MOLECULAR AND CELLULAR BIOLOGY, Feb. 1996, p. 468–474 Vol. 16, No. 2
0270-7306/96/$04.00+0

Repression of Beta Interferon Gene Expression in Virus-Infected Cells Is Correlated with a Poly(A) Tail Elongation

EVA DEHLIN,[1,2] ALEXANDER VON GABAIN,[1,2]* GUNNAR ALM,[3] RUTH DINGELMAIER,[1] AND ORNA RESNEKOV[2]†

Institute of Microbiology and Genetics, Vienna Biocenter, A-1030 Vienna, Austria,[1] and Microbiology and Tumor Biology Center, Karolinska Institutet, S-171 77 Stockholm,[2] and Department of Immunology, Biomedical Center, University of Uppsala, S-751 23 Uppsala,[3] Sweden

Received 19 July 1995/Returned for modification 17 August 1995/Accepted 17 October 1995

Expression of beta interferon (IFN-β) is transiently induced when Namalwa B cells (Burkitt lymphoma cell line) are infected by Sendai virus. In this study, we found that an elongation of the IFN-β mRNA could be detected in virus-infected cells and that such a modification was not observed when the IFN-β transcript was induced by a nonviral agent, poly(I-C). Treatment of the cells with a transcriptional inhibitor (actinomycin D or 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole) resulted in further elongation of the transcript. Characterization of the elongated IFN-β transcript by primer extension and RNase H treatment showed that the modification was a result of an elongated poly(A) tail of up to 400 nucleotides. We conclude that the poly(A) tail elongation of the IFN-β transcript is associated with the viral infection. Furthermore, the presence of the elongated IFN-β transcript correlated with a decrease of IFN-β protein in the medium and in cell extracts. Sucrose gradient analysis of cytoplasmic extracts showed that IFN-β transcripts with elongated poly(A) tails were found in the nonpolysomal fractions, whereas the shorter transcripts could be detected in both polysomal and nonpolysomal fractions. A longer form of the IFN-β mRNA was also found in the nonpolysomal fractions of cells not treated with transcriptional inhibitors. Thus, the observed regulation of IFN-β mRNA is not entirely dependent on the inhibition of transcription. To our knowledge, this study provides the first example of a poly(A) tail elongation in somatic cells that negatively influences gene expression in vivo.

Alpha, beta, and gamma interferons (IFN-α, -β and -γ) are a group of inducible proteins that have diverse biological effects which include antiviral activity, an antiproliferative effect, and the ability to modify the immune response (for comprehensive reviews, see references 15, 16, and 44). IFNs are induced by a variety of biological agents which include viruses and double-stranded RNA (15, 16). The induction is transient and activation of IFN gene expression can be monitored at the RNA level. In the case of the IFN-β mRNA, the first stage of the observed transient induction has been shown to be due to transcriptional activation of the IFN-β gene (42), while repression of the transient induction has been shown to be a combination of both transcriptional repression and the short half-life of the IFN-β transcript (51, 56, 57). The short half-life of the IFN-β mRNA is presumed to be due to the presence of four repeats of a consensus sequence (UUAUUUAU) in the 3′ untranslated region (UTR). This consensus sequence was originally identified in a study which compared the sequences of molecules that are involved in the immune response and characteristically have a short half-life (10, 47). In addition, a less well defined, second region of the IFN-β transcript, 5′ to the translation stop codon, has been implicated in destabilization of the transcript (56).

Sequences within the 3′ UTR of the IFN-β transcript have also been suggested to function as translation-inhibitory elements in *Xenopus* oocytes and reticulocyte lysates (27–29). A physical interaction between the poly(A) tail and a AU-rich region in the 3′ UTR of the IFN-β mRNA has been proposed to lead to translational inhibition in the rabbit reticulocyte lysate system (20).

In this study, we investigated posttranscriptional regulation of IFN-β expression. We show that infection of Namalwa B cells with Sendai virus and subsequent inhibition of transcription leads to a structural change in the induced IFN-β transcript which is associated with a lowered translation of the message and a changed pattern of polysome association. The structural change is due to an elongation of the poly(A) tail of the transcript. We suggest that this posttranscriptional mechanism may repress IFN-β gene expression during viral infection.

MATERIALS AND METHODS

Cells and growth of cell cultures. Namalwa cells (substrain B) were obtained from Eric Lundgren (Umeå University, Umeå, Sweden). The cells were maintained as described previously (8). For IFN production, cultures containing 10^6 cells per ml were primed with IFN-α (100 U/ml) for 1 h and induced with Sendai virus (50 hemagglutinating units/ml). Transcription was inhibited by actinomycin D (ActD; Boehringer) at a final concentration of 5 μg/ml or by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB; Sigma) at a final concentration of 20 μM. In our hands, more than 90% of the IFN produced in Namalwa cells (substrain B) induced by Sendai virus was IFN-β, as assessed by an enzyme-linked immunosorbent assay (ELISA) (data not shown). When IFN production was induced with a nonviral agent, the cells were washed once with serum-free medium (RPMI) and resuspended at 10^7 cells per ml in the same medium. Cells were incubated with a mixture of 100 μg of poly(I-C) (Boehringer) and 300 μg of DEAE-dextran (Pharmacia) per ml at 37°C for 30 min and subsequently diluted to 10^6 cells per ml with prewarmed medium containing 10% fetal calf serum (15). The dilution was set as the time of induction.

Preparation and analysis of RNA. Cytoplasmic RNA was extracted and RNA blot hybridization analysis was performed as described previously (33). Ten

* Corresponding author. Mailing address: Vienna Biocenter, Institute of Microbiology and Genetics, Dr. Bohr-Gasse 9, A-1030 Vienna, Austria. Phone: 43-1-79515 4117. Fax: 43-1-79515 4114. Electronic mail address: alex@gem.univie.ac.at.

† Present address: Department of Cell and Developmental Biology, Biological Laboratories, Harvard University, Cambridge, MA 02138.

micrograms of RNA per sample, unless otherwise noted, was separated on 1% agarose-formaldehyde gels. RNA markers (Bethesda Research Laboratories) were used for size determinations. The RNA was blotted onto Hybond-N filters (Amersham) and processed according to the manufacturer's instructions. Primer extension reactions were performed as described previously (3). Primers were end labelled by using phage T4 polynucleotide kinase. The primers used as probes were 5′-CCA CAG GAG CTT CTG ACA CTG AAA-3′ (5′ primer, designed to recognize only IFN-β, not IFN-α) and a β-actin primer (PC-11; 5′-CAG TCA GGT CCC GGC CAG CCA GGT CCA GAC GCA GGA TGG CAT GGG GGA GG-3′; a gift from the Ludwig Institute, Stockholm, Sweden). For Northern (RNA) analysis a 767-bp *Hin*dII fragment of a subclone of pCosIFN-β (22) was purified and labelled by random priming (Amersham). Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA cloned into the *Pst*I site of pBR322 (52) was used as probe for a housekeeping mRNA. RNase H (Pharmacia) assays were performed as described previously (17, 55), using oligo(dT) (12 to 18 nucleotides [nt]; Pharmacia) for the sizing of the poly(A) tail.

