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Polyadenylylated nuclear RNA encoded by Kaposi sarcoma-associated herpesvirus

(human herpesvirus 8/U RNA/snRNA/mRNA/U1 RNA/Sm antigen/polyadenylylation/splicing)

REN SUN*†‡, SU-FANG LIN*‡, LYN GRADOVILLE§, AND GEORGE MILLER*§¶

Departments of *Molecular Biophysics and Biochemistry, †Genetics, and §Pediatrics and Epidemiology and Public Health, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520

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ABSTRACT **A newly recognized γ herpesvirus known as Kaposi sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8) is present in Kaposi sarcomas and body-cavity-based lymphomas. Here we identify a novel abundant 1.2-kb RNA, polyadenylated nuclear RNA (PAN RNA), encoded by the virus. The majority of cDNAs produced from poly(A)-selected RNA isolated from a human body cavity lymphoma cell line 48 hr after butyrate induction of KSHV lytic replication represented PAN RNA. Within PAN RNA were two 9 and 16 nt stretches with 89% and 94% identity to U1 RNA. A third stretch of 14 nt was 93% complementary to U1. The 5′ upstream region of PAN RNA contained both proximal and distal sequence elements characteristic of regulatory regions of U snRNAs, whereas the 3′ end was polyadenylylated. PAN RNA was transcribed by RNA polymerase II, lacked a trimethylguanosine cap, and did not associate with polyribosomes. PAN RNA formed a speckled pattern in the nucleus typical of U snRNAs and colocalized with Sm protein. Therefore, PAN represents a new type of RNA, possessing features of both U snRNA and mRNA.**

DNA sequences of Kaposi sarcoma-associated herpesvirus (KSHV) have been consistently found in Kaposi sarcoma (KS), including the AIDS-related, African endemic, transplant-related, and classical forms (1–5). Furthermore, the presence of antibodies against KSHV lytic antigens is highly correlated with KS in HIV-infected patients (6). These findings support an etiologic role of KSHV in KS. In addition, the virus has been detected in body-cavity-based B-cell lymphoma and multicentric Castleman disease (7, 8). A B-cell line (BC-1), established from body-cavity-based B-cell lymphoma, contains two latent γ herpesviruses, KSHV and Epstein–Barr virus (EBV). In this cell line lytic gene expression of KSHV can be selectively induced by *n*-butyrate (6). *n*-butyrate treatment results in shut off of host cell and EBV gene expression. Other cell lines from body cavity lymphoma harbor only KSHV, which can also be switched into lytic gene expression by chemical inducing agents (9).

This study was initiated by characterizing cDNAs isolated from the BC-1 cell line after *n*-butyrate induction of the KSHV lytic cycle. Prominent in this library were cDNAs that represented a viral RNA designated PAN RNA with features of both mRNAs and U snRNAs. Here we provide the sequence of PAN RNA and its gene, show that it is nuclear and colocalizes with the Sm antigen, and demonstrate that it is strongly induced during early lytic cycle activation of KSHV.

MATERIALS AND METHODS

Cell Culture. BC-1 cells (7) were grown in RPMI 1640 medium supplemented with 15% fetal bovine serum at 37°C in the presence of 5% CO_2/95% air. HH514-16 cells derived from the African Burkitt lymphoma line, P3J-HR-1, contain only EBV genomes (10). Media for HH514-16 cells was supplemented with 8% fetal bovine serum. Chemically induced cells were harvested after exposure to 3 mM sodium butyrate and/or 20 ng/ml PMA (phorbol 12-myristate 13-acetate).

RNA Preparation and Northern Blot Analysis. RNAs were prepared, fractionated on 1% formaldehyde agarose gels, and transferred to nylon membranes (Nytran, Schleicher & Schuell) by standard procedures (11). DNA probes were labeled by the random-primed method. The DNA fragments used in hybridization reactions were a 575-bp PCR product of the PAN RNA gene amplified by primers 17739 (5′-gcattggattcaatctccaggcc-3′) and 17741 (5′-cattgttacacaacgcttt-tcacc-3′) or PCR-amplified products of the U1 gene (12) or KSHV/dehydrofolate reductase gene (unpublished work). Hybridization was carried out in 50% formaldehyde/5× SSC/5× Denhardt's solution/1% SDS/100 μg/ml ssDNA at 45°C overnight. Filters were washed with 3× SSC/0.1% SDS twice for 15 min each and in 0.1× SSC/0.1% SDS once for 30 min at 68°C. For quantitation of RNA loading, blots were stripped and reprobed with the gene for H1 RNA of RNase P; band intensity was quantitated with a PhosphorImager (Molecular Dynamics).

cDNA Library Construction and Cloning. Total cellular RNA was extracted from BC-1 cells, which had been treated with sodium butyrate for 48 hr. The poly(A) RNA was selected on an oligo(dT) column. The library was cloned in the λZAPII vector (Stratagene) according to the manufacturer's protocol. Isolated clones were plaque purified at least twice before *in vivo* excision.

