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Postinduction Turnoff of Beta-Interferon Gene Expression

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Viral induction of the human beta-interferon (IFN-β) gene leads to a transient accumulation of high levels of IFN-β mRNA. Previous studies have shown that the increase in IFN-β mRNA levels after induction is due to an increase in the rate of IFN-β gene transcription. In this paper, we show that the rapid postinduction decrease in the level of IFN-β mRNA is due to a combination of transcriptional repression and rapid turnover of the mRNA. This transcriptional repression can be blocked with cycloheximide, suggesting that the synthesis of a virus-inducible repressor is necessary for the postinduction turnoff of the IFN-β gene. Analysis of the sequence requirements for IFN-β mRNA instability revealed two regions capable of destabilizing a heterologous mRNA. One destabilizer is an AU-rich sequence in the 3′ untranslated region, and the other is located 5′ to the translation stop codon.

Human beta-interferon (IFN-β) gene expression is highly inducible by virus or double-stranded RNA (for a review, see reference 6). Induction leads to transcriptional activation of the gene and rapid accumulation of IFN-β mRNA for 8 to 10 h, after which the level of IFN-β mRNA rapidly decreases (26; K. Zinn, Ph.D. thesis, Harvard University, Cambridge, Mass., 1983). Although considerable information has been obtained regarding the mechanism of IFN-β gene activation (for reviews, see references 18 and 36), relatively little is known about the mechanisms involved in the postinduction decrease of IFN-β mRNA.

The normal kinetics of IFN-β mRNA induction and decay can be altered by metabolic inhibitors such as actinomycin D (ActD), 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), and cycloheximide (CHX) (4, 25, 30–32). If these inhibitors are present during induction, IFN-β mRNA continues to accumulate instead of undergoing the normal postinduction decrease. This phenomenon, known as superinduction, led to the proposal that the normal postinduction decrease in IFN-β mRNA is controlled at the level of mRNA stability. According to this proposal, a labile RNase whose synthesis is blocked by metabolic inhibitors is responsible for the rapid postinduction turnover of IFN-β mRNA (4, 30–32). However, since superinduction requires the same transcriptional signals as viral induction (7, 20), it is also possible that CHX prevents the synthesis of a repressor required for turning off transcription of the IFN-β gene.

Although superinduction experiments imply a role for both mRNA turnover and transcriptional repression in the regulation of the IFN-β gene, the relative contributions of these mechanisms have not been systematically studied. In this paper, we describe two different approaches to studying the contributions of transcriptional control and mRNA turnover to IFN-β gene regulation. First, we used nuclear transcription experiments to directly demonstrate that IFN-β gene expression is turned off at the level of transcription and that this repression requires protein synthesis. Second, the role of IFN-β mRNA stability was investigated by analyzing mRNA accumulation for fusion genes containing various portions of the IFN-β gene. Reciprocal fusions between the rabbit β-globin (RBG) and IFN-β genes indicated that there are determinants for mRNA degradation in both the 3′ untranslated region and the region 5′ to the translation stop codon of IFN-β mRNA.

Similar observations were made with a fusion gene containing the IFN-β promoter linked to the transcription unit of the human growth hormone (hGH) gene. Nuclear transcription experiments revealed that transcriptional regulation of the hGH fusion gene and the IFN-β gene after viral induction is similar, but RNase protection studies indicated that hGH mRNA is present several hours after its transcription has terminated. Therefore, the difference in the kinetics of mRNA accumulation between hGH mRNA and IFN-β mRNA is due to a difference in the stability of the two mRNAs. This observation provides an assay for the IFN-β mRNA sequences required for normal IFN-β mRNA turnover. Using this assay, we identified two regions of IFN-β mRNA that render hGH mRNA unstable. One of these destabilizers is located in the 3′ untranslated region of IFN-β mRNA and is similar to the AU-rich destabilizer previously identified by Shaw and Kamen (33). The other destabilizer is located 5′ to the translation stop codon of IFN-β mRNA. We conclude that the postinduction decrease of IFN-β mRNA is due to both transcriptional repression and rapid turnover of the mRNA. We also investigated the effect of both IFN-β destabilizers on the deadenylation of mRNA as well as the abilities of ActD and CHX to block the degradation and deadenylation of mRNA.

MATERIALS AND METHODS

Cell culture and transfection. C127, MG63, and NIH 3T3 cells (American Type Culture Collection) were grown in Dulbecco modified Eagle medium (DME) plus 10% fetal calf serum. C127 and NIH 3T3 cells were seeded 1 day prior to transfection and were 30 to 50% confluent at the time of transfection. The cells were transfected and transformants were selected as previously described (10). G418-resistant colonies were selected with 0.4 mg of G418 (GIBCO Laboratories) per ml.