In vitro transcription and polyadenylation. The *Hin*dII fragment of pCosIFN-β was subcloned into pSP64poly(A) (Promega), linearized with *Eco*RI, and transcribed in vitro by SP6 RNA polymerase (Boehringer). The in vitro transcripts served as substrates for polyadenylation by yeast poly(A) polymerase (U.S. Biochemical) as described by Ligner et al. (30, 31). The different lengths of the poly(A) tails were achieved by stopping the adenylation reaction after 0 and 10 min. Aliquots of the products were labelled at the 3′ end with [^{32}P]cordycepin 5′-triphosphate and analyzed by 4% urea-polyacrylamide gel electrophoresis (PAGE) to determine the lengths of the transcripts.

In vitro translation assays. Polysome analyses of in vitro-polyadenylated IFN-β transcripts were performed with a Flexi rabbit reticulocyte lysate system (Promega). The Namalwa B-cell extract was prepared as described for Ehrlich ascites tumor cell extract (13), treated with micrococcal nuclease, and dialyzed against equilibration buffer (90 mM KCl, 2 mM magnesium acetate, 7 mM 2-mercaptoethanol, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.6]). A typical translation mix contained 50 μM amino acid mixture minus methionine, 1 mM ATP, 5 mM creatine phosphate, 0.25 mM GTP, 0.4 mM spermidine, 0.5 mM dithiothreitol, 30 mM HEPES (pH 7.5), 1 to 2 μl of [^{35}S]methionine (1,000 Ci/mmol), and 1 to 2 μg of RNA. Translation products were analyzed either by trichloroacetic acid precipitation or by sodium dodecyl sulfate-PAGE.

Protein analysis. For a quantitative analysis of the IFN-β protein, ELISAs were performed by using medium (from which cells were removed by centrifugation) or cell extracts. Cell extracts were prepared from 10^7 cells, using 1 ml of lysis buffer (150 mM NaCl, 1.0% Nonidet P-40, 50 mM Tris [pH 8.0]). Cells were left on ice for 30 min and centrifuged at 4°C for 10 min at 10,000 × *g*. The supernatant was used for the analysis. The ELISA for IFN-β has been described before (11). In brief, microtiter plates coated with goat antibodies to IFN-β were incubated with samples, washed, incubated with murine monoclonal antibodies to IFN-β, washed, and incubated with peroxidase-labelled affinity-purified goat antibodies to murine immunoglobulin. After a final wash, substrate solution containing tetramethylbenzidine was added, and the plates were read in a spectrophotometer. Purified human IFN-β (Bioferon, Laupheim, Germany) was used as a laboratory standard. Its potency in antiviral units was established with the IFN-β reference Gb23-902-531 (National Institutes of Health, Bethesda, Md.).

Polysome analysis. Namalwa B cells were harvested 6 h after infection with Sendai virus or at 90 min after the addition of ActD and lysed as described by Chen et al. (12). The in vitro transcripts with poly(A) tails of 30 and 500 nt, respectively, were incubated for 20 min with rabbit reticulocyte extracts (Promega) and mixed with lysis buffer (as described above). The cell extracts (corresponding to 5×10^7 cells) and the in vitro translation mixtures were layered on linear 15 to 50% sucrose gradients and centrifuged at 40,000 rpm for 110 min in an SW40 rotor (Beckman) at 4°C. Fractions of 0.7 ml were collected and processed as described earlier (12). The A_{254} was monitored during harvesting of the gradients with an ISCO UA6 absorbance monitor. RNA was extracted from the collected fractions, separated on a 1.2% agarose gel, and analyzed by Northern blot hybridization.

Sequence analysis of IFN-β mRNA. IFN-β mRNA extracted from ActD-treated cells was cloned into the *Hin*dII site of pBluescript II SK, employing using reverse transcription-PCR. A primer-adapter (5′-GATCGGCGCCGGC GT_{15}-3′) was used both for cDNA synthesis and as a 3′ primer in the PCR. The 5′ primers 5′-AAGCTTCTAACTGCAACCTTTCGAAGCC-3′ and 5′-TACA GGTTACCTCCGAAACTGAAG-3′ were designed to hybridize to the 5′ end and close to the translational stop codon of the IFN-β cDNA, respectively. Blunt-ended PCR products were generated by a fill-in reaction with Klenow enzyme. Following restriction enzyme analysis, putative clones obtained with both 5′ primers were sequenced with modified T7 DNA polymerase (Sequenase; U.S. Biochemical).

RESULTS

Transcriptional induction of IFN-β and the effect of transcription inhibition in Namalwa B cells. We chose to use the IFN-β transcript as a model system for studying eukaryotic posttranscriptional control since the transcript can be induced to high levels (e.g., by Sendai virus or by double-stranded RNA), is not spliced, and has a short half-life.

