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The Epstein–Barr virus (EBV) small RNA EBER1 binds and relocalizes ribosomal protein L22 in EBV-infected human B lymphocytes

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ABSTRACT **Epstein–Barr virus (EBV), an oncogenic herpesvirus, encodes two small RNAs (EBERs) that are expressed at high levels during latent transformation of human B lymphocytes. Here we report that a 15-kDa cellular protein called EAP (for EBER associated protein), previously shown to bind EBER1, is in fact the ribosomal protein L22. Approximately half of the L22 in EBV-positive cells is contained within the EBER1 ribonucleoprotein (RNP) particle, whereas the other half resides in monoribosomes and polysomes. Immunofluorescence with anti-L22 antibodies demonstrates that L22 is localized in the cytoplasm and the nucleoli of uninfected human cells, as expected, whereas EBV-positive lymphocytes also show strong nucleoplasmic staining. *In situ* hybridization indicates that the EBER RNPs are predominantly nucleoplasmic, suggesting that L22 relocalization correlates with binding to EBER1 *in vivo*. Since incubation of uninfected cell extracts with excess EBER1 RNA does not remove L22 from preexisting ribosomes, *in vivo* binding of L22 by EBER1 may precede ribosome assembly. The gene encoding L22 has recently been identified as the target of a chromosomal translocation in certain patients with leukemia, suggesting that L22 levels may be a determinant in cell transformation.**

Epstein–Barr virus (EBV) is a lymphotrophic herpesvirus that infects and immortalizes human B lymphocytes *in vitro* (1–3). EBV is the causative agent of infectious mononucleosis and is associated with Burkitt lymphoma and other lymphoproliferative diseases (1). Although most EBV genes are silent in EBV-transformed cells, the genes for two small RNAs called EBER1 and EBER2 (for EBV encoded RNAs) are actively transcribed (up to 5×10^6 per cell) (2–4). Yet *in vitro*, EBV mutants carrying deletions of the EBER genes do not detectably differ from wild-type EBV in their ability to immortalize primary B cells, express latent viral proteins, or undergo the transition to viral replication in either the presence or absence of interferon (5, 6).

EBERs have been reported to reside predominantly in either the nucleoplasm (7, 8) or the cytoplasm (9) of EBV-transformed lymphocytes. The EBERs assemble into ribonucleoprotein (RNP) particles that include the La autoantigen (10). La binds the oligouridylate stretch at the 3′ termini of all mammalian RNA polymerase III transcripts, transiently for most RNAs but stably in the case of the EBERs (see ref. 11 for references). Immunolocalization of the La protein has demonstrated that it is predominantly nucleoplasmic (12–14). We previously identified a second cellular protein, EBER-associated protein (EAP), that quantitatively binds EBER1 (11). EAP also associates with HVP1, a homologous RNA encoded by the baboon *Herpesvirus papio* (11, 15). EAP's RNA binding site has been mapped to stem–loop III of EBER1, a region that is highly conserved in HVP1 (15). Yet, no cellular RNA ligand for this abundant, highly-conserved RNA binding protein could be easily identified.

We show here that EAP is in fact the ribosomal protein L22, which had not been previously sequenced. Experiments characterizing the interaction between L22 and EBER1 are consistent with a model in which L22 is captured by EBER1 in the nucleoplasm before it is assembled into the ribosome in the nucleolus. Intriguingly, a chromosomal translocation t(3;21)(q36;q22) identified in patients with several forms of leukemia has recently been shown to join the acute myeloid leukemia 1 gene *AML1* out of frame with the EAP gene (16). The *AML1* gene encodes one of the subunits of core-binding factor α (CBF-α) and is the human homolog of the mouse gene encoding DNA-binding factor PEBP2αB and the *Drosophila* segmentation gene *runt* (17). This raises the possibility that altering the cellular levels of available EAP, through either mutation or sequestration by EBER1, may facilitate cell transformation *in vivo*.