Inductions. The cells were induced with Sendai virus (SPAFAS, Inc.) or double-stranded RNA [poly(I)-poly(C); Pharmacia LKB] in the absence of CHX as previously described (10). MG63 cells were treated with virus for 2 h, and C127 cells were treated with virus or 100 μg of poly(I)-poly(C) per ml for 4 h. After the treatments with virus or

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double-stranded RNA in serum-free DME, the cells were rinsed in phosphate-buffered saline and incubated in DME plus 2% fetal calf serum. The superinductions were performed the same as the viral inductions, except that 25 μg of CHX per ml was included in the induction medium as well as in the DME plus 2% fetal calf serum. ActD treatment was done by adding 5 μg of ActD (U. S. Biochemical Corp.) per ml in dimethyl sulfoxide to each plate of cells.

RNA analysis. RNA was isolated by the guanidinium thiocyanate procedure (5). Total cellular RNA (10 μg) was assayed by RNase protection as described previously (40). For 5′ mapping, RNA was digested with 40 μg of RNase A (Sigma Chemical Co.) per ml and final samples were denatured and run on a 7% acrylamide-urea gel. For 3′ and poly(A) tail mapping, RNA was digested with 2 μg of RNase T_1 (Calbiochem-Behring) per ml as described by Wilson and Treisman (39). The samples were split before precipitation, and a portion of each sample was denatured and run on a 7% acrylamide-urea gel, while the other portion was run on a 5% native acrylamide gel.

Nuclear transcription reactions. Nuclei were isolated at various times following induction, and nascent transcripts were elongated in the presence of ^{32}P-labeled UTP as described previously (23, 41). Radioactive transcripts were hybridized to excess DNA immobilized on a nitrocellulose filter. The DNA probes included sense and antisense human IFN-β, mouse IFN-β, and hGH; γ-actin as an internal reference; and m13mp8 as a nonspecific control. Cytoplasmic RNA for RNase protection analysis was isolated from the same cells used for nuclear transcriptions.

Plasmid constructions. The control RBG plasmid contains the simian virus 40 enhancer adjacent to the RBG gene (−450 to +1646) and ligated into the *Eco*RI (5′ end) and *Sal*I (3′ end) sites of pSP64 (Promega Biotec). The RBG/IFN fusion plasmid has the IFN-β sequences from +638 to +1300 substituted for the RBG sequences from +1196 to +1646. The control IFN plasmid [pBVIF(Δ−77)+277C] was provided by H. Burstein and contains human IFN-β sequences from −77 to +1300, marked at +277 with a *Cla*I linker and inserted into the *Bam*HI site of the bovine papillomavirus-BV1 vector. The IFN/RBG fusion plasmid has the RBG sequences from +1196 to +1646 substituted for the IFN sequences from +638 to +1300.

The control fusion plasmid, IRE/hGH, was derived from IRE/TK/HGH (9) by inserting a second copy of the IFN regulatory element (IRE) (−77 to −37 of human IFN-β) adjacent to and in the same direction as the existing copy of the IRE and inserting an *Xho*I linker at +1532 of the hGH gene. The linker is in the 3′ untranslated region of the hGH gene and does not affect expression of the fusion gene. All of the inserts were ligated into the *Xho*I linker of IRE/hGH and were flanked by linkers to allow directional insertion. Inserts include 150 base pairs of *lac*Z DNA from m13mp18 (*Hin*dIII to *Fsp*I) and human IFN-β sequences from −10 to +638, +638 to +727, +638 to +760, +638 to +794, +638 to +837, +760 to +794, and +780 to +807. Inserts are oriented such that the RNA produced from the fusion gene has the same sequence as the appropriate portion of IFN-β mRNA. In addition, sequences from −10 to +638 and +780 to +794 were inserted in the reverse orientation. Plasmids were named by including, in parenthesis, the coordinates of the insert. For example, the plasmid containing the IFN-β sequence from −10 to +638 was named IRE/hGH(IFN−10/638).