Genomic Library. A cosmid library was constructed in the Supercos-1 vector (Stratagene) using total genomic DNA from BC-1 cells.

DNA Sequence Analysis. The sequences of PAN RNA cDNA and genomic clones were determined in both directions via primer walking. DNA sequence data were compiled and analyzed using GELASSEMBLE, TESTCODE, BLAST, FRAMES of the Wisconsin Sequence Analysis Package, Version 8 (GCG). Sequence data reported in this paper have been deposited in the GenBank data base.

Nuclear Run-On. One microgram of each plasmid DNA containing the EBV EBER1 and EBER2 genes, KSHV small viral capsid antigen gene (S.-F.L., R.S., H. Lee, L.G., D. Shedd, K. Haglund and G.M. unpublished work), or KSHV PAN RNA gene were denatured and cross-linked to nitrocellulose paper. Nuclei were prepared from BC-1 cells induced by 3 mM

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Abbreviations: KS, Kaposi sarcoma; KSHV, Kaposi sarcoma-associated herpesvirus; EBV, Epstein–Barr virus; FISH, fluorescence *in situ* hybridization.

Data deposition: The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U51039 (PAN RNA cDNA) and U51040 (PAN RNA genomic DNA)].

‡R.S. and S.-F.L. contributed equally to this work.

¶To whom reprint requests should be addressed.

butyrate for 12 hr. Nuclei were incubated with [α-^{32}P]dUTP for 30 min and the RNA was extracted (11).

Sedimentation of PAN RNA on a Sucrose Gradient. The procedure was based on published methods with some modifications (13, 14). BC-1 cells (5×10^7) treated with 3 mM sodium butyrate for 24 hr were resuspended in 1 ml HKM buffer (20 mM Hepes, pH 7.5/100 mM KCl/1.5 mM $MgCl_2$) containing 0.5% Triton X-100, and homogenized at 4°C with a Teflon–glass homogenizer. The nuclei were removed by centrifugation at 12,000 × *g* for 5 min; supernatants were loaded onto a gradient of 10–40% sucrose (wt/wt) prepared in HKM buffer containing 3 mM 2-mercaptoethanol with a 0.5-ml 60% sucrose cushion. Gradients were centrifuged at 41,000 rpm in a Beckman SW-41 rotor for 2 hr at 4°C. When the procedure was performed with EDTA, $MgCl_2$ was replaced by 10 mM EDTA in the HKM buffer. Gradients were fractionated into 18 fractions of 0.6 ml each. Twenty-five microliters of 20% SDS was added and fractions were extracted with phenol and chloroform. RNA was precipitated with 0.5 M NaCl and 1 vol of isopropanol and pelleted by centrifugation, washed with 70% ethanol, dried, and suspended in 10 μl of diethylpyrocarbonate-treated distilled water. Samples were subjected to Northern blot analysis as described above.

Relative Abundance of PAN RNA. Twofold dilutions of samples of RNA from untreated BC-1 cells and from BC-1 cells that had been treated with *n*-butyrate for 48 hr were analyzed by Northern blotting. The same filter was hybridized simultaneously with 4×10^6 cpm of PAN RNA and U1 RNA probes. The probes were in excess. The signals from this blot were quantitated by PhosphorImager. The ratio of PAN RNA to U1 RNA was calculated by adjusting for the specific activity of the probes and the probe lengths. The calculation assumed that both probes have similar hybridization efficiency.

