, washed in 6× SSC (0.9 *M* NaCl, 0.09 *M* Na_3-citrate), and baked for 2 hr at 80°. Two filters were hybridized in 100 μl 65% formamide (BRL), 0.4 *M* NaCl, 10 m*M* PIPES (Sigma), pH 7.0, containing 100 μg total cellular RNA for 2.5 hr at 52°. The filters were washed extensively with 1× SSC, 0.5% SDS (62°), and 2 m*M* EDTA. Bound mRNA was eluted by boiling the filters for 75 sec in 300 μl double-distilled water, and was ethanol precipitated together with 20 μg calf liver tRNA (Boehringer-Mannheim).

In vitro translation, immunoprecipitation, and SDS-PAGE. The RNA was *in vitro* translated with a mRNA-dependent rabbit reticulocyte lysate prepared according to Pelham and Jackson (1976). [^{35}S]Methionine (NEN) was used as radioactive amino acid. The probes were either loaded directly onto 10 or 12.5% SDS-polyacrylamide gels (Bayliss and Wolf, 1981) after heating with electrophoresis sample buffer (50 m*M* Tris, pH 7.0, 2% SDS, 5% 2-mercaptoethanol, 30% sucrose) or mixed with immunoprecipitation buffer (1% Triton X-100, 0.1% SDS, 0.137 *M* NaCl, 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 10% glycerol, 20 m*M* Tris, pH 9.0; 0.01% NaN_3 and 1 m*M* PMSF), and used for immunoprecipitation (Bayliss *et al.*, 1983). Five microliters of a pool of human sera from patients with nasopharyngeal carcinoma

(EBNA 1:400, EA 1:1200, VCA 1:6600) was used for one assay after preincubation with a protein extract from 5×10^6 unlabeled EBV-negative BJA-B cells. The immune complexes were bound on protein A-Sepharose (Pharmacia), washed, eluted by boiling the beads in electrophoresis sample buffer, and loaded onto the SDS-polyacrylamide gels. These were stained, fixed, dried, and exposed to ^3H ultrofilm (LKB).

RESULTS

Mapping of Proteins in Induced P3HR1 Cells

Total cellular RNA from induced P3HR1 cells was isolated and translated *in vitro* with rabbit reticulocyte lysate. The translation products were immunoprecipitated and EBV-specific proteins were identified on SDS-polyacrylamide gels. Immunoprecipitated translation products with RNA from EBV-negative BJA-B cells were used as control. A large number of *in vitro* translated EBV proteins ranging from 150 to 18 kDa were identified.

After a first comparison with immunoprecipitations of *in vivo* labeled induced P3HR1 cells most proteins seemed to comigrate with a protein of the same molecular weight (Fig. 1). However, using a panel of different sera for comparative immunoprecipitation we could not confirm the identity of the *in vivo* labeled and the *in vitro* translated proteins of the same molecular weight with the exception of the proteins 150, 143, 138, and 90 kDa. The latter proteins were recognized or not recognized by all sera we have tested in a coordinated way from the *in vitro* translation products and the *in vivo* labeled cell lysates. All other proteins failed to react with all sera in this strict sense. Either monospecific sera or monoclonal antibodies are necessary to prove immunologically the relationship of the *in vitro* translated proteins and their *in vivo* labeled counterparts.

After hybridization of the P3HR1 RNA to cloned EBV-DNA fragments and *in vitro* translation of the bound mRNA all identified EBV proteins could be mapped to discrete regions of the genome (Fig. 2).