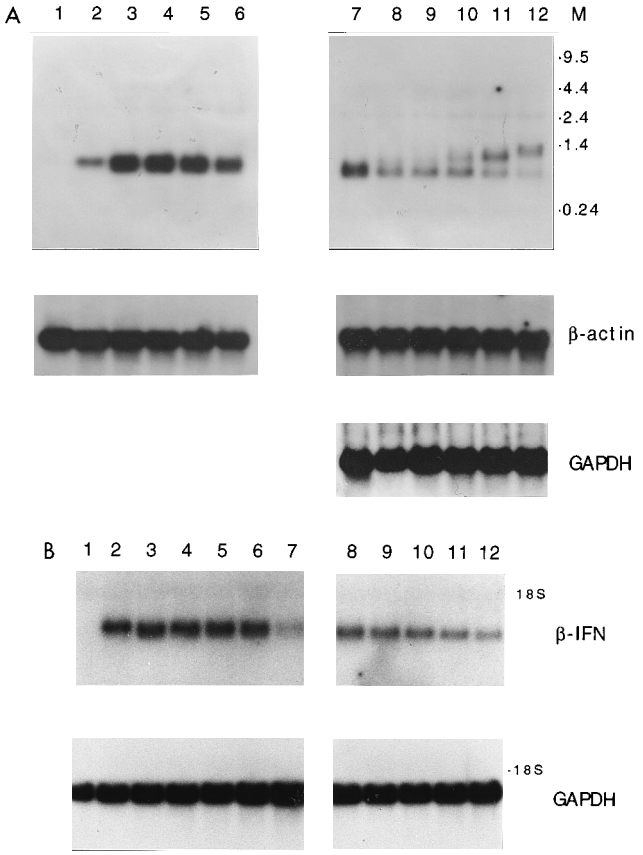

FIG. 1. (A) Sendai virus induction of IFN-β mRNA in Namalwa B cells and the effect of a transcriptional inhibitor, ActD. Northern hybridization was performed with cytoplasmic RNA isolated from cells at different time points after virus induction or ActD addition as described in Materials and Methods. (Top) Northern hybridization, using a ^{32}P-labelled IFN-β-specific probe (Materials and Methods), was performed on total cytoplasmic RNA (10 μg) isolated at 0, 3, 4.5, 6, 8, and 10 h (lanes 1 to 6, respectively) after induction with Sendai virus or on cytoplasmic RNA (10 μg) isolated at 10, 30, 60, 90, 120, and 240 min (lanes 7 to 12, respectively) after the addition of ActD (5 μg/ml) at 6 h postinfection. Positions and lengths (in kilobases) of the RNA size marker (M) are indicated at the right. (Bottom) The same filters stripped and rehybridized with probes for β-actin or GAPDH (see Materials and Methods). (B) Nonviral induction of IFN-β mRNA. Poly(I-C) was used together with DEAE-dextran as the inducer as described in Materials and Methods. (Top) The kinetics of induction was examined by extracting cytoplasmic RNA at 0, 3, 6, 9, 12, 15, and 24 h after transfection (lanes 1 to 7, respectively). The effect of ActD treatment after 9 h of poly(I-C) induction can be seen in lanes 8 to 12. Samples for RNA extraction were taken at 15, 30, 60, 120, and 240 min after addition of ActD and separated on a 1.2% agarose gel. Northern analysis was performed with the IFN-β-specific probe. (Bottom) The same blot rehybridized with GAPDH as a probe. The position of 18S rRNA is indicated.

Northern analysis of cytoplasmic RNA prepared from Namalwa B cells which had been induced with Sendai virus detected a transcript of about 1 kb in length corresponding to IFN-β mRNA (Fig. 1A, top, lanes 1 to 6). The transcript began to appear 3 h after induction and was maximally induced at 6 h, with the cellular abundance of the IFN-β transcript declining thereafter. A control transcript, actin-β, was present in essentially equivalent amounts at all time points (Fig. 1A, bottom, lanes 1 to 6). Addition of the transcriptional inhibitor ActD to the cultures 6 h after infection with Sendai virus

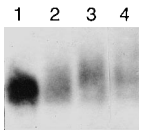

FIG. 2. Northern blot hybridization analysis of cytoplasmic RNA extracted from Sendai virus-induced Namalwa B cells and subsequently treated with the transcriptional inhibitor DRB for 10 min (lane 1), 60 min (lane 2), 120 min (lane 3), and 240 min (lane 4). A *Hin*dII fragment of a genomic clone of IFN-β was used as a probe.

resulted in the appearance of a second, longer IFN-β transcript. This longer transcript appeared 90 min after the addition of the drug, and by 240 min it was the predominant RNA species present (Fig. 1A, top, lanes 7 to 12). The two control transcripts, GAPDH and β-actin, did not vary in length after exposure to ActD (Fig. 1A, bottom, lanes 7 to 12).

When the cells were induced by transfection with double-stranded RNA [poly(I-C)], IFN-β mRNA was induced from 3 until 15 h after transfection (Fig. 1B, top, lanes 1 to 7). The IFN-β transcript was present in approximately equal amounts in all samples which were induced by poly(I-C); however, the maximum level of induction was lower than that seen with Sendai virus. A control transcript, GAPDH, was present in the uninduced samples and at all time points after induction in approximately equal amounts (Fig. 1B, bottom, lanes 1 to 7). In contrast to what was seen in Sendai virus-induced cells, the addition of ActD to poly(I-C)-induced cells caused no change in length of the IFN-β transcript (Fig. 1B, top, lanes 8 to 12).

ActD causes a general transcriptional shutoff by interchalating between the base pairs of a DNA helix, thereby preventing RNA polymerases I to III from binding to the promoter sequences. This causes a perturbation of cellular metabolism that may affect the regulation of posttranscriptional control (18, 23). To address this question, DRB, an adenosine analog that inhibits specific RNA polymerase II-mediated initiation, was used as a transcriptional inhibitor (59). Figure 2 shows a Northern analysis of RNA extracted from Sendai virus-infected and subsequently DRB-treated Namalwa B cells. By 60 min of DRB treatment, the IFN-β mRNA became more heterogeneous (Fig. 2, lane 2), and by 120 min, two discrete populations of the transcript were formed and were maintained up to 240 min after the addition of DRB (Fig. 2, lanes 3 and 4). An elongation of IFN-β can thus be detected in the presence of two transcriptional inhibitors with different modes of action. An elongated transcript can also be detected in the absence of transcriptional inhibitors (see below).

Nature of the structural change to the IFN-β transcript. It seemed likely that the longer transcript detected after ActD treatment of the virally induced cells was due to a change at the 3′ and/or the 5′ end of the transcript because the IFN-β transcript is not spliced (22). Furthermore, no alternative polyadenylation sites are used when IFN-β mRNA is induced by Sendai virus in Namalwa cells (41).

We first examined the integrity of the 5′ end of the transcript by primer extension analysis using a primer specific for IFN-β mRNA and corresponding to the 5′ end of the coding region. On the basis of the known sequence and the primer used, the extension product was expected to be 202 nt long. Cytoplasmic RNA prepared from Namalwa B cells which had been induced with Sendai virus for 0, 4.5, and 6 h and a cytoplasmic RNA sample from cells which had been induced with Sendai virus for 6 h and then treated with ActD for 2 h were used in the assay. As can be seen in Fig. 3, all RNA samples which contained an induced IFN-β transcript resulted in an extension

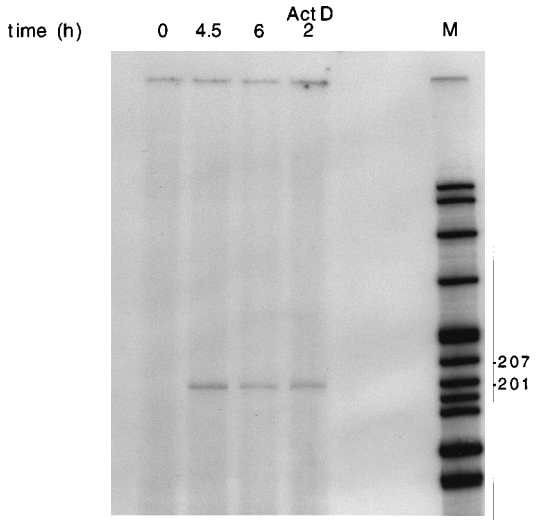

FIG. 3. Structural change in the 5′ end of the IFN-β transcript was examined by primer extension from a ^{32}P-labelled primer (24-mer) which was specific for the 5′ end of the transcript, using avian myeloblastosis virus reverse transcriptase. The first three lanes show the extension product from total cytoplasmic RNA, isolated at 0, 4.5, and 6 h, respectively, after Sendai infection. Lane 4 shows the extension product from a cytoplasmic RNA sample taken 6 h after infection and 2 h after the addition of ActD. Lane M, size marker (pBR322 digested with *Hpa*II). Sizes are indicated in nucleotides.

product of the predicted 202 nt, while the uninduced cells produced no extension product, as expected. We therefore conclude that the observed elongation of the IFN-β transcript as a result of the incubation with ActD was not due to a change in the 5′ end of the transcript.