MATERIALS AND METHODS

Immunoprecipitations, Electrophoresis, and Blotting. Immunoprecipitation extracts were prepared by centrifuging tissue culture cells at $1100 \times g$ for 5 min, washing in TBS (50 mM Tris/150 mM NaCl, pH 7.5), and resuspending at 10^7 cells per ml in either NET (50 mM Tris/150 mM NaCl, pH 7.5) or TR buffer (150 mM NaCl/20 mM Tris/10 mM magnesium acetate/5 mM EGTA, pH 7.5) containing 0.05% Nonidet P-40 and 10 mM dithiothreitol. Cells were disrupted with 10–20 strokes of a Kontes dounce homogenizer and a tight pestle (pestle B). Nuclei were removed by centrifugation at $10,000 \times g$ for 10 min. Postnuclear extracts were immunoprecipitated and washed in the appropriate buffer as described (11, 15). All steps were carried out at 4°C. Electrophoresis, Northern (RNA) blotting and Western blotting (immunoblotting) were performed as described (11, 15).

***In Vitro* Translation, Ribosomal Protein Isolation, and Two-Dimensional (2-D) Gel Electrophoresis.** An RNA encoding human EAP was transcribed *in vitro* with T7 RNA polymerase (11) and translated in wheat germ translation extract (Promega) in the presence of [^{35}S]methionine according to the manufacturer's specifications. Ribosomal proteins were prepared from immunoprecipitation extracts described above, starting with 2×10^8 cells per ml in TR buffer. The supernatants were adjusted to 0.5% deoxycholate and recentrifuged at $15,000 \times g$ for 15 min. This extract was fractionated on a 10–60% (vol/vol) glycerol gradient in TR buffer con-

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Abbreviations: EBV, Epstein–Barr virus; EBER, EBV-encoded RNAs; EAP, EBER-associated protein; RNP, ribonucleoprotein; 2-D, two dimensional; DAPI, 4′,6-diamidino-2-phenylindole.

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taining 2 mM dithiothreitol by using either an SW 41 rotor for 3 hr at 40,000 rpm or an SW 28 rotor for 5 hr at 28,000 rpm. Extract (0.3 or 1.0 ml) was loaded onto a single SW 41 or SW 28 gradient, respectively. Polysome fractions were pooled, diluted 1:1.5 to 1:2 with TR buffer and pelleted in an SW 41 rotor for 10 hr at 40,000 rpm or in an SW 28 rotor for 12 hr at 28,000. Pellets were resuspended by homogenizing in 20 mM Tris/5 mM magnesium acetate, pH 7.5, and brought to 100 mM magnesium acetate. Radiolabeled *in vitro* translation products were added to unlabeled ribosomal proteins at this point (for Fig. 1). Two volumes of acetic acid were added, and the samples were incubated at 4°C for 45 min. After spinning for 15 min at 15,000 rpm in a Sorvall SS-34 rotor, the supernatant was dialyzed for 6 hr against two changes of 100 mM acetic acid/10 mM 2-mercaptoethanol, and lyophilized. The sample was then fractionated on a basic-SDS 2-D gel (18).

For quantitation, polysomal proteins isolated from BJAB, BJAB-B1, and Raji cell extracts were subjected to 2-D gel electrophoresis, stained with Coomassie brilliant blue, and quantitated by densitometry on a Visage 2000 scanning system using Bio Image 2-D software (Millipore). Intensity values for >35 ribosomal spots were determined on two or more individual gels for each tissue culture cell type. After normalizing each gel for loading differences, the intensity of each spot was compared for each cell type studied.