The 5′ RBG probe, pSP65RBG, contains the RBG sequences from −96 (*Pst*I) to +267 (*Dde*I) ligated into the *Pst*I and *Sma*I sites of pSP65 (Promega). pSP65RBG was linearized with *Hin*dIII and transcribed with SP6 polymerase to give a 390-nucleotide (nt) transcript which protects 145 nt from the 5′ end of the RBG gene. The 5′ IRE/hGH probe, pSP6IRE/hGH, was derived from IRE/TK/HGH (9) by ligating a fragment containing the thymidine kinase promoter TATA box and hGH gene to +203 into pSP64. pSP6IRE/hGH was linearized with *Eco*RI in the polylinker and transcribed with SP6 polymerase to give a 347-nt transcript. This antisense transcript protects 126 nt from the 5′ end of the fusion gene mRNA to the end of the first exon. The 3′ hGH probe, pSP7hGH3′, was constructed from IRE/hGH (IFN638/837) by ligating the fragment from +1360 to +1660 of hGH into pSP73 (Promega). pSP7hGH3′ was cut with *Bst*NI at +1569 of the hGH gene and transcribed with T7 polymerase to give a 103-nt transcript. This antisense transcript protects 62 nt at the 3′ end of the hGH mRNA.

RESULTS

Role of transcriptional regulation in IFN-β gene expression. In order to examine the contribution of transcriptional regulation to the kinetics of IFN-β induction and decay, we compared the levels of nuclear transcription to the levels of mRNA accumulation at different times after viral induction. Human MG63 cells were treated with Sendai virus, and the nuclei were prepared at various times after induction for in vitro nuclear transcription studies. In parallel, total RNA was prepared for analysis of IFN-β mRNA by RNase protection. The level of IFN-β mRNA reached a maximum approximately 8 h after induction and decreased during the next 8 h (Fig. 1A). Virtually identical kinetics were observed with nuclear transcription experiments (Fig. 1B), indicating that the IFN-β gene is turned off at the level of transcription. We have obtained similar results for both viral and double-stranded-RNA induction of mouse C127 cells (data not shown).

To determine whether superinduction acts at the level of transcription, MG63 cells were induced with virus in the presence of CHX and the levels of mRNA accumulation and nuclear transcription were measured. In the presence of CHX, the level of IFN-β mRNA reached a plateau 8 h after induction and remained high for at least 20 h (Fig. 2A). The level of nuclear transcription also remained high for at least 20 h after induction (Fig. 2B), indicating that the high level of IFN-β mRNA observed in the absence of protein synthesis was due, at least in part, to the continued synthesis of IFN-β mRNA. We conclude that the normal postinduction turnoff of IFN-β mRNA transcription requires protein synthesis, which suggests that the synthesis of a virus-inducible repressor is responsible for turning off the IFN-β gene.

Role of mRNA stability in IFN-β gene regulation. Although the IFN-β gene is clearly turned off at the level of transcription, IFN-β mRNA turnover may also play a role. We investigated this possibility with reciprocal fusions between RBG and IFN-β genes. The intact RBG gene and a fusion gene containing the 3′ untranslated region of IFN-β were stably transfected into NIH 3T3 cells by cotransfection with a neomycin plasmid and selection for G418 resistance. Although high levels of RBG mRNA accumulated in pools of transformants containing the intact RBG gene, very little fusion gene mRNA accumulated in pools of transformants containing the RBG/IFN fusion gene (Fig. 3A), suggesting that the sequences in the 3′ untranslated region of IFN-β mRNA contain a destabilizing sequence and thereby prevent mRNA accumulation. We found similar results with the

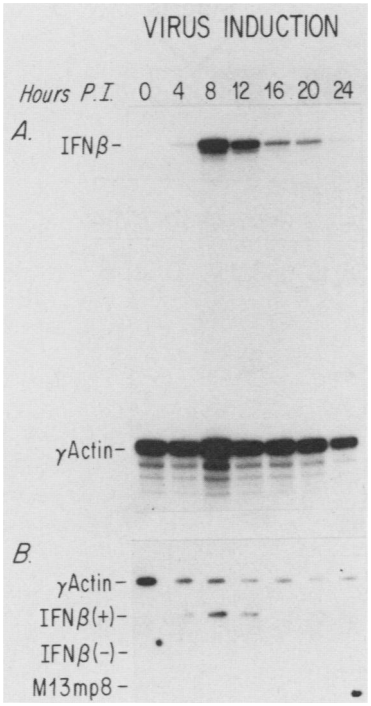

FIG. 1. Comparison of kinetics of mRNA accumulation and in vitro nuclear transcription of the endogenous human IFN-β gene after viral induction of MG63 cells. (A) RNase protection analysis of total cytoplasmic RNA. Hours P.I., Number of hours after addition of virus. RNA was probed with a 5′ human IFN-β probe, pSP65′IF (40), as well as a human γ-actin probe, pSP6γ-actin (8). (B) Nuclear transcription experiments with virus-induced human MG63 cells. DNA probes, indicated to the left, were as follows: γActin, double-stranded DNA containing the γ-actin gene; IFNβ(+) and IFNβ(−), phage m13 single-stranded DNA containing the human IFN-β gene in either orientation (provided by S. Goodbourn); M13mp8, nonspecific single-stranded vector DNA.