We reasoned that the change in length of the IFN-β mRNA might be explained by an elongation of the poly(A) tail. This possibility was examined by using an RNase H assay in which total cytoplasmic RNA was hybridized to oligo(dT) and then treated with RNase H. RNase H digests the RNA of an RNA-DNA hybrid and in this case selectively removed poly(A) tails from RNA, which were then analyzed by Northern analysis using an IFN-β-specific probe. As can be seen in Fig. 4, lane 6, the presence of both oligo(dT) and RNase H caused a reduction in length of the two IFN-β transcripts to a smaller size of double intensity when we used RNA isolated from virus-in-

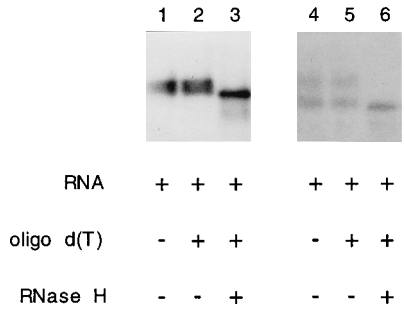

FIG. 4. Northern analysis of RNase H-treated cytoplasmic RNA isolated 6 h after Sendai virus induction or 120 min after ActD addition. A ^{32}P-labelled *Hin*dII fragment of the IFN-β gene was used as probe. Lanes 1 to 3 show RNA isolated 6 h after Sendai infection and not treated (lane 1), hybridized to oligo(dT) (lane 2), and hybridized to oligo(dT) and subsequently treated with RNase H (lane 3). Lanes 4 to 6 show RNA isolated 6 h after Sendai virus induction following incubation with ActD for 120 min and not treated (lane 4), hybridized to oligo(dT) (lane 5), and hybridized to oligo(dT) and subsequently treated with RNase H (lane 6).

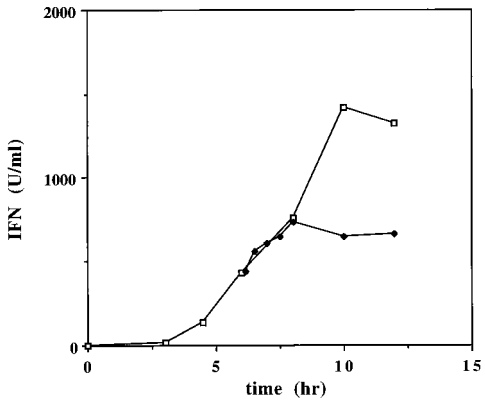

FIG. 5. Analysis of IFN-β protein secreted into the medium. Samples were taken at 3, 4.5, 6, 8, 10, and 12 h after Sendai infection (squares) and at 0, 30, 60, 90, 120, 240, and 360 min after ActD addition (diamonds). The cells were pelleted, and the supernatant was assayed in an ELISA as described in Materials and Methods.

duced, ActD-treated cells. In the absence of ActD, the IFN-β species was shortened to a length which corresponds to that of the mature transcript minus a poly(A) tail (Fig. 4, lane 3). IFN-β RNA from virus-induced cells either with or without ActD treatment was shortened to the same length (Fig. 4, lanes 3 and 6). In control experiments which contained only RNA and oligo(dT), the IFN-β transcripts were not shortened (Fig. 4, lanes 1, 2, 4, and 5). The size of a ^{32}P-labelled, in vitro-synthesized IFN-β transcript was unchanged after hybridization to oligo(dT) and incubation with RNase H (data not shown), indicating that the IFN-β mRNA does not contain any internal sequences that can form stable hybrids with oligo(dT). In addition, IFN-β mRNA extracted from ActD-treated cells was cloned by using reverse transcription-PCR and then sequenced. Comparison with the published IFN-β sequence revealed no changes in either the 5′ or 3′ UTR that could explain the elongation of the IFN-β transcript seen in the Northern analyses (data not shown). These results are consistent with the interpretation that the longer transcript observed after treatment of the cells with ActD is due to the presence of a longer poly(A) tail.

Elongation of the poly(A) tail of the IFN-β mRNA is associated with an arrested IFN synthesis. We wished to determine whether we could detect a physiological effect which could be associated with the presence of the IFN-β transcript containing an elongated poly(A) tail. Since IFN is a secreted molecule, we were able to analyze the medium of cells which had been used for RNA analysis for the presence of the IFN-β protein and then correlate this with the RNA analysis. The presence of IFN-β in the medium was monitored by an ELISA. As can be seen in Fig. 5, the amount of IFN-β in the medium steadily increased from 3 h after the induction with Sendai virus until about 10 h after induction. In contrast, accumulation of the IFN-β protein in the medium ceased about 2 h after the Sendai virus-induced cells were treated with ActD. Two hours after ActD addition, the elongated IFN-β transcript becomes the predominant mRNA species (Fig. 1A, lane 11). This result indicated that the elongation of the IFN-β transcript negatively affects the accumulation of the IFN-β protein in the medium.

To test whether secretion of the IFN-β protein was affected by treatment of the cells with ActD, we measured the amount of IFN-β protein in cell extracts and in the medium by using the ELISA. If the cells continued to translate the IFN-β message after the addition of ActD but were unable to secrete the protein, the amount of IFN-β in the cell extracts should increase over time, while the amount of IFN-β in the medium should remain constant. If, on the other hand, the secretion of IFN-β was not affected by treatment of the cells with ActD but translation of the IFN-β message was affected, the amount of IFN-β in the cell extracts would be expected to decrease over time, and the amount of IFN-β in the medium would increase as long as the cells had IFN-β to export. As can be seen in Fig. 6, the amount of IFN-β in the cell extracts decreased over time after the addition of ActD, while the IFN-β in the medium increased until 7.5 h (90 min after addition of ActD), after which there was no further increase in the amount of IFN-β seen in the medium. Without the addition of ActD to the cultures, the amount of IFN-β in the cell extracts increased up to 7.5 h and then remained fairly constant, while the amount of IFN-β in the medium continued to increase up to 9 h. No effect on bulk cellular translation was seen until 4 h after the addition of ActD, as measured by the incorporation of [^{35}S]methionine (data not shown). The results are consistent with the explanation that ActD does not inhibit the secretion of IFN-β from these cells. In the absence of ActD, IFN-β is translated and is secreted from the cells up to at least 9 h after induction with Sendai virus. Translation of IFN-β (as assessed by the intracellular level of IFN-β) appears to stop shortly after the addition of ActD to the cultures, while secretion of the protein continues, and by 9 h the cells are essentially depleted of the IFN-β protein.