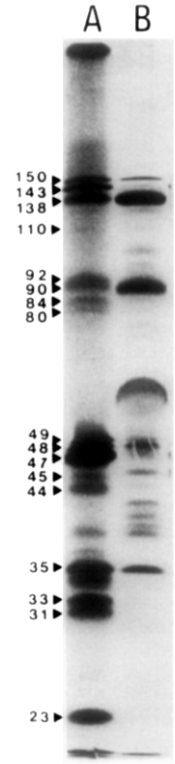
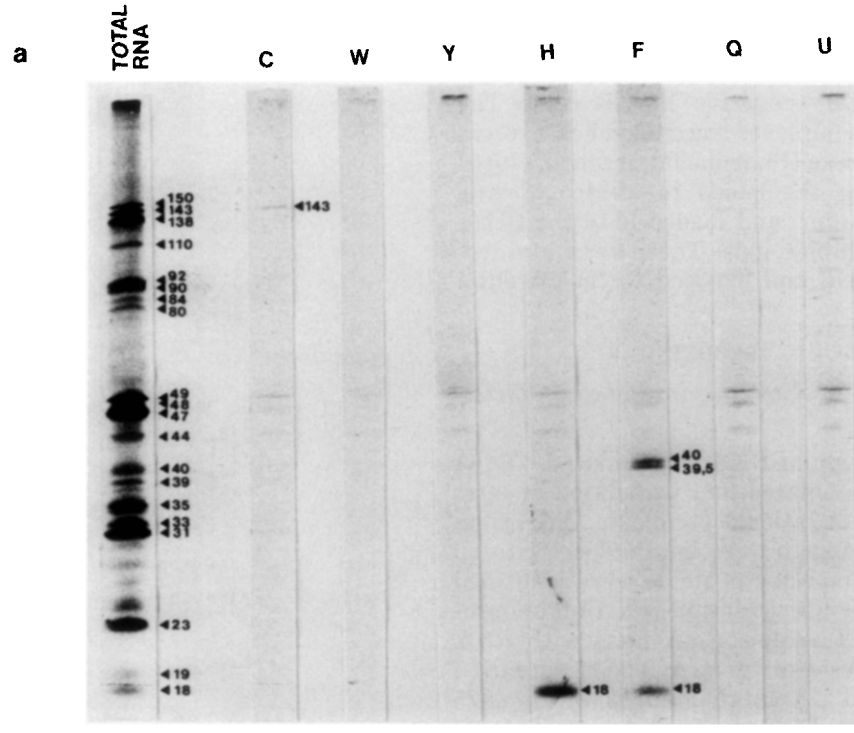


FIG. 1. Comparison of *in vivo* labeled and *in vitro* translated EBV proteins. A: *In vitro* translation of RNA from P3HR1 cells. B: Extract of *in vivo* labeled P3HR1 cells. The proteins were immunoprecipitated and resolved on a SDS-polyacrylamide gel. The apparent molecular weights are given in daltons $\times 10^{-3}$.

As compared to translation from unseparated RNA more EBV proteins could be identified because some mRNA species were enriched by the hybridization procedure and a few translated proteins were only visible after separation from the most prominent bands. Moreover different proteins of the same molecular weight seem to exist which are resolved only after mapping to different parts of the genome. Using the *Bam*H1 fragments, two fragments which subdivide the *Bam*H1 A fragment and the overlapping sheared fragments, the coding regions of most proteins could be mapped to narrow regions of the genome.