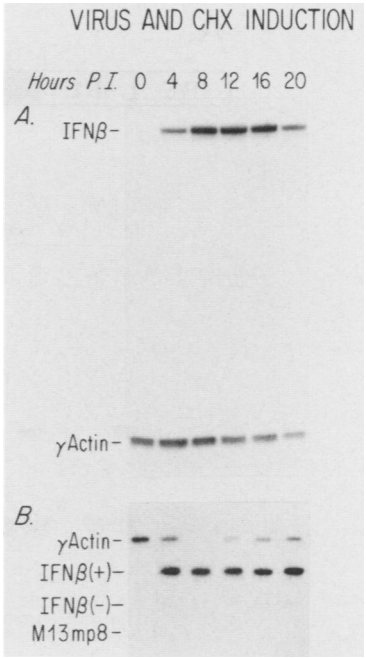

FIG. 2. Effect of CHX on IFN-β mRNA accumulation and nuclear transcription after viral induction of MG63 cells: RNase protection analysis (A) and nuclear transcriptions (B) following viral induction in the presence of CHX. Probes are the same as those described in the legend to Fig. 1. Consistent with previous results (41), we observed a low level of inducible antisense IFN-β transcription.

transient expression of the RBG and RBG/IFN genes in NIH 3T3 and HeLa cells (data not shown).

If the only destabilizing sequence in IFN-β mRNA is in the 3′ untranslated region, hybrid mRNAs in which this region has been replaced should be stabilized relative to intact IFN-β mRNA. To test this possibility, we compared the levels of mRNA that accumulated from an intact IFN-β gene and a fusion gene in which the 3′ untranslated region of IFN-β was replaced with the 3′ untranslated region of RBG. Each of these constructs was placed on a bovine papillomavirus vector, transfected into C127 cells, and induced with double-stranded RNA. The transcript levels and kinetics of induction and decay for the fusion gene were indistinguishable from the transcript levels and kinetics of induction and decay for the intact IFN-β gene (Fig. 3B). Similar results were obtained with virus and CHX inductions (data not shown). Thus, replacement of the 3′ untranslated region of IFN-β with the 3′ untranslated region of a known stable transcript did not lead to an increase in mRNA stability. This observation suggests that coding or 5′ untranslated regions of IFN-β mRNA also contain destabilizing sequences.

The role of mRNA stability in IFN-β gene expression was further investigated by studying the induction and decay kinetics of a heterologous transcript driven by the IFN-β gene promoter. Two copies of the IRE (10) were placed upstream of the thymidine kinase promoter TATA box and the hGH-coding and 3′ noncoding regions. This fusion gene, called IRE/hGH, was placed on a bovine papillomavirus vector and transfected into mouse C127 cells. Pools of foci were induced with Sendai virus, and total RNA was analyzed by RNase protection. The IRE/hGH gene was inducible by virus, but in contrast to endogenous mouse IFN-β

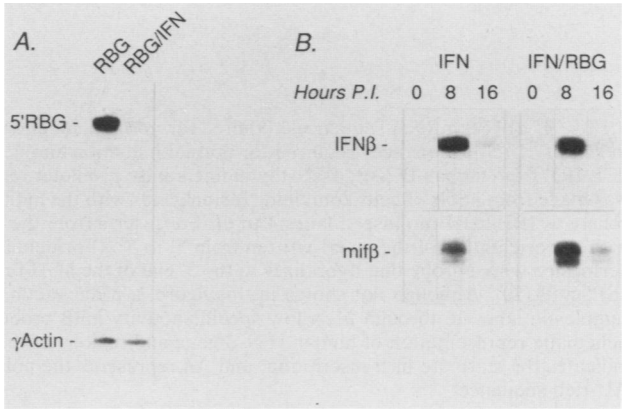

FIG. 3. Analysis of RBG and IFN-β fusion transcripts. (A) RNase protection analysis of RBG and RBG/IFN transcripts from pools of stably transformed NIH 3T3 cells. RNA was probed with a 5′ RBG probe, pSP65RBG, and a human γ-actin probe, pSP64γ-actin (8). (B) RNase protection analysis of IFN and IFN/RBG transcripts in pools of C127 transformants following double-stranded-RNA induction. RNA was probed with a 5′ human IFN-β probe, pSP65′IF (40), and an internal mouse IFN-β probe, pSP6mifβ (10). The mouse IFN-β signal (mifβ) is a 15-fold-lower exposure than the human IFN-β (IFNβ) signal. P.I., Postinduction.