The elongated IFN-β mRNA is not associated with polysomes and can also be detected without inhibition of transcription. The limited accumulation of the IFN-β protein in the medium and the decrease of IFN-β in the cytoplasm following ActD addition could be due to inefficient translation of the IFN-β mRNA. To address this question, we fractionated cytoplasmic extracts on linear sucrose gradients (12) from Sendai virus-infected cells treated and not treated with ActD.

A typical UV absorbance profile is shown in Fig. 7A. The actively translated mRNAs were found in fractions 10 to 19, which contained the polysomes, while fractions 1 to 9 contained ribosomal subunits and monosomes (as judged by methylene blue staining of the 18S and 28S rRNAs on the hybridization filters). RNA was extracted from each fraction and subjected to Northern analysis (Fig. 7B).

The majority of the IFN-β mRNAs from Sendai virus-induced cells not treated with ActD cosedimented with the poly-

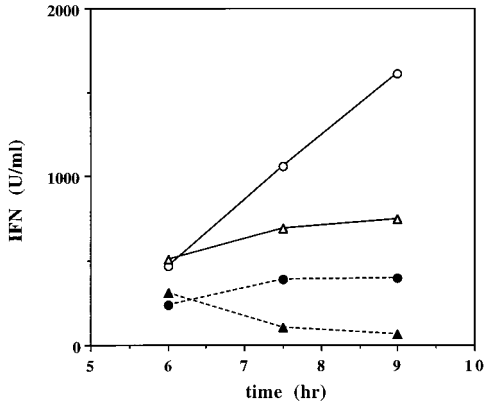

FIG. 6. ELISA analysis of IFN-β protein both in the medium and in cell extracts. Cell extracts were prepared as described in Materials and Methods. Open symbols, IFN-β protein secreted into the medium; filled symbols, IFN-β protein in cell extracts; triangles, the amount of IFN-β protein found after ActD treatment; circles, IFN-β protein in controls.

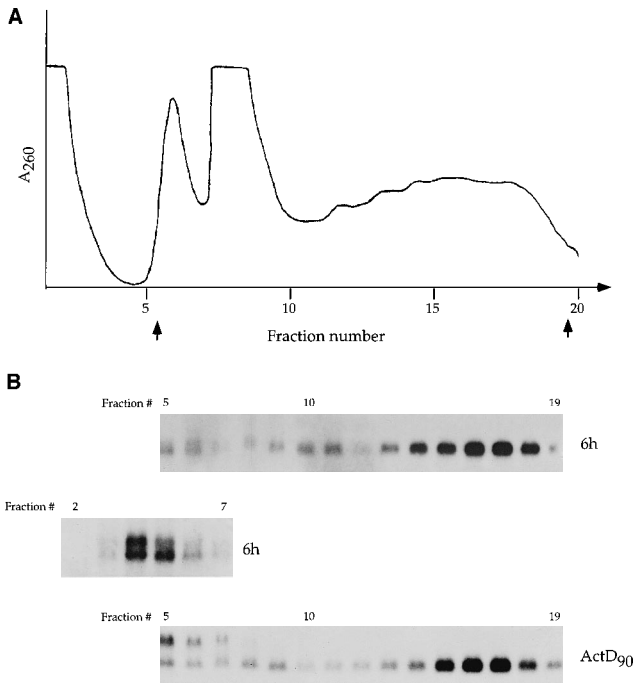

FIG. 7. (A) Absorbance profile of a typical sucrose gradient fractionation of cytoplasmic extracts from Sendai virus-infected Namalwa B cells. Fractions 10 to 21 contained the bulk polysomes, and fractions 1 to 9 included monosomes and ribosomal subunits (as judged by staining of rRNA). The first and last fractions which were analyzed by Northern hybridisation are indicated by arrows. (B) Distribution of the IFN-β transcript after sucrose gradient fractionation. The cell extracts were prepared as described in Materials and Methods and layered on 15 to 50% linear sucrose gradients. RNA was extracted from the collected fractions and separated on a 1.2% agarose gel for Northern analysis. (Top) IFN mRNA distribution 6 h after Sendai virus infection; (middle) separation of the long and short forms of the IFN-β transcript on an agarose gel run for additional 2 h (fractions 2 to 7); (bottom) IFN-β mRNA distribution 6 h after virus infection and after 90 min of ActD treatment.

somal fractions. Strikingly, a smaller amount sedimented more slowly and appeared to be separated into longer and shorter forms (Fig. 7B, top [6 h], fractions 5 to 7). To better separate these two RNA species, RNAs from fractions 2 to 7 were run on a 1.2% agarose gel to obtain a resolution better than that obtained previously. Two forms could easily be separated and were even more pronounced in fractions 4 and 5 (Fig. 7B, lower panel [6 h]). These data suggest that the extension of the IFN-β transcript is Sendai virus specific and does not solely depend on transcriptional inhibitors. It is, however, apparent that the fraction of the elongated form in the absence of a transcriptional inhibitor is relatively small (less than 10% of total IFN-β mRNA), which explains why it cannot be seen in the Northern analysis shown in Fig. 1A and 4, in which nonfractionated total RNA was analyzed.

The elongated IFN-β mRNA was found exclusively in the nonpolysomal fractions when the ActD-treated cell extracts were fractionated (Fig. 7B, bottom, fractions 5 to 8). In fact, it was detected in the fractions preceding the monosome peak, suggesting that too long poly(A) tails interfere with the assembly of the 80S initiation complex.

The shorter IFN-β transcript from either treatment (with or without ActD) appeared to be of the same length in all fractions, whereas the longer species in the nonpolysomal fractions became more extended upon ActD treatment. This is consistent with the idea that the extra IFN-β species seen without

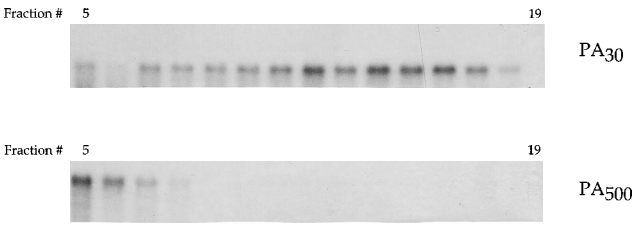

FIG. 8. Distribution of the in vitro-polyadenylated IFN-β transcripts after sucrose gradient fractionation. The length of the added poly(A) tail is indicated at the right (30 nt [PA_{30}] or 500 nt [PA_{500}]).