Slide Preparation and Immunofluorescence. Approximately 2.5×10^5 cells per slide were removed from culture and spun onto Vectabond-treated (Vector Laboratories) slides by using a cytocentrifuge (Shandon, Pittsburgh). Slides were fixed in Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS) containing 4% paraformaldehyde for 10 min, followed by permeabilization at −20°C for 10 min with methanol containing EGTA (0.5 mM final) and rinsing three times for 5 min each in PBS. Alternative permeabilization agents such as acetone, Nonidet P-40, or Triton X-100 were found to be either too harsh (cell morphology was altered) or too variable (some cells were permeabilized, while adjacent cells were not). Monolayer HeLa and HEp-2 cells (used as negative controls) were prepared as described (19). After blocking in PBS containing 3% bovine serum albumin (BSA), affinity-purified rabbit anti-L22 and mouse anti-rRNA ($Y_{10}B$; see ref. 20) antibodies were diluted 1:250 in the same solution and allowed to bind for 30 min at 25°C. The slides were washed three times in PBS for 5 min each and treated with tetramethylrhodamine B isothiocyanate (TRITC)-conjugated goat anti-mouse IgG or fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Sigma). Slides were again washed in PBS, counterstained with 4′,6-diamidino-2-phenylindole (DAPI), and mounted (19). Confocal microscopy confirmed that the nucleoplasmic relocalization of L22 in the EBV-infected cell types was not due to out-of-focus light from the cytoplasm.

Oligonucleotides and *in Situ* Hybridization. The antisense oligomers used in this study were: EBER1-(136–151), 5′-CAGCUGGUACUUGACC(dC*)$_4$dG-3′; EBER1-(13–28), 5′-ICAAAACCUCUAIIIC(dC*)$_4$dA-3′; anti-28Sa, 5′-AIAICCAAUCCUUAUBBBBdT-3′; anti-28Sa2, 5′-d(CT*TAGAGCCAAT*CCT*TAT*C)-3′; EBER2-(107–124), 5′-CCUIACUUICAAAUICUC(dC*)$_4$dT-3′; U2-(25–43), 5′-d(ACAGAT*ACT*ACACT*TGAT*C)-3′. Residues preceded by a lowercase d are deoxyribose (DNA) linkages; A, G, C, U and I are 2′-*O*-Me-RNA [except for anti-28Sa, which is 2′-*O*-allyl-RNA (21)]. Residues marked with an asterisk are amine-modified, whereas B residues mark sites of biotinylation with an abasic biotin phosphoramidite. Equivalent results were obtained when the haptenation of the EBER1-(136–151) and 28S oligonucleotides was switched and when the EBER1-(13–28) oligomer was used. *In situ* hybridization was carried out as described (19). The images shown in Fig. 4, *J–O* were acquired with digoxigenin-labeled EBER1-(136–151) and biotinylated anti-28Sa oligonucleotides. Digoxigenin was visualized by using fluorescein-conjugated anti-digoxigenin Fab fragments (Boehringer Mannheim), while biotin was detected with Texas red-conjugated avidin DCS (Vector Laboratories). Alternatively, fixation in methanol containing EGTA without paraformaldehyde as described (9) gave no detectable EBER1 hybridization signal, and that of an antisense U2 small nuclear RNA control probe was drastically reduced. Attempts to colocalize anti-L22 and the EBER1 oligonucleotides in the same experiment were unsuccessful because no salt conditions were found that retained both of the signals; in high salt (0.75 M) anti-L22 was not detectable, and in low salt (0.15 M) the oligomer signal was significantly reduced.

Glycerol Gradients. Sample preparation and analytical glycerol gradients were run as described for ribosomal protein preparation (employing the SW 41 rotor), except that 0.25 ml of extract from 2.5×10^7 cells was loaded on each gradient. Prior to loading, certain samples were incubated with 100 μg of either *Escherichia coli* tRNA (Sigma) or EBER1 for 15 min at 25°C. EBER1 was generated as described (15). Gradients were dripped from the bottom and collected as 15 fractions (approximately 1 ml each) into tubes containing 10 μl of 2-mercaptoethanol and 100 μl of 20% SDS. A 50-μl aliquot of each fraction was then boiled for 5 min and loaded directly onto a 15% SDS/polyacrylamide gel, and Western-blotted. Each fraction (125 μl) was extracted with phenol, and extracted material was precipitated with ethanol, loaded onto a 8.3 M urea/polyacrylamide gel, and Northern-blotted (15).