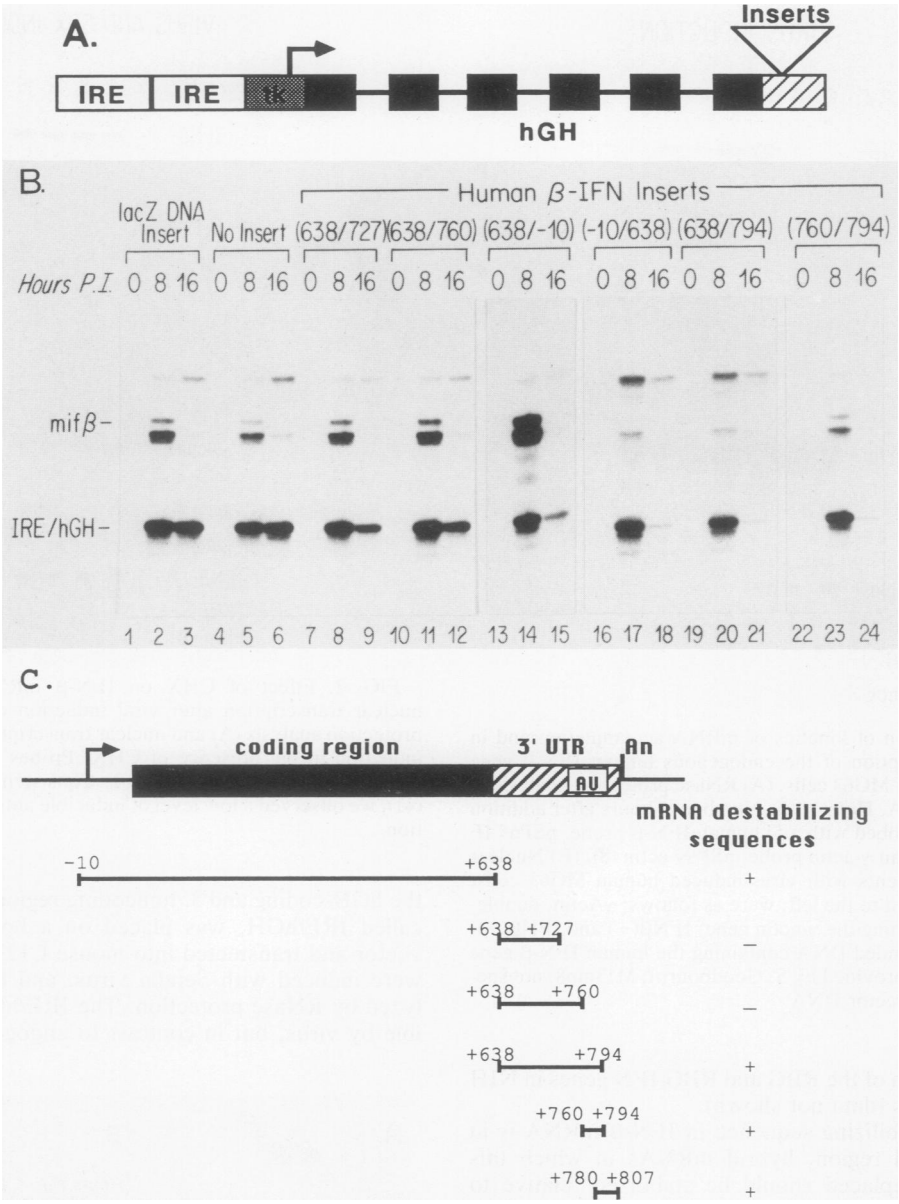

FIG. 4. IFN-β mRNA sequences required for normal induction and decay kinetics. (A) Schematic representation of the fusion gene used to identify IFN-β sequences required for normal induction and decay kinetics. The arrow indicates the start site of transcription. Symbols: ▭, IRE from human IFN-β; ▩, thymidine kinase promoter region; ■, hGH coding region; ▨, hGH 3′ untranslated region. (B) RNA was made from pools of cells containing fusion genes with the indicated inserts at 0, 8, and 16 h after viral induction (Hours P.I.). The control gene was IRE/hGH (no insert; lanes 4 to 6). For inserts from the IFN-β gene, numbers indicate nucleotides after the start of transcription, with the orientation of the insert written from 5′ to 3′. (For details of DNA constructs, see Materials and Methods.) RNase protection was performed with a probe that hybridizes to the 5′ end of the hGH fusion gene, pSP6IRE/hGH, and an internal probe to the mouse IFN-β gene, pSP6mifβ (10). Although not shown in this figure, a pSP6γ-actin probe indicated that the level of mRNA in each lane is comparable. For samples in lanes 16 through 21, a low-specific-activity mifβ probe was used; therefore, the mifβ signal is not visible on this exposure. (C) Schematic representation of human IFN-β gene and regions of the gene used as inserts in assay for the destabilizing sequences. The arrow indicates the start site of transcription, and An represents the poly(A) tail. Symbols: ■, coding region; ▨, 3′ untranslated region; ▭, AU-rich sequence.