***In Situ* Hybridization and Immunofluorescence.** *In situ* hybridization procedures for detection of RNA were modified from published methods (15, 16). Cells were pelleted and resuspended in PBS to a density of 3×10^6 cells/ml. Ten microliters of cell suspension were placed onto fluorescent antibody slides (Bellco Glass). Air-dried slides were fixed with freshly made 4% paraformaldehyde in PBS (pH 7.4) for 15 min at room temperature. One group of slides was pretreated with 0.2% Triton X-100 for 5 min at 4°C to remove the cytoplasm, and the hybridization signals were compared. Biotin-11-dUTP-labeled probes were prepared by nick translation of 2 μg of pBKCMV (Stratagene) or pBKCMV-PAN plasmid DNA (11). Products with an average length of 200 bp were purified through G-50 Sephadex. Ten micrograms each of *Escherichia coli* t-RNA and denatured salmon sperm DNA were added to 150 ng of nick-translated biotin-11-dUTP-labeled probe, and dried in a Speed Vac. The dried pellet was resuspended in 10 μl of formamide, denatured by heating for 10 min at 70°C and immediately chilled on ice. The probe was resuspended in 20 μl of hybridization mixture containing 10% dextran, 0.2% BSA, 2× SSC, and 0.2 mM vanadyl adenosine. The hybridization mixture was layered onto slides that were covered with a coverslip, sealed with mineral oil, and incubated in a humid chamber at 37°C for 3–18 hr. Slides were washed at 37°C for 30 min each in 50% formamide in 2× SSC, 2× SSC, and 1× SSC. The hybridization signal was detected by incubation for 1 hr at 37°C with fluorescein-conjugated avidin DN (Vector Laboratories) at 2 μg/ml in 4× SSC containing 1% BSA. Slides were rinsed at room temperature for 10 min each in 4× SSC, 4× SSC with 0.1% Triton X-100, and 4× SSC. Cells were examined in antifade medium containing propidium iodide.

For colocalization studies, BC-1 cells that had been hybridized for PAN RNA, but not mounted in antifade medium, were stained with monoclonal antibody Y-12, which is specific for Sm antigen. The primary antibody was detected with rhodamine-conjugated goat anti-mouse antibodies. Confocal microscopic images were taken on a Bio-Rad MRC-600 confocal image system with a Zeiss Axiovert 10 microscope. The fluorescein image and rhodamine image were processed and superimposed with NIH IMAGE 1.6 and ADOBE PHOTOSHOP 2.5 programs.

RESULTS

Cloning of PAN RNA cDNA. To study gene expression of KSHV, a cDNA library was constructed with poly(A)-selected RNA from BC-1 cells induced 48 hr previously with butyrate. Surprisingly, more than 80% of cDNA clones in this library contained overlapping sequences representing the same RNA. The predominance of this RNA is likely to reflect its abundance and the shut off of host cell and EBV mRNAs, which accompany treatment of BC-1 cells with butyrate (G.M., L. Heston, E. Grogan, L.G., M. Rigsby, R.S., D. Shedd, V.M. Kushnaryov, S. Grossberg and Y. Chang, unpublished work). The three longest cDNAs started at the same position, designated nucleotide 1 in Fig. 1*A*. The 3′ end of this RNA was mapped by sequencing six independent cDNA clones, which had the same polyadenylylation site and different numbers of adenylate residues. There was no extensive homologous sequence in the data base. Computer-assisted analysis indicated that PAN RNA was unlikely to encode protein. Fifty-seven stop codons were distributed randomly in all three reading frames. The largest possible open reading frame (nt 582–765) was located in a region deemed least likely to encode protein according to eukaryotic codon usage determined by the GCG TESTCODE program (Fig. 1*B*). Of 21 methionines only 3 met Kozak's consensus for initiators. These three potential open reading frames would be 37, 25, and 51 amino acids. PAN RNA was U rich (33%), a characteristic of some U RNAs (17, 18). PAN RNA contained a 16-nt sequence in which 15 nt were identical to U1 RNA and a 14-nt sequence complementary to U1 RNA with a 1 bp mismatch (Fig. 1*C*). Both sequences were located in the third stem-loop of U1 RNA (12). Furthermore, PAN RNA contained a sequence identical to the first 7 nt at the 5′ end of U1 RNA.

PAN RNA Gene in the KSHV Genome. The PAN RNA gene was identified on three different cosmid clones each of which also contained the KSHV uracil–DNA glycosylase gene. Comparison of the sequence of genomic DNA with the cDNA sequence (Fig. 1*A*) showed that there was no RNA splicing or RNA editing during or after the synthesis of PAN RNA. The 3′ end of PAN RNA was located 18 or 19 bp downstream of a polyadenylylation signal (AATAAA). Sequences typical of U snRNA promoters and enhancers were found at −57 and −184 upstream of PAN RNA (Fig. 1*D*) (19). In cellular U snRNA genes a loosely conserved promoter element at −51 to −53 is the reference point that determines the site for transcription initiation (19). The highly conserved enhancer element further upstream is responsible for a 100-fold increment in U snRNA expression (20). No U snRNA specific 3′ end transcriptional termination signal sequence was found (21–23).