RESULTS

EAP Is the Ribosomal Protein L22. We had previously prepared rabbit anti-EAP antibodies and had observed that they immunoprecipitated a small but higher-than-background amount of rRNA (15), suggesting that EAP might be a ribosomal protein. To explore this possibility, we utilized 2-D gel electrophoresis. A T7 RNA polymerase transcript encoding EAP was translated *in vitro* in the presence of [^{35}S]methionine, and the translation products were combined with a preparation of human ribosomes. Upon 2-D gel electrophoresis, the radiolabeled EAP spot comigrated with the 60S subunit ribosomal protein L22 in two different basic-SDS systems (Fig. 1, and data not shown) and a basic-acidic system (J. Olvera and I. Wool, personal communication). Recognition of L22 by anti-EAP antibodies was further demonstrated by immunoblotting of 2-D gels (J. Olvera and

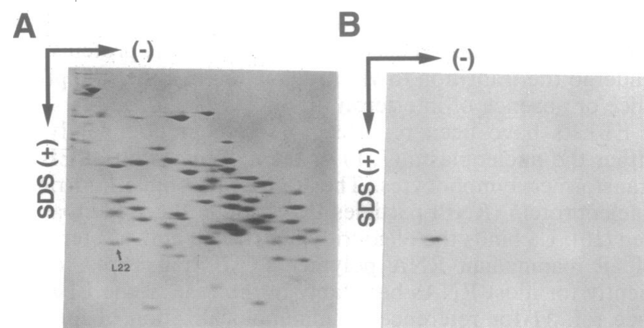

FIG. 1. EAP is the large subunit ribosomal protein L22. EAP message was translated *in vitro* in the presence of [^{35}S]methionine. The translation product was mixed with an unlabeled preparation of human ribosomal proteins and run on a basic-SDS 2-D gel. The gel was stained with Coomassie brilliant blue, photographed (*A*), and subsequently dried and exposed to film (*B*). Alignment of the autoradiograph and the dried gel confirmed that the spot in *B* comigrated with the L22 spot (arrow in *A*). Polysomal proteins run without radiolabeled EAP gave a pattern indistinguishable from that of *A*.

I. Wool, personal communication). Although human L22 had not been previously sequenced, the amino acid composition determined for rat L22 (39) closely matches that predicted from the cDNA sequence of human EAP (11).

Availability of L22 in the Ribosome and the EBER1 RNP. We next asked what fraction of ribosomal protein L22 (EAP) is bound by EBER1 in EBV-transformed B cells by immunoprecipitating cell extracts with excess anti-La antibodies and subjecting the pellets and La-depleted supernatants to Western blotting with anti-EAP antibodies (henceforth called anti-L22). In BJAB (a human EBV-negative B-cell line) cell extract, EBER1 is not present to tether L22 to La, and thus anti-La did not immunoprecipitate L22 (Fig. 2, lane 1). In contrast, in BJAB-B1 (an EBV-positive line derived from BJAB) and Raji (a human EBV-positive B-cell line) cells, approximately 30% and 50% of the L22 was present in the immunoprecipitates, respectively (Fig. 2, lanes 2–3). Control Northern blot analyses showed that EBER1 was quantitatively immunoprecipitated by the anti-La serum in both cases (Fig. 2, compare lanes 2 and 3 with 5 and 6).

To understand why experiments with anti-L22 showed only a small amount of rRNA immunoprecipitation (15), we examined anti-L22 precipitates obtained under various conditions. Our previous analyses had used buffers lacking Mg^{2+}, which are known to unfold ribosomes. Cell extracts from BJAB, BJAB-B1, and Raji were prepared in immunoprecipitation buffers with and without Mg^{2+}. In the absence of Mg^{2+}, we observed that anti-L22 immunoprecipitated both L22 and EBER1 very efficiently, while only a small percentage of the cellular rRNA (represented by 5S rRNA) was precipitated (Fig. 2, compare lanes 7–9 with 10–12). In contrast, when a Mg^{2+}-containing buffer (designed to stabilize ribosomes) was used, L22 was not quantitatively precipitated (Fig. 2, compare lanes 13–15 with 16–18). This could