Using fragments from the M-ABA strain spanning the deletion in the B95-8 strain and a plasmid containing the *Eco*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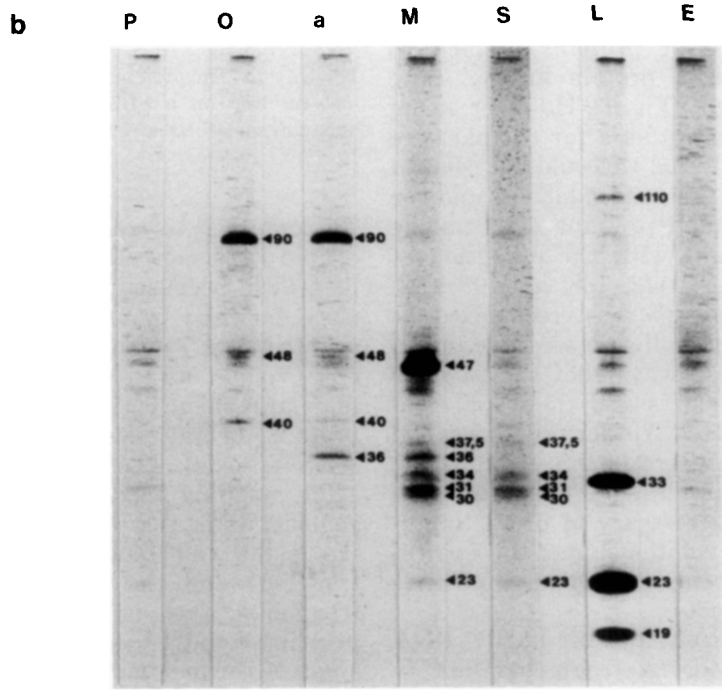
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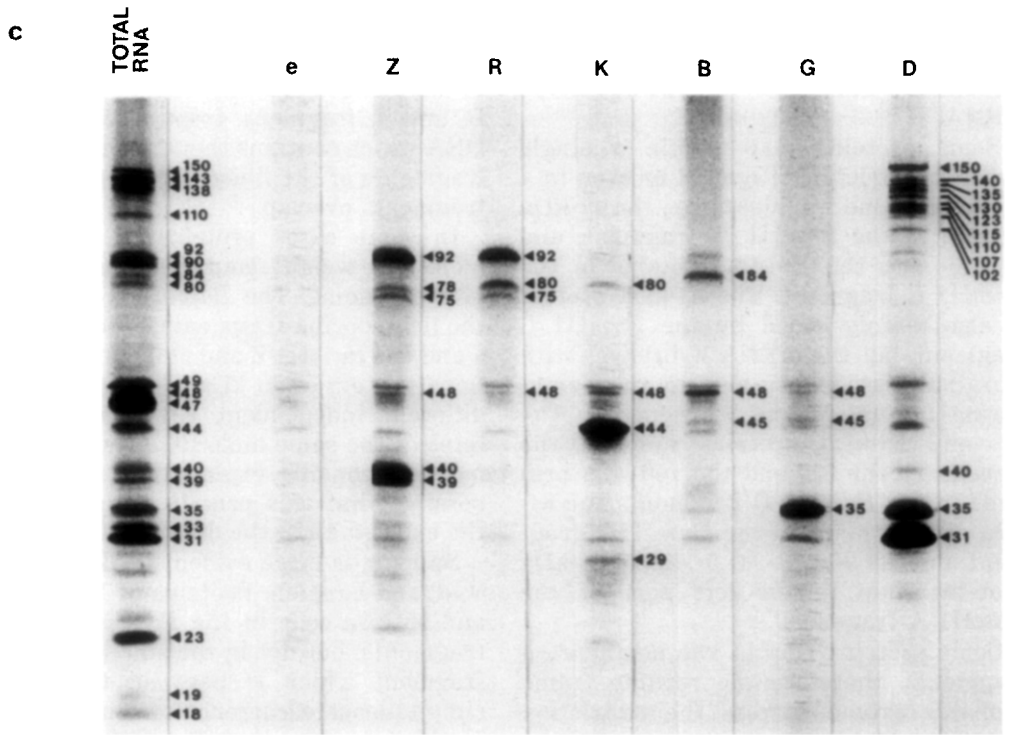
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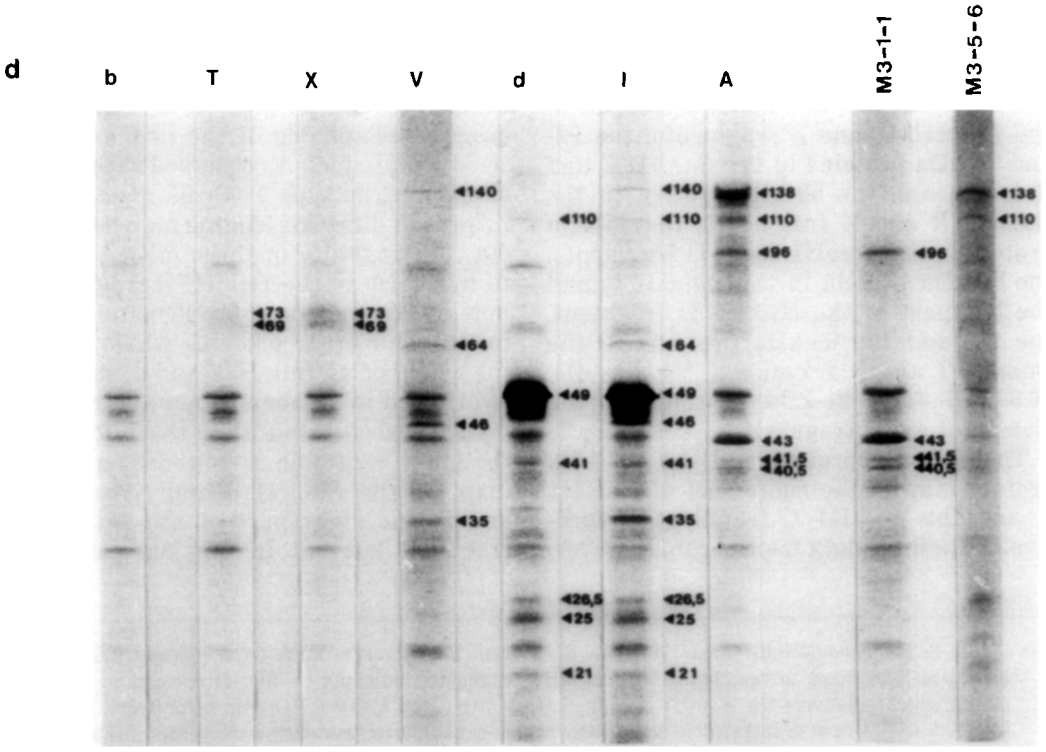
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D and *Eco* I fragment from circular M-ABA DNA no additional protein could be detected by translation of hybrid-selected mRNA.