PAN RNA Is Transcribed by RNA Polymerase II. A nuclear run-on experiment was employed to ascertain which RNA polymerase transcribes PAN RNA. α-amanitin (24), at a concentration of 5 μg/ml, reduced transcription elongation of PAN RNA 83-fold. In the same experiment transcription of a typical viral mRNA (encoding KSHV small viral capsid protein) was reduced 63-fold, but transcription of EBV encoded RNAs (EBERs), which are RNA polymerase III transcripts, was reduced only 4-fold. This result showed that PAN RNA was transcribed by RNA polymerase II (Fig. 2*A*). Most mature U snRNAs carry a trimethylguanosine cap at the 5′ end (26). In an immunoprecipitation experiment PAN RNA was not found to have a trimethylguanosine cap, whereas U1 RNA was capped as expected (Fig. 2*B*).

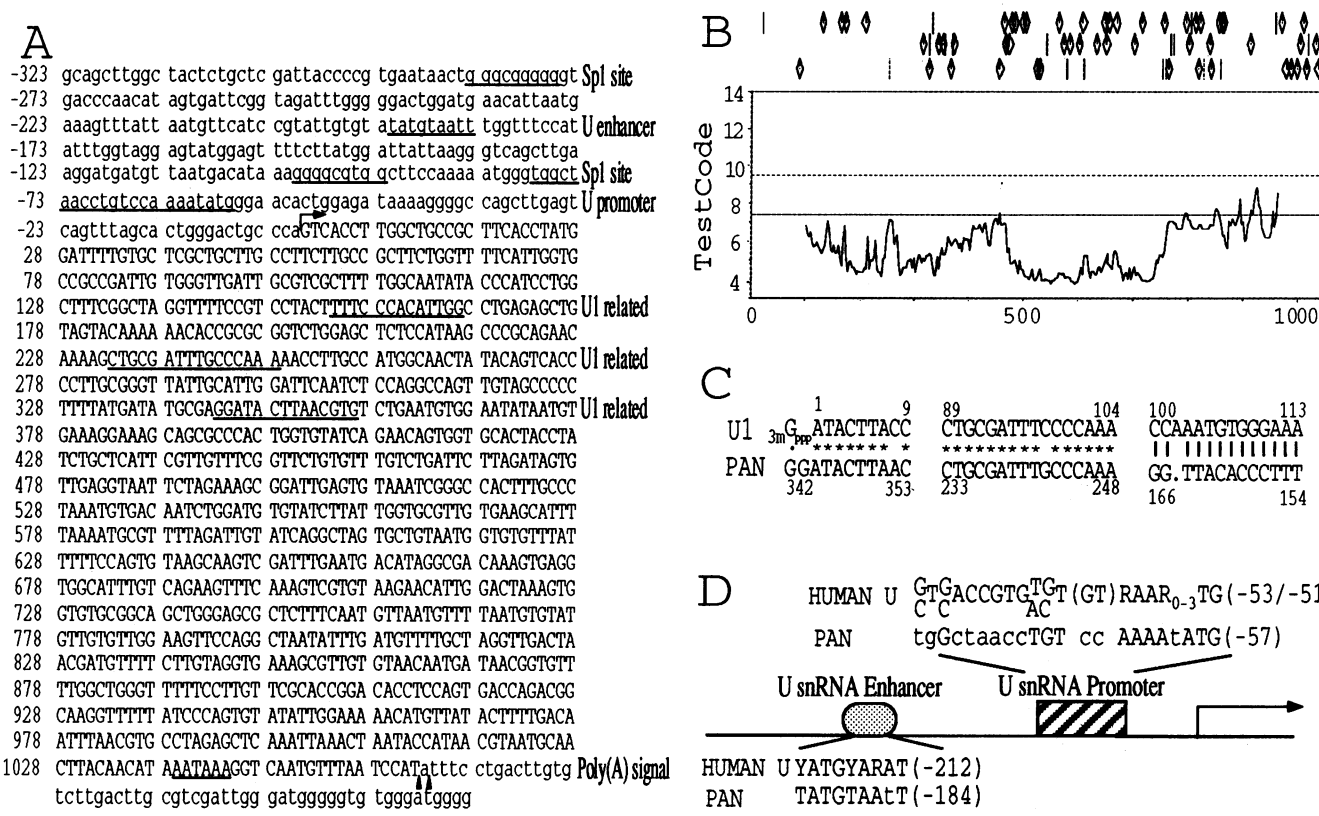

FIG. 1. Sequence analysis of the PAN RNA gene. (*A*) Nucleotide sequence of PAN cDNA and genomic DNA. cDNA sequence is shown in uppercase letters; 5′ and 3′ flanking genomic sequences are shown in lowercase letters. The first nucleotide of the cDNA, arbitrarily assigned as position 1, is indicated with an arrow. Sequences homologous to the enhancer and promoter of U snRNA genes, sequences related to U1 RNA, and the polyadenylylation recognition sequence are underlined and indicated on the right side of these sequences. Two possible sites for addition of poly(A) are indicated by arrowheads. (*B*) Evaluation of the coding capacity of PAN RNA. The GCG TESTCODE program was used. At the top of the graph, potential start codons and stop codons are indicated by vertical lines and diamonds, respectively. The value above the line of 9.5 indicates the transcript being a coding RNA with 95% confidence. The value below the line of 7.5 indicates the RNA being noncoding with the same confidence. (*C*) Sequences related to U1 RNA. Two regions of PAN RNA (nt 342–356 and 233–248) were homologous (*) to human U1 RNA. One stretch of PAN RNA from nt 154–166 was complementary (|) to human U1 RNA. (*D*) Comparison of 5′ upstream sequences of U snRNA genes and the PAN RNA gene. Homologous sequences in the KSHV PAN gene are shown in uppercase letters and nonhomologous sequences in lowercase letters. The GT dinucleotides, conserved in human U snRNA genes but not in U RNA genes in other species, are in parentheses. Arrow represents start point of transcription.