ActD treatment is an elongation and not a shortening (deadenylation or degradation) of the transcript. Moreover, these results indicate that an extension of the poly(A) tail also occurs in virus-infected cells not treated with ActD.

Effect of the elongated poly(A) tail on polysome association and translation in vitro. To confirm that an elongation of the poly(A) tail is sufficient to repress translation of IFN-β mRNA, in vitro-transcribed IFN-β RNA with a short poly(A) tail was further polyadenylated by using yeast poly(A) polymerase PAP (30, 31). RNAs with tails containing 30 and ~500 adenosines, were incubated with rabbit reticulocyte extracts and fractionated on the same type of sucrose gradients as used for the cellular extracts. Figure 8 shows that the IFN-β transcript with a poly(A) tail of ~500 nt was not associated with polysomes, while the transcript with a short poly(A) tail of 30 nt was predominantly found in the polysomal fractions. These results match our in vivo data (Fig. 7) and confirm the observations of Grafi et al. (20). They show that an IFN-β transcript with an extended poly(A) tail is able to physically interact with translation-inhibitory sequences in the 3′ UTR, sequences that first were identified by Kruys et al. (27–29). Our data not only support this model but also suggest that this mechanism occurs in somatic cells.

We also investigated the significance of the extended poly(A) tails of IFN-β mRNA on translation in Namalwa B-cell extracts. IFN-β mRNA from the nonpolysomal fractions containing predominantly the elongated transcripts was selected by hybridization to immobilized IFN-β DNA. After elution of the selected RNA, half of the sample was annealed to oligo(dT) and treated with RNase H prior to translation in vitro. The translational efficiency of the shortened IFN-β transcript was significantly higher than that of the RNA with an elongated poly(A) tail (data not shown). We propose that it is the shorter IFN-β transcript which is translationally active in vivo and that the elongation of the poly(A) tail is sufficient to account for the observed inhibition of IFN-β expression.

DISCUSSION

In this report, we have shown that elongated poly(A) tails are involved in the down-regulation of IFN-β protein production in virus-infected cells. Extended forms of the IFN-β transcript were preferentially found in nonpolysomal fractions, and the reduction of IFN-β production occurred in synchrony with the poly(A) tail elongation. The elongated forms of IFN-β mRNA were not detected in cells induced by poly(I-C). Furthermore, an elongation of housekeeping mRNA species was not observed. Thus, we suggest that poly(A) tail aberrations are part of a virus-mediated strategy to lower the expression of defense-specific proteins.

The effect of polyadenylation on the mechanism or control of translation has been considered for some years, and a final

picture has not yet emerged (26, 45, 58). In several instances, an increase in the length of the poly(A) tail has been reported to be associated with an increase in the translation of a particular mRNA (24, 36, 38, 43, 50), while in other instances deadenylation of a transcript has been associated with translational activation (4). Our results show that an in vivo elongation of the poly(A) tail of the IFN-β transcript is associated with an inhibition of translation and therefore confirm the in vitro data of Grafi et al. (20). They have shown that poly(A) tails longer than 30 to 50 nt have an inhibitory effect on the translation of IFN-β in rabbit reticulocytes, and Kruys et al. (28, 29) demonstrated that sequence elements in the 3′ UTR of IFN-β mediate inhibition of translation. It has been shown that in *Xenopus* oocytes, a poly(A) tail increase from 50 to 150 nt allowed for polysome recruitment, while messages which contained a poly(A) tail increase from 150 to 400 nt were not recruited onto polysomes (39). Our results are consistent with the idea that IFN-β mRNA with a relatively long poly(A) tail is translated inefficiently if at all. Several cell lines which produce long IFN-β transcripts and relatively little IFN-β protein have been described (14, 21, 46, 48). However, some of these longer IFN-β transcripts may be attributed to alternative usage of poly(A) addition sites (41).

An elongation of the IFN-β transcript poly(A) tail was not observed when the cells were induced with poly(I-C), but such an elongation did occur when the cells were infected by Sendai virus alone (Fig. 7B, lower panel [6 h]) or, more apparently, when the infected cells were treated with the transcriptional inhibitors (Fig. 1A, lanes 7 to 12, and Fig. 2). We cannot exclude the possibility that the extension of the poly(A) tail takes place in the nucleus, but if this is so, we interpret our data to mean that the elongated transcript is transported into the cytoplasm. Small but reproducible changes in size distribution of cytoplasmic poly(A)-containing RNAs (in particular IFN mRNA) have previously been observed in Namalwa cells infected by Newcastle disease virus (a paramyxovirus) (5). Furthermore, an accumulation of the elongated IFN-β transcript was detected in the nonpolysomal fractions when extracts from Sendai virus-infected cells, treated or not treated with ActD, were fractionated over a sucrose gradient. Therefore, we suggest that virus-mediated functions participate in the mechanism of IFN-β mRNA poly(A) tail regulation. In this context, it is worth mentioning that all known replicative steps of paramyxoviruses are reported to be restricted to the cytoplasm of the host cell.

The transcriptional inhibitors ActD and DRB blocks transcription of host-encoded but not virus-encoded genes (18, 59); thus, a virus-mediated poly(A) tail elongation of the IFN-β transcript may be facilitated by a transcriptional block of a labile, host-encoded deadenylation activity (1, 2, 25, 40, 54). Since similar modifications of housekeeping mRNA species were not found, one would predict that the IFN-β transcript contains sequences that specify the observed changes.

Sequences identified to date which are required for cytoplasmic polyadenylation lie within 3′ UTRs and include the poly(A) motif (AAUAAA) and a *cis* element (cytoplasmic polyadenylation element; UUUUUUAU) (6, 34, 35, 39). Additional sequence elements, 5′ of the cytoplasmic polyadenylation element and 3′ of the poly(A) motif, inhibit polyadenylation and promote deadenylation during *Xenopus* development (49). The IFN-β mRNA 3′ UTR is extremely AU rich. It contains four repetitions of the motif UUAUUUAU, which is thought to be a determinant of mRNA instability (47). One of the repetitions is 3′ to the IFN-β poly(A) motif, while the other three are 5′ to the poly(A) motif. The motif UUAUUUAU differs by only one nucleotide from the sequence that has been reported to be required for cytoplasmic polyadenylation. In the specific case of IFN-β, this sequence has also been shown to function as a translation-inhibitory element in *Xenopus* oocytes and reticulocyte lysates (27–29). Several different factors which recognize such elements have been described (7, 9, 19, 32, 35, 37, 53). The possibility exists that the *cis* and *trans* elements involved in the regulation of mRNA stability, cytoplasmic polyadenylation, and translational regulation overlap. One might envision that in a case such as IFN-β, a molecule with complex biological functions, a set of overlapping posttranscriptional control mechanisms might operate to govern the ultimate expression of the protein as well as strict transcriptional control. It is also possible that one of these mechanisms is a target used by Sendai virus to escape the IFN defense system. Future studies will address the regulation and hierarchy of these mechanisms in response to viral infection.