Some proteins map within a single *Bam*H1 restriction fragment or even in a segment of one fragment, e.g., the 47-kDa protein in the *Bam*H1 M fragment, and the 33- and the 19-kDa proteins in the *Bam*H1 L fragment. The 23-kDa protein is also mainly coded by the *Bam*H1 L fragment, but the mRNA hybridizes with marginal significance also to the neighboring *Bam*H1 M and S fragments. The 44- and 29-kDa proteins map in the *Bam*H1 K, the 31- and the 150-kDa proteins in the *Bam*H1 D fragment. The 84-kDa protein maps in the *Bam*H1 B fragment and the 96-, 43-, 41.5-, and 40.5-kDa proteins map in the left part of the *Bam*H1 A fragment.

Some proteins map in two neighboring fragments, therefore the reading frame probably extends across the restriction site. The 18-kDa protein maps in the *Bam*H1 H and the left part of the *Bam*H1 F fragment, the 90-kDa protein in the *Bam*H1 O and a fragments, the 36-kDa protein in the *Bam*H1 a and M fragments, the 37.5-, 34-, 31-, and 30-kDa proteins in the *Bam*H1 M and S fragments, the 92- and 75-kDa proteins in the *Bam*H1 Z and R fragments, the 80-kDa protein in the *Bam*H1 R and K fragments, the 45-kDa protein in the *Bam*H1 B and G fragments, the 35-kDa protein in the *Bam*H1 G and the left part of the *Bam*H1 D fragment, the 73- and the 69-kDa proteins in the *Bam*H1 T and X fragments, the 49-, 41-, 26.5-, 25-, and 21-kDa proteins in the *Bam*H1 d and I fragments.

The 138-kDa protein and the 143-kDa protein map in the right part of *Bam*H1 A and the *Bam*H1 C fragment, respectively. Their reading frames can, however, expand to the terminal *Bam*H1 fragments which we have not tested. It is not possible to answer this question using the *Eco*R1 D and I fragment from circular EBV DNA which contains the terminal *Bam*H1 fragments of the linear DNA because the fragments overlap.

In some cases proteins of the same molecular weight map in different parts of the genome. The *Bam*H1 F fragment, the neighboring fragments *Bam*H1 O and a and the *Bam*H1 Z and D fragments code for a protein of 40 kDa. The existence of different independent EBV-specific proteins of the same molecular weight is the most reasonable explanation. It is also possible that this protein is spliced and the exons map in the different fragments.

Splicing is more evident in the *Bam*H1 V, d, and I region. Proteins of 140, 64, 46, and 35 kDa code in the *Bam*H1 V and I fragments, but not in the small *Bam*H1 d fragment which is between them. The simultaneous occurrence of four proteins in the two separated *Bam*H1 fragments suggests a splicing event. The four proteins may be either the result of incorrect termination or initiation of the translation or due to the presence of partly and completely spliced mRNAs or due to heterogeneous splicing of the primary transcription product. As observed with herpes simplex virus type 1 (Frink *et al.*, 1983), introns of different lengths may be spliced out. The inability of these mRNA species to hybridize to the *Bam*H1 d fragment is not due to technical problems since the mRNAs for other proteins which map in the neighboring *Bam*H1 I and d fragments hybridized in reasonable quantities.

It is also possible that the mRNA for the 110-kDa protein is spliced. The protein maps in the *Bam*H1 d and I fragments and in the right part of the *Bam*H1 A fragment but not in the left part. The

FIG. 2. Hybrid-selected translation with RNA from P3HR1 cells. RNA from induced P3HR1 cells was hybridized to the cloned EBV-*Bam*H1 fragments indicated by the letters and to two clones which subdivide the *Bam*H1 A fragment (M 3-1-1 and M 3-5-6, see Fig. 6 for map). The selected mRNA was translated *in vitro* with rabbit reticulocyte lysate. The translated proteins were immunoprecipitated and analyzed on SDS-polyacrylamide gels. The EBV proteins are indicated in each fragment with their molecular weights in daltons $\times 10^{-3}$.

110-kDa protein from the *Bam*H1 L fragment is probably independent.

The mRNA for the 48-kDa protein also seems to be spliced. *Bam*H1 restriction fragments O, a, Z, R, K, B, and G code for a protein of this size. If the 48-kDa protein encoded by *Bam*H1 Z, R, K, B, and G is a single protein the mRNA has to be spliced, the 48-kDa protein mapping in the *Bam*H1 O and a fragments may be unrelated to this protein.