mRNA, the level of hGH mRNA remained high 16 h after induction (Fig. 4B, lanes 4 to 6). When nuclear transcription experiments were carried out with cells containing the IRE/hGH gene, we found that like transcription of the intact IFN-β gene, transcription of IRE/hGH decreased significantly by 16 h after induction (Fig. 5, lanes 1 to 3). Thus, the IRE alone conferred normal transcriptional turnoff on a heterologous transcript. Moreover, the high levels of hGH gene mRNA at late times after induction must reflect the greater stability of hGH mRNA relative to IFN-β mRNA.

In order to identify the IFN-β sequences required for mRNA instability, we inserted various portions of the IFN-β gene into the 3′ untranslated region of the IRE/hGH gene and determined whether normal IFN-β induction and decay kinetics could be conferred upon the IRE/hGH fusion gene. Two segments of the IFN-β gene caused the level of fusion

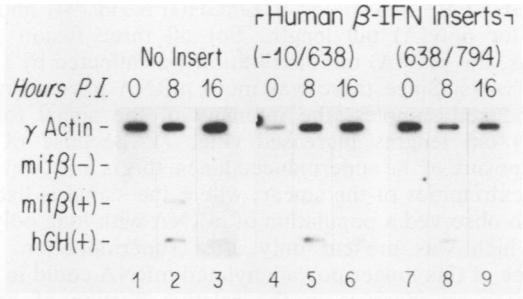

FIG. 5. In vitro nuclear transcriptions of C127 cells containing IRE/hGH, IRE/hGH(IFN−10/638), or IRE/hGH(IFN638/794). Nuclei were isolated 0, 8, and 16 h after viral induction as indicated (Hours P.I.). DNA probes, indicated to the left, were as follows: γActin, double-stranded DNA containing the γ-actin gene; mifβ(−) and mifβ(+), phage m13 single-stranded DNA containing the mouse IFN-β gene in either orientation (provided by S. Goodbourn); hGH(+), phage m13 single-stranded DNA containing the hGH gene in the orientation which hybridizes to hGH mRNA (provided by S. Goodbourn). We did not observe hybridization to nonspecific m13 vector DNA but did see a consistent strong signal with a probe to antisense hGH transcripts.

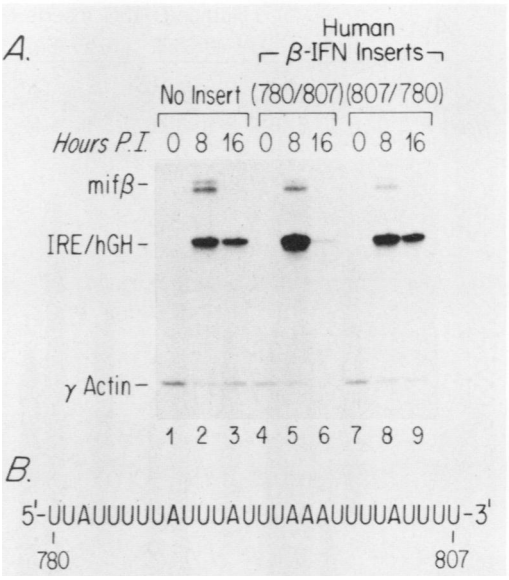

FIG. 6. An AU-rich sequence from the IFN-β transcript destabilized the hGH fusion gene mRNA in one orientation but not in the reverse orientation. (A) Induction and RNase protection as described in the legend to Fig. 4. The pSP6γ-actin probe is fivefold lower in specific activity than the pSP6mifβ and pSP6IRE/hGH probes. (B) Sequence of human IFN-β gene from +780 to +807.