ACKNOWLEDGMENTS

We thank G. Adolf (Bender + Co., Vienna, Austria) for valuable information about nonviral induction of Namalwa cells, H. Strander, M. Baccarini, and T. Decker for stimulating discussions, Margret Wahlström for excellent technical assistance, and Cathal Wilson for critically reading the manuscript.

This work was supported by grants from the Swedish Cancer Society, the Birthe Meyer Fund, and the Austrian Science Foundation. Orna Resnekov was supported during a portion of this work by an EMBO postdoctoral fellowship, the Swedish Cancer Society, and a Science Scholar Fellowship from the Bunting Institute.

REFERENCES

1. **Åström, J., A. Åström, and A. Virtanen.** 1992. In vitro deadenylation of mammalian mRNA by HeLa cell 3′ exonuclease. EMBO J. **10:**3067–3071.
2. **Åström, J., A. Åström, and A. Virtanen.** 1992. Properties of a HeLa cell 3′ exonuclease specific for degrading poly(A) tails of mammalian mRNA. J. Biol. Chem. **267:**18154–18159.
3. **Ayer, D. E., and W. S. Dynan.** 1988. Simian virus 40 major late promoter: a novel tripartite structure that includes intragenic sequences. Mol. Cell. Biol. **8:**2021–2033.
4. **Ballantine, J. E. M., and H. R. Woodland.** 1985. Polyadenylation of histone mRNA in *Xenopus* oocytes and embryos. FEBS Lett. **180:**224–228.
5. **Berger, S. L., M. J. M. Hitchcock, K. C. Zoon, C. S. Birkenmeier, R. M. Friedman, and E. H. Chang.** 1980. Characterization of interferon messenger RNA synthesis in Namalwa cells. J. Biol. Chem. **255:**2955–2961.
6. **Bilger, A., C. A. Fox, E. Wahle, and M. Wickens.** 1994. Nuclear polyadenylation factors recognize cytoplasmic polyadenylation elements. Genes Dev. **8:**1106–1116.
7. **Bohjanen, P. R., B. Petryniak, C. H. June, C. B. Thompson, and T. Lindsten.** 1991. An inducible cytoplasmic factor (AU-B) binds selectively to AUUUA multimers in the 3′ untranslated region of lymphokine mRNA. Mol. Cell. Biol. **11:**3288–3295.
8. **Borg, H., I. Nordensson, T. Ny, and E. Lundgren.** 1985. Evidence for IFN-β heterogeneity in a substrain of Namalwa cells. Appl. Biochem. Biotechnol. **11:**111–122.
9. **Brewer, G.** 1991. An A+U rich element RNA-binding factor regulates c-*myc* mRNA stability in vitro. Mol. Cell. Biol. **11:**2460–2466.
10. **Caput, D., B. Beutler, K. Hartog, R. Thayer, S. Brown-Shimer, and A. Cerami.** 1986. Identification of a common nucleotide sequence in the 3′-untranslated region of mRNA molecules specifying inflammatory mediators. Proc. Natl. Acad. Sci. USA **83:**1670–1674.
11. **Cederblad, B., and G. Alm.** 1991. Interferons and the colony-stimulating factors IL-3 and GM-CSF enhance the IFN-α response in human blood leucocytes induced by herpes simplex virus. Scand. J. Immunol. **34:**549–555.
12. **Chen, X., J. D. Sparks, Z. Yao, and E. A. Fisher.** 1993. Hepatic polysomes that contain apoprotein B mRNA have unusual physical properties. J. Biol. Chem. **268:**21007–21013.
13. **Clemens, M. J.** 1984. Translation of eucaryotic messenger RNA in cell-free extracts, p. 231–270. *In* B. D. Hames and S. J. Higgins (ed.), Transcription and translation: a practical approach. IRL Press Limited, Oxford.
14. **Content, J., L. De Wit, L. Tavernier, and W. Fiers.** 1983. Human fibroblast interferon RNA-transcripts of different sizes in poly(I)·poly(C) induced cells. Nucleic Acids Res. **11:**2627–2637.
15. **DeMaeyer, E.** 1981. Translation of mouse interferon mRNA in heterologous cells. Methods Enzymol. **79:**99–103.
16. **DeMaeyer, E., and J. DeMaeyer-Guinard.** 1988. Interferons and other regulatory cytokines, p. 39–66. John Wiley & Sons, Inc., New York.