Mapping of Proteins in Induced Raji Cells

All proteins mapped with RNA from induced Raji cells are assumed to be early proteins. RNA from induced Raji cells was hybridized to cloned EBV-DNA fragments. The bound mRNA was eluted and translated *in vitro* with rabbit reticulocyte lysate. The translation products were analyzed in SDS-polyacrylamide gels without immunoprecipitation to identify even early proteins against which antibodies are not present in the human sera used. Thirteen proteins could be identified (Fig. 3). The 18-kDa polypeptide in the *Bam*H1 fragments H and F and the 40- and 39.5-kDa proteins in the *Bam*H1 F fragment are probably identical to the corresponding proteins in induced P3HR1 cells. Similarly, the 47-kDa protein in Charon 4A EB 38-47 and EB 45-54, the 90-kDa protein in EB 38-47, the 48-kDa protein in EB 61-72 and EB 69-79, and the 73- and 69-kDa proteins in EB 75-84 and EB 83-93 seem to be identical to corresponding, more precisely mapped proteins in induced P3HR1 cells.

The 47-, 41-, and 35-kDa proteins in the *Bam*H1 F fragment, the 96-kDa protein in Charon 4A EB 53-61, and the 105-kDa protein in Charon 4A EB 83-93 could not be identified among the proteins in induced P3HR1 cells because the early mRNAs for these proteins are enriched in induced Raji cells. The 105-kDa protein is the only one of them which could not be immunoprecipitated. None of these five proteins is visible after hybrid selected translation with RNA from P3HR1 cells and direct resolution of the translation products on SDS-polyacrylamide gels without immunoprecipitation.

Identification of a Translatable mRNA in EBV-Negative BJA Cells, Which Hybridizes to EBV DNA

RNA from EBV-negative BJA-B cells was hybridized to cloned EBV-DNA fragments, and the bound mRNA was eluted and translated *in vitro*. The translation products were analyzed on SDS-polyacrylamide gels. The EBV *Bam*H1 K fragment, a part of the Charon 4A EB 61-72, selects cellular mRNA which can be translated *in vitro* into two proteins of 92 and 84 kDa (Fig. 4). This fragment is the only one which selects a translatable cellular mRNA (Fig. 5). These two proteins were also translated with mRNA from P3HR1 and Raji cells transcribed from the EBV-*Bam*H1 K fragment or the homologous cellular sequences. Translation of hybrid-selected RNA from highly induced P3HR1 or Raji cells, however, failed to detect the respective proteins.

DISCUSSION

A map summarizes the identified EBV proteins in induced Raji and induced P3HR1 cells (Fig. 6). Coding regions are drawn with maximum exclusion of nonhybridizing DNA. With the exception of slight differences in the molecular weight most proteins have also been mapped with RNA from B95-8 cells (Hummel and Kieff, 1982b). We have mapped most polypeptides more precisely using smaller restriction fragments and overlapping sheared fragments covering the entire EBV genome. Some additional proteins could be identified, others were not detected in our experiments. This could be due to different expression of the EBV genome in the cell lines. P3HR1 and Raji cells are human lymphoblastoid cell lines, whereas B95-8 is an *in vitro* transformed marmoset cell line. The pattern of EBV proteins in P3HR1 and B95-8 cells shows some differences (Bayliss and Wolf, unpublished observations). This is well documented for the major glycoproteins. B95-8 cells produce mostly gp350 whereas P3HR1 cells produce mostly gp220. The unglycosylated precursor for the gp350 is 135 kDa, that