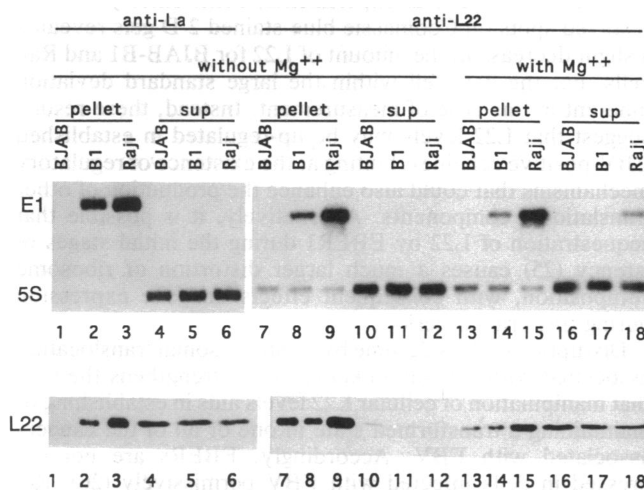

FIG. 2. Immunoprecipitation of L22. Extracts from BJAB, BJAB-B1 (B1), or Raji cells prepared in either Mg^{2+}-lacking NET or Mg^{2+}-containing TR buffer were immunoprecipitated with an excess of anti-La (lanes 1–6) or affinity-purified anti-L22 (lanes 7–18) antibodies. Equivalent portions of immunoprecipitation pellets (lanes 1–3, 7–9, and 13–15) and supernatants (lanes 4–6, 10–12, and 16–18) were electrophoresed on a 15% SDS/polyacrylamide gel, blotted, and probed with affinity-purified anti-L22 antibodies. RNAs extracted from these pellets and supernatants were run on a 6% polyacrylamide/8.3 M urea gel, Northern-blotted, and probed using EBER1 (E1) and 5S rRNA (provided by B. Peculis) antisense RNA probes. Lanes 1–6 and 7–18 represent different experiments (see text). Approximately 5–10%, 10–20%, and 30–40% of the total L22 were immunoprecipitated by anti-L22 for BJAB, BJAB-B1, and Raji, respectively, in multiple experiments. Silver staining of RNAs from anti-L22 immunoprecipitates confirmed the fact that 5S is representative of the other rRNAs.

be explained if L22 were unavailable to the antibody when contained in intact ribosomes but released when ribosomes are unfolded in the absence of Mg^{2+}. The observation that a higher percentage of L22 can be immunoprecipitated by anti-L22 antibodies from EBV-positive versus EBV-negative cells when ribosomes are stabilized in the presence of a Mg^{2+}-containing buffer (Fig. 2; compare lanes 14 and 15 with 13) is expected, since a large fraction of the L22 in infected cells is bound to EBER1 instead of being sequestered in ribosomes.

Localization of L22 in EBV-Positive and EBV-Negative Cells. As shown above, an appreciable portion of the L22 in infected cells is not associated with ribosomes. Thus, immunofluorescence and *in situ* hybridization were performed to establish the subcellular locations of L22 and EBER1 in EBV-positive and -negative cells. Both HEp-2 cells (an EBV-negative human epithelial cell line) and HeLa cells showed an identical nucleolar and cytoplasmic staining pattern with mouse monoclonal anti-rRNA antibodies (20) and affinity-purified anti-L22 antibodies (Fig. 3*A*, and data not shown). Only background fluorescence was detected with the rabbit preimmune serum or with antiserum that had been depleted of anti-L22 antibodies (Fig. 3 *B* and *C*) (15). BJAB cells (also EBV-negative) showed the same exclusively nucleolar and cytoplasmic distribution of L22 seen in HEp-2 cells (Fig. 3 *D* and *E*). Strikingly, EBV-positive cells (Raji and BJAB-B1) exhibited significant nucleoplasmic immunofluorescence in addition to staining of the nucleolus and cytoplasm (Fig. 3 *F*–*H*). This redistribution was specific for L22; anti-rRNA antibodies produced an indistinguishable pattern in EBV-negative and -positive B-cell lines (Fig. 3*I*, and data not shown).