PAN RNA Is a Viral Lytic Early Transcript. There was a low level of PAN RNA expression in untreated BC-1 cells (Fig. 2*C*, lanes 4 and 12), but expression of the 1.2-kb PAN RNA was dramatically induced within 20 hr after addition of butyrate. PAN RNA was also induced by phorbol 12-myristate 13-acetate, but with slower kinetics (Fig. 2*C*). PAN RNA was detected in BC-1 cells, but not in KSHV-negative, EBV-positive cells such as HH514-16. The same RNA signal was seen on Northern blots whether a single-stranded oligonucleotide or double-stranded DNA was used as probe (not shown). This result confirmed the orientation of the cDNA and suggested that there was no significant transcription from the opposite strand. PAN RNA expression was blocked by addition of cycloheximide before butyrate; PAN RNA was resistant to inhibitors of KSHV DNA synthesis such as phosphonoacetic acid and phosphonoformic acid (data not shown). These experiments classified PAN RNA as a viral lytic early transcript.

Relative Abundance of PAN RNA. In uninduced cells the ratio of PAN RNA to U1 RNA was 0.006. After treatment with butyrate for 48 hr the ratio of PAN RNA to U1 RNA was 0.25. If one assumes that the copy number of U1 RNA is 10^6 per cell (12) and that 50% of the BC-1 cells were induced to express PAN RNA (see below), there are 5×10^5 copies of PAN RNA per BC-1 cell induced into the KSHV lytic cycle. A similar estimate was obtained when the level of PAN RNA was compared with the level of H1 RNA of RNase P, which is present at about 10^5 copies per cell (12). Thus, PAN RNA is highly abundant when compared with two different cellular noncoding RNAs.

Sedimentation of PAN RNA. A cellular extract prepared from butyrate-treated BC-1 cells was fractionated on a sucrose gradient. The sedimentation of PAN RNA was compared with that of KSHV dihydrofolate reductase mRNA, another early lytic cycle transcript similar in length to PAN RNA. Dihydrofolate reductase mRNA was detected in the bottom fractions of the gradients where polysomes were located (Fig. 3*A Upper*, fractions 1–5). The presence of EDTA, which causes disruption of polysomes by chelating Mg^{2+}, shifted the sedimentation of dihydrofolate reductase mRNA to the non-polysomal region (Fig. 3*A*). In contrast, PAN RNA was not detected in fractions containing polysomes whether or not Mg^{2+} or EDTA were present (Fig. 3*B*). The sedimentation of PAN RNA was similar to that of the small ribosomal subunit and one to two fractions faster than U1 RNP (Fig. 3 *C* and *D*). These data were consistent with the idea that PAN RNA was noncoding and was associated with proteins other than mature ribosomes or polysomes.