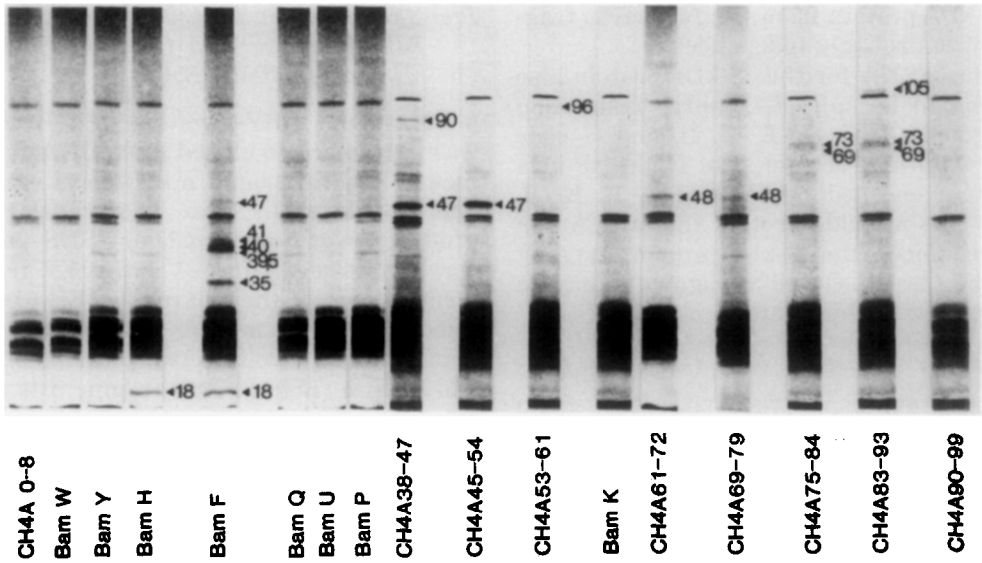

FIG. 3. Hybrid-selected translation with RNA from chemically induced Raji cells. RNA from induced Raji cells was hybridized to cloned EBV-*Bam*H1 fragments and to sheared DNA fragments cloned in Charon-4A as indicated (for map, see Fig. 6). The selected mRNA was translated *in vitro* with rabbit reticulocyte lysate and the translation products were analyzed on an SDS-polyacrylamide gel. The identified proteins were indicated in each fragment with their molecular weights in daltons $\times 10^{-3}$.

for the gp220 is less than 120 kDa, but they share amino acid sequences (Edson and Thorley-Lawson, 1983). By hybrid-selected translation with RNA from B95-8 cells, a 135-kDa protein, the precursor of gp350, and a 100-kDa protein, the precursor of gp220, was mapped in the *Bam*H1 L fragment (Hummel *et al.*, 1984). With RNA from P3HR1 cells, we could map a 110-kDa protein—the potential precursor of gp220—to the *Bam*H1 L fragment.

We could not select translatable RNA with the *Bam*H1 W, Y, Q, U, and P fragments. The *Bam*H1 Y fragment is deleted in P3HR1 DNA, so no protein is expected. No transcript could be mapped to the *Bam*H1 Q, U, and P fragment (Hummel and Kieff, 1982a; Weigel and Miller, 1983) and to the *Bam*H1 W fragment no polypeptide could be mapped in producing B95-8 cells (Hummel and Kieff, 1982b). However, we could map proteins in the *Bam*H1 S fragment, which seems to be silent in B95-8 cells.

It was not possible to identify gene products of EBV expressed during latency with hybrid-selected translation. No EBV-specific protein could be mapped to the EBV genome using RNA from uninduced Raji cells and none of the mapped proteins from P3HR1 cells and induced Raji cells could be correlated to EBV-specific proteins expressed during latency.

In some restriction fragments the coding capacity is too small to code for all the proteins we mapped. There are several possible explanations.

(i) Some proteins may be artificial products of the *in vitro* translation due to incorrect termination or internal initiation. This may have happened preferentially with the mRNA of the *Bam*H1 D fragment coding for the 150-kDa protein. At least some of the protein ladder 140–102 kDa are shorter fragments of the 150-kDa protein because they were not visible in other experiments.

(ii) Unspliced and partly spliced mRNA species may be present in the RNA preparation and may produce longer or shorter (if a present intron contains a stop codon)