17. **Donis-Keller, H.** 1979. Site specific enzymatic cleavage of RNA. Nucleic Acids Res. **7:**179–192.
18. **Dreyfuss, G., S. A. Adam, and Y. D. Choi.** 1984. Physical change in cytoplasmic messenger ribonucleoproteins in cells treated with inhibitors of mRNA transcription. Mol. Cell. Biol. **4:**415–423.
19. **Gershon, P. D., B.-Y. Ahn, M. Garfield, and B. Moss.** 1991. Poly(A) polymerase and dissociable polyadenylation stimulatory factor encoded by vaccinia virus. Cell **66:**1269–1278.
20. **Grafi, G., I. Sela, and G. Galili.** 1993. Translational regulation of human beta interferon mRNA: association of the 3′ AU-rich sequence with the poly(A) tail reduces translational efficiency in vitro. Mol. Cell. Biol. **13:**3487–3493.
21. **Gross, G., W. Bruns, U. Mayr, and J. Collins.** 1982. The identity of interferon-β mRNA transcripts in human fibroblasts and Namalwa cells. FEBS Lett. **139:**201–204.
22. **Gross, G., U. Mayr, W. Bruns, F. Grosveld, H.-H. Dahl, and J. Collins.** 1981. The structure of a thirty-six kilobase region of the human chromosome including the fibroblast interferon gene IFN-β. Nucleic Acids Res. **9:**2495–2507.
23. **Hentze, M. W.** 1991. Determinants and regulation of cytoplasmic mRNA stability in eucaryotic cells. Biochim. Biophys. Acta **1090:**281–292.
24. **Huarte, J., D. Belin, A. Vassalli, S. Strickland, and J.-D. Vassalli.** 1987. Meiotic maturation of mouse oocytes triggers the translation and polyadenylation of dormant tissue-type plasminogen activator mRNA. Genes Dev. **1:**1201–1211.
25. **Huarte, J., A. Stutz, M. L. O'Connell, P. Gubler, D. Belin, A. L. Darrow, S. Strickland, and J.-D. Vassalli.** 1992. Transient translational silencing by reversible mRNA deadenylation. Cell **69:**1021–1030.
26. **Jackson, R. J., and N. Standart.** 1990. Do the poly(A) tail and the 3′ UTR region control mRNA translation? Cell **62:**15–24.
27. **Kruys, V., O. Marinz, G. Shaw, J. Deschamps, and G. Huez.** 1989. Translational blockade imposed by cytokine-derived UA-rich sequences. Science **245:**852–854.
28. **Kruys, V., M. Wathelet, P. Poupart, R. Contreras, W. Fiers, J. Content, and G. Huez.** 1987. The 3′ untranslated region of the human interferon-β mRNA has an inhibitory effect on translation. Proc. Natl. Acad. Sci. USA **84:**6030–6034.
29. **Kruys, V. I., M. G. Wathelet, and G. A. Huez.** 1988. Identification of a translation inhibitory element (TIE) in the 3′ untranslated region of the human interferon-β mRNA. Gene **72:**191–200.
30. **Lingner, J., and W. Keller.** 1993. 3′-end labeling of RNA with recombinant yeast poly(A) polymerase. Nucleic Acids Res. **21:**2917–2920.
31. **Lingner, J., J. Kellermann, and W. Keller.** 1991. Cloning and expression of the essential gene for poly(A) polymerase from *S. cerevisiae*. Nature (London) **354:**496–498.
32. **Malter, J. S.** 1989. Identification of an AUUUA-specific messenger RNA binding protein. Science **246:**664–666.
33. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **McGrew, L. L., E. Dworkin-Rastl, M. B. Dworkin, and J. D. Richter.** 1989. Poly(A) elongation during *Xenopus* oocyte maturation is required for translational recruitment and is mediated by a short sequence element. Genes Dev. **3:**803–815.
35. **McGrew, L. L., and J. D. Richter.** 1990. Translational control by cytoplasmic polyadenylation during *Xenopus* oocyte maturation: characterization of *cis* and *trans* elements and regulation by cyclin/MPF. EMBO J. **9:**3743–3751.
36. **Muschel, R., G. Khoury, and L. M. Reid.** 1986. Regulation of insulin mRNA abundance and adenylation: dependence on hormones and matrix substrata. Mol. Cell. Biol. **6:**337–341.
37. **Ohno, S., and T. Tanigushi.** 1981. Structure of a chromosomal gene for human interferon beta. Proc. Natl. Acad. Sci. USA **78:**5305–5309.
38. **Paek, I., and R. Axel.** 1987. Glucocorticoids enhance stability of human growth hormone mRNA. Mol. Cell. Biol. **7:**1496–1507.
39. **Paris, J., and J. D. Richter.** 1990. Maturation-specific polyadenylation and translational control: diversity of cytoplasmic polyadenylation elements, influence of poly(A) tail size, and formation of stable polyadenylation complexes. Mol. Cell. Biol. **10:**5634–5645.
40. **Peppel, K., and C. Baglioni.** 1991. Deadenylation and turnover of interferon-β mRNA. J. Biol. Chem. **266:**6663–6666.
41. **Raj, N. B. K.** 1988. Human fibroblast but not lymphoid cells have unusually long polyadenylated interferon-β_1 mRNAs. J. Interferon Res. **8:**179–192.
42. **Raj, N. B. K., and P. M. Pitha.** 1983. Two levels of regulation of β-interferon gene expression in human cells. Proc. Natl. Acad. Sci. USA **80:**3923–3927.
43. **Robinson, B. G., D. M. Frim, W. J. Schwartz, and J. A. Majzoub.** 1988. Vasopressin mRNA in the suprachiasmatic nuclei: daily regulation of polyadenylate tail length. Science **241:**342–344.
44. **Romeo, G., G. Fiorucci, and G. B. Rossi.** 1989. Interferons in cell growth and development. Trends Genet. **5:**19–24.
45. **Sachs, A., and E. Wahle.** 1993. Poly(A) tail metabolism and function in eucaryotes. J. Biol. Chem. **268:**22955–22958.
46. **Sagar, A. D., L. A. Pickering, P. Sussman-Berger, W. E. Stewart II, and P. B. Sehgal.** 1981. Heterogeneity of interferon mRNA species from Sendai virus-induced human lymphoblastoid (Namalwa) cells and Newcastle disease virus-induced murine fibroblast (L) cells. Nucleic Acids Res. **9:**149–160.
47. **Shaw, G., and R. Kamen.** 1986. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective degradation. Cell **46:**659–667.
48. **Shuttleworth, J., J. Morser, and D. C. Burke.** 1983. Expression of interferon-α and interferon-β genes in human lymphoblastoid (Namalwa) cells. Eur. J. Biochem. **133:**399–404.
49. **Stebbins-Boaz, B., and J. D. Richter.** 1994. Multiple sequence elements and a maternal mRNA product control cdk2 RNA polyadenylation and translation during early *Xenopus* development. Mol. Cell. Biol. **14:**5870–5880.
50. **Strickland, S., J. Huarte, D. Belin, A. Vassalli, R. J. Rickles, and J.-D. Vassalli.** 1988. Antisense RNA directed against the 3′ noncoding region prevents dormant mRNA activation in mouse oocytes. Science **241:**680–684.
51. **Taniguchi, T.** 1989. Regulation of interferon-β gene: structure and function of *cis*-elements and *trans*-acting factors. J. Interferon Res. **9:**633–640.
52. **Tso, J. Y., X.-H. Sun, T.-H. Kao, K. S. Reece, and R. Wu.** 1985. Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. Nucleic Acids Res. **13:**2485–2502.
53. **Vakalopoulou, E., J. Schaack, and T. Shenk.** 1991. A 32-kilodalton protein binds to AU-rich domains in the 3′ untranslated regions of rapidly degraded RNAs. Mol. Cell. Biol. **11:**3355–3364.
54. **Varnum, S. M., and W. M. Wormington.** 1990. Deadenylation of maternal mRNAs during Xenopus oocyte maturation does not require specific cis-sequences: a default mechanism for translational control. Genes Dev. **4:**2278–2286.
55. **Vournakis, J. N., A. Efstratiadis, and F. C. Kafatos.** 1975. Electrophoretic patterns of deadenylated chorion and globin mRNAs. Proc. Natl. Acad. Sci. USA **72:**2959–2963.
56. **Whittemore, L.-A., and T. Maniatis.** 1990. Postinduction turnoff of beta-interferon gene expression. Mol. Cell. Biol. **10:**1329–1337.
57. **Whittemore, L.-A., and T. Maniatis.** 1990. Postinduction repression of the β-interferon gene is mediated through two positive regulatory domains. Proc. Natl. Acad. Sci. USA **87:**7799–7803.
58. **Wormington, M.** 1993. Poly(A) and translation: development control. Curr. Opin. Cell Biol. **5:**950–954.
59. **Zandomeni, R., B. Mittleman, D. Bunick, S. Ackerman, and R. Weinman.** 1982. Mechanism of action of dichloro-β-D-ribofuranosyl-benzimidazole: effect on in vitro transcription. Proc. Natl. Acad. Sci. USA **79:**3167–3170.