PAN RNA Is Located in Nucleoplasm. Cellular localization of PAN RNA was defined by fluorescence *in situ* hybridization (FISH). Specific FISH signals with a speckled pattern were observed in 1–2% of untreated BC-1 cells and in 40–50% of

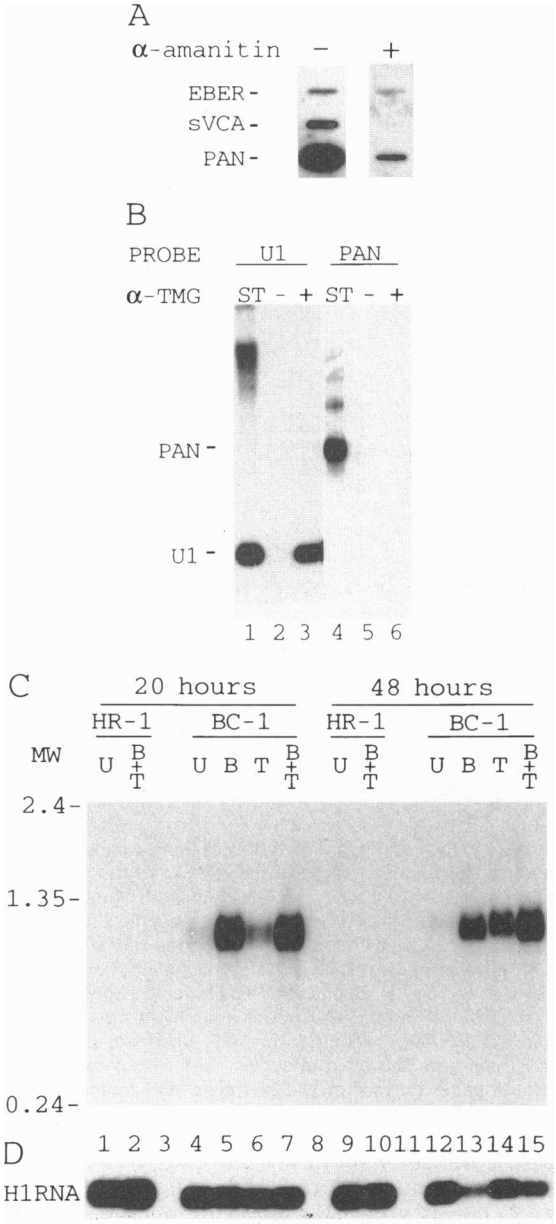

FIG. 2. Transcription of PAN RNA. (*A*) Sensitivity to α-amanitin. Nuclei were isolated from BC-1 cells induced with 3 mM *n*-butyrate for 12 hr. ^{32}P-labeled nuclear run-on transcripts were synthesized in the presence (+) and absence (−) of α-amanitin. DNAs containing sequences of EBERs, KSHV-encoded small viral capsid antigen (sVCA), and PAN RNA were cross-linked to nitrocellulose paper and hybridized with labeled transcripts. (*B*) Immunoprecipitation with monoclonal antibody against trimethylguanosine cap (α-TMG). Total cellular RNAs prepared from induced BC-1 cells were incubated with (lanes 3 and 6) or without (lanes 2 and 5) α-TMG, and precipitated by rabbit anti-mouse antibody conjugated protein A-Sepharose. Starting materials (lanes 1 and 4) and precipitated RNAs were detected on Northern blots by probes for U1 RNA and PAN RNA, respectively. (*C*) Inducibility of PAN RNA. U, B, T, or B+T represent cells that were uninduced, induced with butyrate, induced with PMA, or induced with butyrate and PMA. Cells (2×10^6) were used in each sample. No RNA was loaded in lanes 3, 8, and 11. (*D*) Quantitation of RNA loading. The same filter was hybridized with DNA encoding H1 RNA of human RNase P (25).