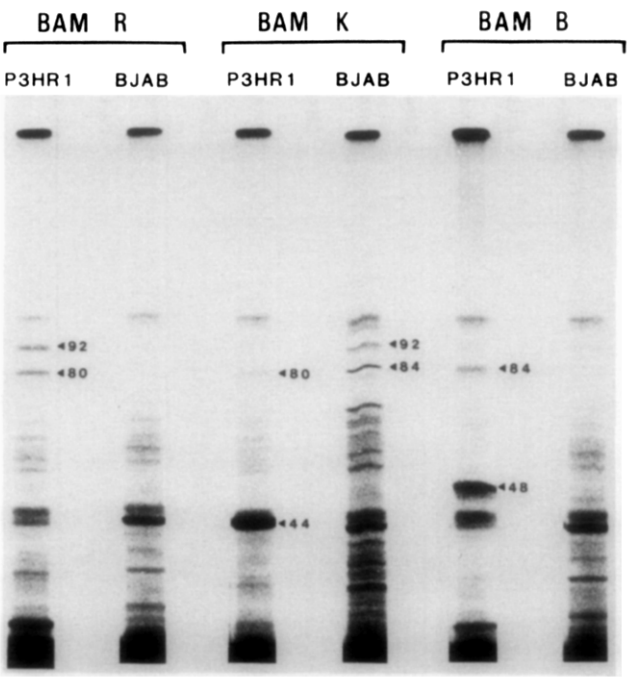

FIG. 4. Hybrid-selected translation with RNA from P3HR1 and BJA-B cells. RNA from EBV-negative BJA-B cells and from induced P3HR1-cells was hybridized to the EBV-*Bam*H1 fragments R, K, and B, and the selected mRNAs were analyzed as described in Fig. 3. From induced P3HR1 cells, the same population of mRNAs as in Fig. 2 hybridized to the cloned *Bam*H1 fragments. Due to the higher background without immunoprecipitation, not all proteins are visible. The *Bam*H1 K fragment, but not the neighboring fragments, selects mRNAs which can be translated *in vitro* into proteins of 92 and 84 kDa. These proteins are not visible after hybrid-selected translation with RNA from induced P3HR1 cells due to the effects of the inducing agents.

proteins than the completely spliced mRNA. For example, the 140-, 64-, 46-, and 35-kDa proteins are probably related to each other and represent parts of the 140-kDa protein.

(iii) If the mRNA is spliced, only a short stretch in the DNA fragment in question has to be represented in the transcript to bind the mRNA, which is translated into the entire protein.

(iv) Overlapping mRNA species which are translated into different proteins and mRNA species which include the coding regions for different independent polypeptides may result in a particular protein pattern in some fragments. These phenomena have been described for herpes simplex virus type 1 (Anderson *et al.*, 1981; Draper *et al.*, 1982; McLauchlan and Clements, 1983; Frink *et al.*, 1983; Costa *et al.*, 1983). Some proteins with slightly differing molecular weights coding in the same fragment (e.g., 40- and 39.5-kDa proteins in the *Bam*H1 F, 40- and 39-kDa proteins in the *Bam*H1 Z, and 73- and 69-kDa proteins in the *Bam*H1 T and X fragments) may be a result of initiation of translation at different sites. This is also documented for herpes simplex virus type 1 (Marsden *et al.*, 1983).

Recent S1-mapping data (T. Gibson, P. Stockwell, M. Ginsberg, and B. Barell, personal communication) suggest that mRNA species containing the information for two proteins are also transcribed from EBV DNA. Early in infection, an open reading frame of 2.7 kb is transcribed rightward from a promotor in the *Bam*H1 O fragment to a polyadenylation signal in the *Bam*H1 a fragment. The same reading frame is also transcribed from a second

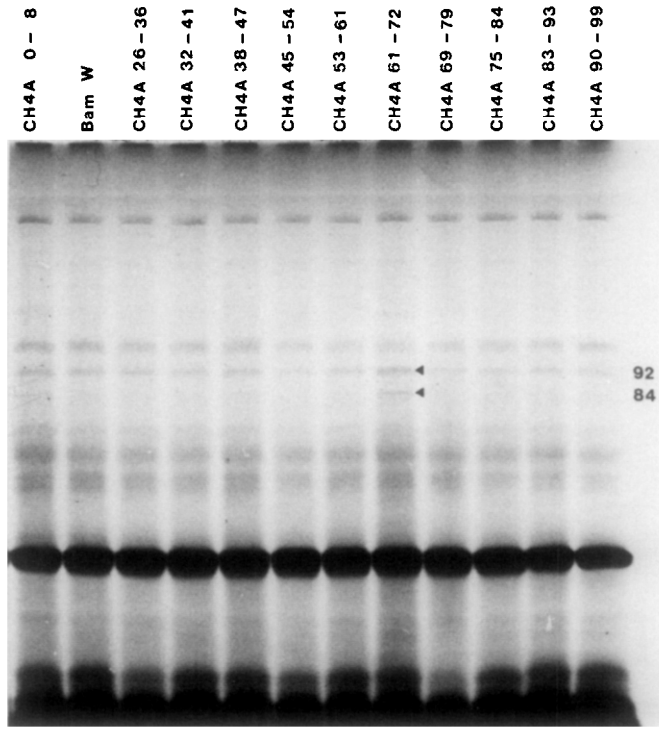

FIG. 5. Hybrid-selected translation with RNA from BJA-B cells. RNA from EBV-negative BJA-B cells was hybridized to sheared EBV-DNA fragments cloned in Charon 4A and to a segment of the large internal repeat cloned as *Bam*H1 fragment in pBR 322. The selected mRNA was analyzed as described in Fig. 3. Only the Charon 4A EB 61-72, which contains the *Bam*H1 K fragment selects mRNA, which can be translated into proteins of 92 and 84 kDa.