Since EBER1 had been reported to reside predominantly in either the nucleoplasm (7, 8) or cytoplasm (9), we reexamined the location of EBER1 RNA by *in situ* hybridization. We used an oligonucleotide complementary to nucleotides 136–151 of EBER1, which was shown to be specific by *in vitro* selection from total RNA (data not shown); a previously characterized 28S antisense oligonucleotide (21) provided an internal control for permeabilization and hybridization. BJAB, BJAB-B1, and Raji cells all showed exclusively nucleolar and cytoplasmic hybridization with the 28S oligonucleotide (Fig. 3 *L* and *O*, and data not shown), while hybridization with the EBER1 oligomer showed predominantly nucleoplasmic fluorescence, with slight staining of the cytoplasm in BJAB-B1 and Raji cells (Fig. 3*N* and data not shown). Furthermore, an antisense EBER2 oligomer also showed primarily nucleoplasmic hybridization in EBV-infected cells (not shown). As anticipated, the EBER1 oligonucleotide produced only background hybridization with BJAB cells, which do not contain EBERs (Fig. 3*K*). We conclude that the unusual nucleoplasmic location of L22 in EBV-positive cells coincides with the presence of EBER1, suggesting that EBER1 is the active agent in the relocalization process.

EBER1 Is Unable To Associate with Ribosome-Bound L22. The nucleoplasmic location of the EBER1 RNP suggests that the formation of EBER1–L22 complexes *in vivo* could occur either prior to L22 assembly into the ribosome or by removal of L22 from preexisting ribosomes and subsequent sequestration in the nucleus. To address this question, we compared a Mg^{2+}-containing uninfected cell extract (BJAB) incubated with excess EBER1 RNA to an EBV-positive cell extract (Raji) by glycerol gradient fractionation. Western and Northern blot analyses (Fig. 4*A*) revealed that in the control BJAB extracts, the vast majority of L22 and 5S rRNA copeaks with monosomes (lanes 6–8) and polysomes (lanes 10–13), as expected. Preincubation of BJAB extract with EBER1 (40-fold more than is present in Raji cell extract) did not shift a large fraction of L22 to the top of the gradient (Fig. 4*C*), nor