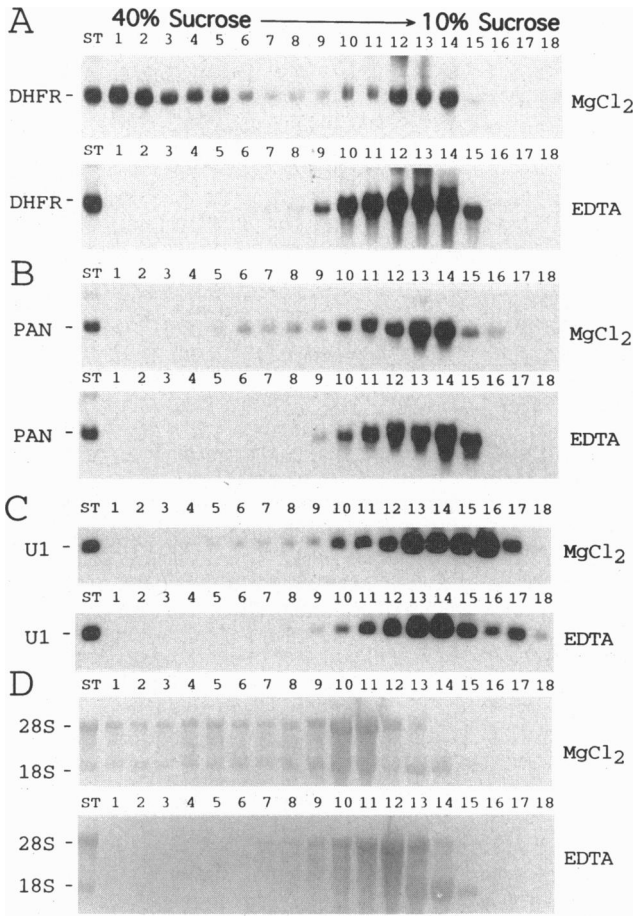

FIG. 3. Sedimentation of PAN RNA in sucrose gradients. RNA from each fraction of sucrose gradient was extracted, evenly divided onto two gels, and detected by Northern blot analysis. The filters were hybridized to the dihydrofolate reductase (DHFR) gene (*A*) and the PAN RNA gene (*B*). Filter B was reprobed with the U1 gene (*C*), then stained with methylene blue for ribosomal RNAs (*D*). Fraction 1 represents the bottom of the gradient, and fraction 18 represents the top. ST is a marker RNA lane representing the starting material before centrifugation. $MgCl_2$ and EDTA indicate gradients were prepared in the presence of 1.4 mM $MgCl_2$ or 10 mM EDTA, respectively.

butyrate-induced BC-1 cells (Fig. 4*D*). The increase in the number of FISH-positive cells induced by butyrate correlated with the magnitude of the increase of RNA signal on Northern blot (Fig. 2 *C* and *D*) and with a comparable increase in the number of cells producing KSHV lytic cycle polypeptide antigens as assessed by immunofluorescence (6). No FISH signal was seen in HH514-16 cells, which contained EBV and not KSHV (Fig. 4*B*). Vector sequences did not hybridize with induced BC-1 cells (data not shown). Propidium iodide counter-staining of the nuclei demonstrated that the hybridization signals were located in nuclei. The same pattern of hybridization was observed in the nuclei whether or not the cytoplasm had been removed with Triton X-100. These results indicated that PAN RNA was predominantly, if not exclusively, nuclear. When induced BC-1 cells were examined under a confocal microscope, the FISH signal was excluded from nucleoli. The speckled pattern of PAN RNA in the nucleoplasm is a characteristic of U snRNAs (27). Like U snRNAs, PAN RNA colocalized with Sm antigens (Fig. 4 *E–G*). Nevertheless, PAN RNA may not directly associate with Sm antigens since we have not been able to coimmunoprecipitate PAN RNA with antibodies against Sm (unpublished results).

DISCUSSION

PAN RNA is similar to U snRNAs and to mRNAs. It contains sequences in its 5′ upstream region that resemble promoter and enhancer sequences of U snRNAs. In common with U snRNAs it lacks long open reading frames, is abundant, is