promotor at least 1.0 kb further upstream from the first one. The additional sequence contains a second open reading frame of about 1.2 kb. The 2.7-kb reading frame is presumably translated into a 90-kDa polypeptide, as we mapped a protein of 90 kDa to the *Bam*H1 O and a fragments. The 1.2-kb reading frame is with marginal significance translated into a polypeptide of 40 or 48 kDa. Though the 1.2-kb reading frame is contained completely within the *Bam*H1 O fragment, the described mRNA would hybridize both to the *Bam*H1 O and *Bam*H1 a fragments. Therefore, after hybrid-selected translation, a protein of 40 or 48 kDa would map in the *Bam*H1 O and a fragments as detected in our experiments. The observation that cytoplasmic polyadenylated mRNAs of 4.5 and 3.1 kb mapped in the *Bam*H1 O and a fragments (Hummel and Kieff, 1982a) support our suggestion.

By hybridization of RNA from EBV-negative BJA-B cells to the cloned *Bam*H1 K fragment we could select a cellular mRNA which is translatable *in vitro* to proteins of 84 and 92 kDa. This fragment contains an unusual repeat which is transcribed in latent and productive infection (Heller *et al.*, 1982a) and hybridizes to cellular DNA (Heller *et al.*, 1982b). Our results indicate that the cellular equivalent of the repeat region is also transcribed in BJA-B cells.

Due to the high GC content (74%) of the repeat region, hybridization results should be interpreted carefully. Further experiments are necessary to strengthen the supposition that transcription of the homologous region in the cellular genome occurs.

Maitland *et al.* (1981) reported that certain restriction fragments of HSV 2 hybridized to human placental RNA under

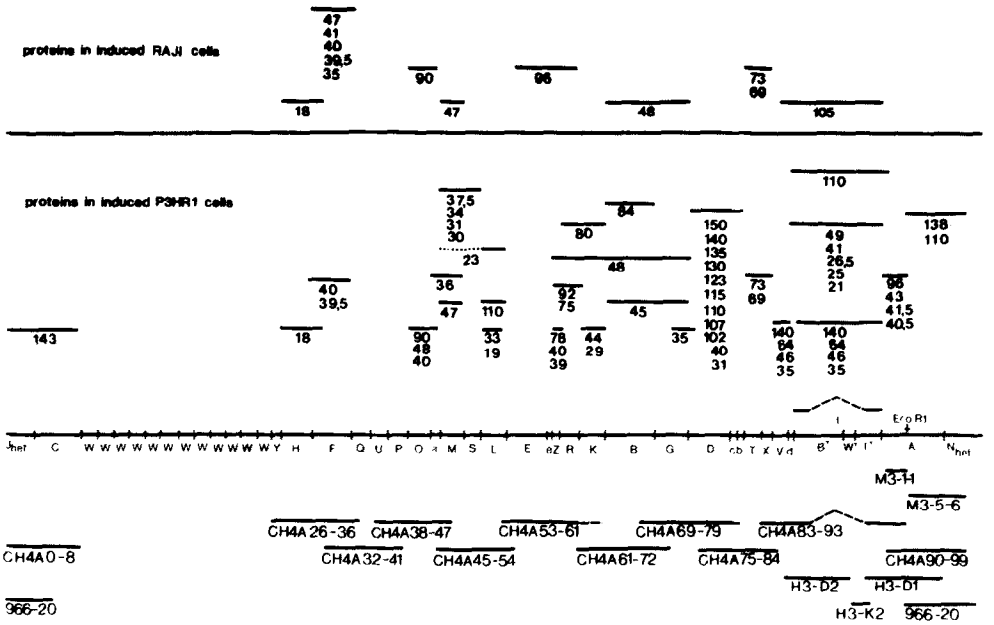

FIG. 6. Map of the polypeptides translated after hybrid selection. The coding regions of the proteins are drawn as maximal exclusion of noncoding sequences relative to the *Bam*H1 restriction fragments of EBV DNA and the DNA clones used for hybridization. The deletion in B95-8 cells combining the *Bam*H1 fragments B', W', and I' to the *Bam*H1 fragment I is indicated as a dotted line. The proteins are indicated with their molecular weights in daltons $\times 10^{-3}$.

high stringency. The map locations of these fragments coincide with the inverted repeats where Peden *et al.* (1982) reported homology with repetitive mammalian sequences. Immediate early mRNAs code in this region (Clements *et al.*, 1977; Easton and Clements, 1980). If EBNA is regarded as an immediate early protein, the agreement between HSV and EBV is remarkable.

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