gene transcript to decrease by 16 h after induction and thus conferred normal IFN-β decay kinetics on the fusion gene. One segment, referred to as the 5′ region, was located between −10 and +638 of the IFN-β gene and included the entire coding and 5′ untranslated regions (Fig. 4B, lanes 16 to 18). The other segment was located within the 3′ untranslated region (Fig. 4B, lanes 19 to 24). Nuclear transcription experiments revealed that the rate of transcription of IRE/hGH was not affected by IFN-β insertions, indicating that the insertions act as mRNA destabilizers (Fig. 5). Insertions of similar sizes from other portions of the IFN-β gene or from *lac*Z DNA did not lead to a significant decrease in mRNA levels by 16 h after induction (Fig. 4B, lanes 1 to 12).

We conclude that there are at least two distinct mRNA destabilizers in IFN-β mRNA. One is located within the 5′ region of the transcript, and the other is in the 3′ untranslated region. Either destabilizer is sufficient to confer normal IFN-β decay kinetics on the fusion gene. The destabilizer in the 3′ untranslated region (+760 to +794) is similar to the AU-rich destabilizer described by Shaw and Kamen (33). However, the best match in IFN-β mRNA to the AU-rich mRNA destabilizer is an overlapping sequence that includes nucleotides +780 to +807. We therefore inserted an oligonucleotide containing the IFN-β sequence from +780 to +807 into the IRE/hGH gene and analyzed, by RNase protection, pools of induced transfectants. This oligonucleotide conferred normal IFN-β decay kinetics on the fusion gene if placed in the sense orientation but did not destabilize the hGH transcript when placed in the reverse orientation (Fig. 6). Therefore, the specific sequence and not simply AU composition is necessary for destabilization.

A homology search of the 5′ region indicated that there are no matches to the AU-rich destabilizer which are greater than 6 nt long. Therefore, we assume that the destabilizer in the 5′ region of the transcript is different from the destabilizer in the 3′ untranslated region. The 5′ region destabilized transcripts regardless of whether it was within the coding region of the test gene mRNA (this paper and our unpublished results), indicating that the coding sequence does not have to be translated to confer mRNA instability. When the 5′ region was inserted into IRE/hGH in the reverse orientation, it also destabilized the transcript, but to a lesser extent than in the correct orientation (Fig. 4, lanes 13 to 15). This instability could be the result of a destabilizing sequence which is recognized in either orientation, or in the case of the reverse orientation, it could be a nonspecific effect due to the size of the insert. However, since an IFN-β transcript with its 3′ untranslated region replaced by the RBG 3′ untranslated region was as unstable as the intact IFN-β transcript (Fig. 3B), there must be a specific destabilizer in the 5′ region which acts independently of the destabilizer in the 3′ untranslated region.

Effect of IFN-β mRNA destabilizers on deadenylation. A number of studies have shown that mRNA degradation is preceded by deadenylation (for a review, see reference 1). In the case of c-*fos* mRNA, the AU-rich mRNA destabilizer in the 3′ untranslated region is required for an increase in the rate of deadenylation in vivo (39). In order to determine whether one or both of the mRNA destabilizers in the IFN-β gene affect the rate of deadenylation, we examined by RNase T_1 protection the size distribution of poly(A) tails on fusion gene transcripts following virus induction of IRE/hGH, IRE/hGH(IFN−10/638), and IRE/hGH(IFN638/794). By 8 h after virus induction, there was a significant accumulation of fusion gene mRNA for all three constructs, and there was a slight decrease in the average length of the poly(A) tail when either destabilizer was present (Fig. 7). After assaying poly(A) tail lengths at 2-h intervals following viral induction, we found that the effect of either destabilizing sequence on poly(A) tail length was never greater than the slight decrease seen 8 h after induction (data not shown) and that the poly(A) tail lengths for all three fusion gene mRNAs decreased at later times. We found similar results for other constructs which contained the IFN-β destabilizing region and found that the patterns of polyadenylation for constructs which contain nondestabilizing inserts were indistinguishable from that of the control (data not shown).