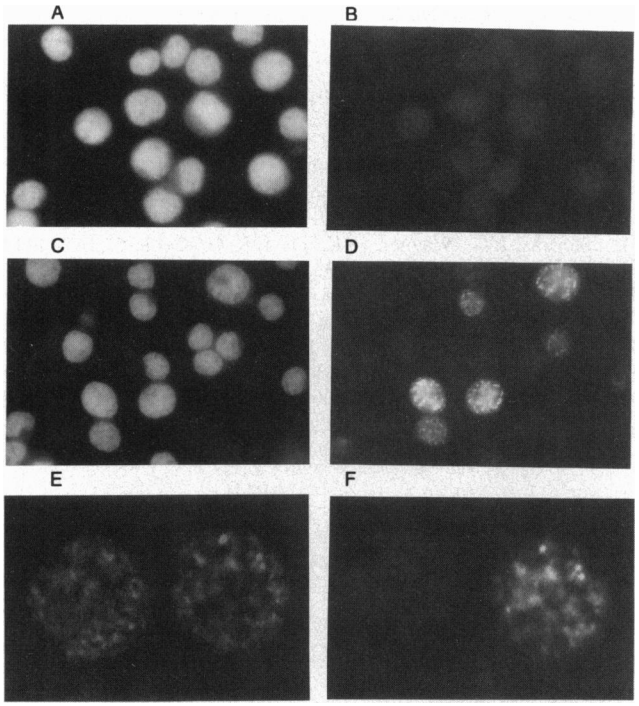

FIG. 4. Cellular localization of PAN RNA. Induced HH514-16 cells (*A* and *B*) and induced BC-1 cells (*C* and *D*) were hybridized with biotinylated pBKCMV-PAN and detected with fluoresceinated avidin. (*A* and *C*) Propidium–iodide staining. (*B* and *D*) Hybridization signals. (*E* and *F*) Colocalization studies using confocal microscopy. In *E* the cells were reacted with anti-Sm antibody followed by rhodamine conjugated anti-mouse Ig. In *F*, the same cells were reacted with a biotinylated probe for PAN RNA followed by fluoresceinated avidin.

predominantly nuclear, and it co-localizes with Sm proteins. Furthermore, it shares several stretches of homology with U1 RNA. One region is homologous to the first 7 nt of U1, which interact with the splice donor site, an early step in spliceosome formation. Two regions are located in the third stem-loop in an area that is of unknown function. None of the regions of U1 that are homologous to PAN RNA are known to be involved in interaction with snRNP-associated proteins. In common with mRNAs are its polyadenylylation signal and its poly(A) tail, as well as a GATAAA box in the promoter region that might function in initiation of transcription. These features of PAN RNA are unexpected because initiation and termination signals for transcription of U snRNAs and mRNAs of higher eukaryotic cells are not considered to be interchangeable (21–23, 26).

Other DNA viruses have been shown to specify noncoding RNAs. These include virus-associated RNAs in adenovirus, EBERs in EBV, herpesvirus saimiri U RNA in herpesvirus saimiri, and latency associated transcript in herpes simpex virus (28–31). The biologic functions of many of these viral noncoding RNAs are obscure. PAN RNA, like noncoding RNAs of other herpesviruses, is found in the nucleoplasm. However, PAN RNA differs from noncoding RNAs of other herpesviruses in two important respects. It is expressed during the viral early lytic phase, whereas EBERs, latency associated transcript and herpesvirus saimiri U RNA are expressed during latency. PAN RNA is polyadenylylated, whereas EBERs, herpesvirus saimiri U RNA, and mature latency associated transcript are not. Possession of traits of both U snRNAs and mRNA also distinguishes PAN RNA from other viral noncoding RNAs. Some apparently noncoding large cellular RNAs have been described to be transcribed by RNA polymerase II. Among these, genetic imprinting RNA H19 and inactive X chromosome specific transcript (Xist) are related to gene inactivation (32, 33). Xist is located in the nucleus, but little is known about how H19 or Xist RNAs inactivate gene expression.

PAN RNA may also selectively inactivate gene expression by interfering with splicing of pre-mRNA. While transcripts of latent and some early lytic genes of herpesviruses are spliced, transcripts of late viral genes are not spliced. One possible function of PAN RNA could be to interfere with splicing of cellular pre-mRNAs, and perhaps latent and early viral pre-mRNAs, while enhancing expression of unspliced mRNAs of late viral genes. The abundance of PAN RNA at about 5×10^5 copies per cell and its persistent expression into the late viral cycle (Fig. 2*C*) are consistent with this role. PAN RNA might achieve this goal by binding to the intron portion of the splice donor site by the stretch of sequence homology to the 5′ end of U1 RNA (reviewed in ref. 34). The two other regions of PAN RNA that are related to U1 may assist this function. Recently the ICP27 protein of herpes simplex virus, which redistributes snRNPs and impairs pre-mRNA splicing, has been found to bind Sm proteins and to change their phosphorylation state (35). The function of ICP27 is essential for herpes simplex virus replication by increasing expression of late viral genes. Thus, herpesviruses may have evolved several means of selective inactivation of gene expression.

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