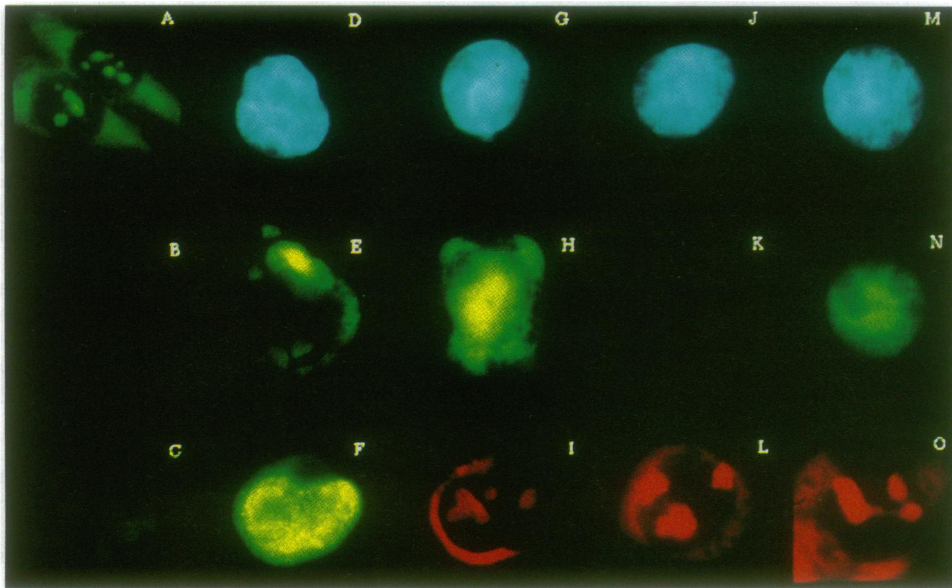

FIG. 3. The intracellular location of L22 is altered in EBV-infected cells. (*A–C*) Immunofluorescence of anti-L22 antibodies in commercially prepared HEp-2 cells. In *A*, affinity-purified rabbit anti-L22 serum stains both the nucleoli and cytoplasm, excluding the nucleoplasm; equivalent results were obtained with HeLa cells (data not shown). *B* shows that only background fluorescence is detected when using rabbit preimmune serum, whereas *C* demonstrates that passage of the antiserum over a β-galactosidase-L22 fusion column (15) reduces the signal to near-background levels. (*D–I*) Immunofluorescence of anti-L22 in three different B-lymphoblastoid cell lines. In *D* and *E*, the same EBV-negative BJAB cell is stained with DAPI (a DNA intercalator) and affinity-purified anti-L22, respectively. *G* (DAPI) and *H* (anti-L22) show a BJAB-B1 cell in which L22 is distributed throughout the cell, including the nucleoplasm. In a Raji cell, anti-L22 (*F*) and anti-rRNA (*I*) reveal that this relocalization of ribosomal protein L22 into the nucleoplasm does not also involve ribosomal RNA. (*J–O*) *In situ* colocalization of digoxigenin-labeled EBER1-(136–151) and biotinylated anti-28Sa oligomers in a BJAB cell (*J*, DAPI; *K*, EBER1; and *L*, 28S) and a BJAB-B1 cell (*M*, DAPI; *N*, EBER1; and *O*, 28S).

did incubation with a nonspecific RNA (*E. coli* tRNA) (data not shown). However, in Raji cell extracts (Fig. 4*B*), about 50% of L22 copeaked with EBER1 (lanes 2 and 3) at the top of the gradient. We conclude that *in vivo* the EBER1–L22 interaction occurs either prior to ribosome assembly or through some active process that is not reproduced *in vitro*.

DISCUSSION

The identification of EAP as the ribosomal protein L22 raises the question of whether L22 binds directly to rRNA, mimicking its well-characterized interaction with stem-loop III of EBER1 (15). Unfortunately, the bacterial homolog of L22 is not known, although a partial N-terminal sequence of the yeast large subunit protein YL31 (22) exhibits approximately 40% identity to the N terminus of L22 (11). Experiments designed to detect a direct interaction between L22 and rRNA also have not been successful (data not shown), nor has visual inspection of the large subunit rRNA sequence revealed candidate regions similar to the binding site mapped in EBER1 (15). Mutually exclusive binding of L22 to the ribosome versus EBER1 would be expected if L22 has only a single RNA binding site and is a direct rRNA-binding protein. Alternatively, L22 may be assembled into the ribosome via protein–protein interactions, leaving its RNA-binding site free to recognize some other RNA (e.g., mRNA or EBER1). However, our inability to detect EBER1 in association with ribosomes in Raji cell extracts (Fig. 4*B*) argues against the availability of such a binding site once L22 is incorporated into the ribosome. Furthermore, the idea that ribosomal proteins must cross the nucleoplasm to reach the nucleolus (23, 24) is consistent with our hypothesis (see above) that *in vivo* EBER1 may intercept L22 in the nucleoplasm prior to ribosome assembly.

One potential consequence of complex formation with EBER1 would be a depletion of L22 from ribosomes in EBV-positive B cells. Thus, we have quantitated the amounts of L22 relative to approximately 35 other ribosomal proteins in polysomes from BJAB versus BJAB-B1 and Raji cells (data not shown). In several experiments, densitometry of well-resolved spots on Coomassie blue-stained 2-D gels revealed a slight decrease in the amount of L22 for BJAB-B1 and Raji cells, but the data fell within the large standard deviation inherent in this type of measurement. Instead, these results suggest that L22 levels may be up-regulated in established EBV-positive cell lines, hinting at the existence of regulatory mechanisms that could also enhance the production of other translational components. Alternatively, it is possible that sequestration of L22 by EBER1 during the initial stages of latency (25) causes a much larger distortion of ribosome composition, with consequent effects on gene expression during this critical period.

Disruption of the L22 gene by a chromosomal translocation associated with several leukemias (16) strengthens the idea that manipulation of cellular L22 levels aids in establishing or maintaining a transformed state in one or all of the cancers associated with EBV. Accordingly, EBERs are not expressed in cells infected with EBV permissively (26). Recently, the misexpression of several other components of the translational apparatus has been linked to various disease states. Deletion of one allele of the human ribosomal protein S4 gene is implicated in Turner syndrome (27, 28). Overexpression of eukaryotic initiation factor 4E (eIF-4E) (29, 30) or the expression of a dominant mutant of the eIF-2 kinase DAI (30) results in malignant transformation of mouse NIH 3T3 cells. Inactivation of one allele of the gene encoding ribosomal protein S6, which becomes phosphorylated in response to mitogenic stimuli, results in hematopoietic neoplasia in *Drosophila* (31, 32).

Like EBV, other viruses encode small RNAs designed to interact with and subvert the functioning of important host proteins. Three of the small RNAs encoded by *Herpesvirus saimiri* bind a cellular 32-kDa protein implicated in rapid mRNA degradation and therefore may act to stabilize mes-

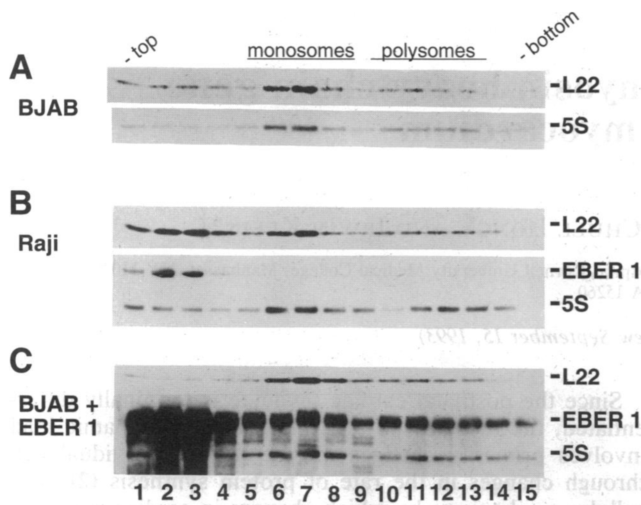

FIG. 4. L22 sediments with ribosomes and endogenous EBER1. Extracts prepared from BJAB cells (*A*) or Raji cells (*B*) in magnesium-containing TR buffer were fractionated on a glycerol gradient. Fractions were Western- or Northern-blotted and probed for L22 or 5S rRNA and EBER1, respectively. (*C*) Another sample of BJAB cell extract was incubated with EBER1 before gradient fractionation and analyzed as for *A* and *B*. Since 40-fold more EBER1 is present in *C*, a false impression that some EBER1 copeaks with monosomes is created by streaking of a small fraction of EBER1 down the gradient combined with degradation in lane 9; this apparent copeaking was not observed in other experiments. Northern blots for *A*, *B*, and *C* were probed, washed, autoradiographed, and photographed together so that the intensity of the EBER1 signals can be directly compared. Although the chemiluminescence used to develop the Western blots is not amenable to quantitation, multiple experiments demonstrated insignificant amounts of L22 at the top of gradients of BJAB compared with Raji cell extracts.

sages for cytokines, lymphokines, and protooncogenes in transformed T lymphocytes (33). Adenovirus VA1 RNA rescues protein synthesis by binding the interferon-induced double-stranded RNA-dependent kinase DAI, thereby blocking the autophosphorylation required for DAI activation (34). A similar function has been proposed for the EBERs (9); however, addition of the EBER genes does not fully rescue adenovirus mutants that lack the VA1 gene (35). Moreover, *in vitro* experiments have indicated that inhibition of the DAI kinase requires much higher concentrations of either EBER than it does of VA1 (7, 36). Our results indicate that, while it is possible that the EBERs act on DAI in EBV-infected cells, EBER1 may also act to alter translation by a distinctly different mechanism than that used by VA1. The function of all these viral small RNAs is reminiscent of that of many viral proteins (e.g., simian virus 40 large T antigen and adenovirus E1A), which interact specifically with host antioncogene products (e.g., p53, Rb) to achieve cell transformation (37, 38). Our identification of ribosomal protein L22 as the target of EBER1 adds to a growing list of such viral strategies.

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