We conclude that both IFN-β mRNA destabilizers are associated with only a slight increase in the rate of mRNA

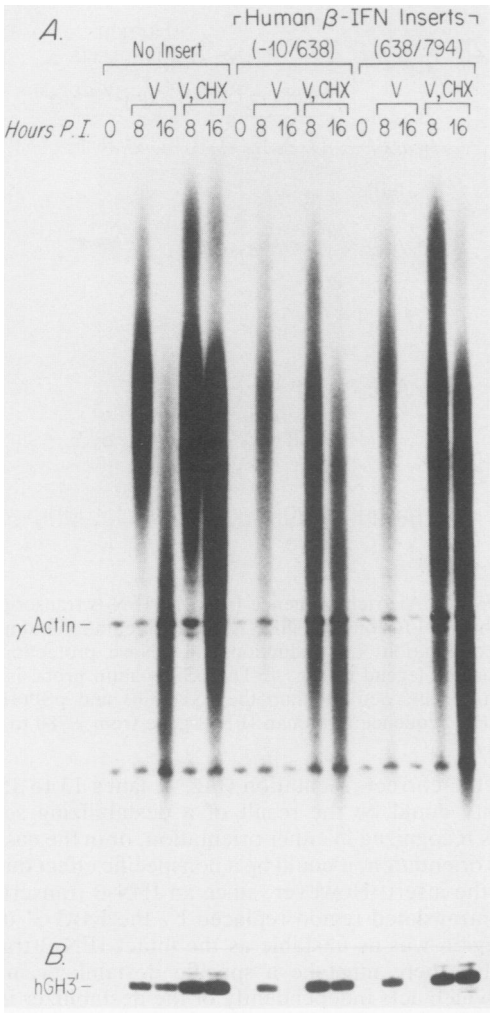

FIG. 7. Effect of IFN-β mRNA destabilizers on deadenylation of mRNA: RNase T_1 protection of fusion transcripts following virus (V) or virus plus CHX (V, CHX) induction with a hGH 3′ probe, pSP7hGH3′, and an internal γ-actin probe, pSP6γ-actin (8). The pSP6γ-actin probe is 100-fold lower in specific activity than the pSP7hGH3′ probe. (A) A portion of each sample was run on a 5% native acrylamide gel. The smear results from differing lengths of poly(A) tail attached to the protected 62-nt fragment. γ-Actin runs as a discrete band, since the probe protects an internal fragment. There is also a lower, nonspecific band in all of the lanes. (B) A portion of each sample was denatured and run on a 7% acrylamide-urea gel, which results in a discrete band from the pSP7hGH3′ probe.

deadenylation. It is possible that, although different in sequence, both destabilizers contain specific signals for deadenylation. However, it is also possible that deadenylation is associated with mRNA degradation regardless of the mechanism of degradation. This latter possibility is strengthened by the fact that all three transcripts, including the hGH transcript which did not contain a IFN-β destabilizing sequence, underwent poly(A) tail shortening by 16 h after induction.

Accelerated deadenylation of c-*fos* mRNA is blocked in the presence of CHX (39). Although one mechanism by which CHX superinduces the IFN-β gene is transcriptional (Fig. 2), CHX could also increase the level of steady-state mRNA by a posttranscriptional mechanism. To test this possibility, we superinduced cells containing IRE/hGH, IRE/hGH(IFN−10/638), or IRE/hGH(IFN638/794) and assayed for poly(A) tail length. For all three fusion gene mRNAs, the poly(A) tail distribution was affected by CHX in two ways. Since there was more mRNA present in the superinduced samples, the intensity of the signal for all poly(A) tail lengths increased (Fig. 7). Because of the overexposure of the superinduced lanes, this is most obvious in the extremities of the smear, where the signal is lighter. We also observed a population of mRNA with long poly(A) tails which was present only after superinduction. The presence of this longer polyadenylated mRNA could in part be due to an increase in the relative portion of newly transcribed mRNA in the presence of CHX. However, we feel that because none of this longer polyadenylated mRNA was detected in the virus-induced samples, its presence was probably the result of a CHX-specific posttranscriptional effect, such as the saturation of degradation or deadenylation factors or the direct blocking of deadenylation. This posttranscriptional effect of CHX is not specific for IFN-β mRNA, since the poly(A) tail length of hGH mRNA containing no IFN-β sequences was also affected.

Effect of ActD on IFN-β destabilizers. Like the IFN-β gene, c-*fos* contains two destabilizers, one AU-rich sequence in the 3′ untranslated region and another within the coding region of the gene (13, 34, 37). These two c-*fos* destabilizers are thought to work through distinct pathways, since they respond differentially to the transcriptional inhibitors ActD and DRB (34). Transcripts containing the c-*fos* AU-rich destabilizer are significantly stabilized in the presence of ActD or DRB, while transcripts containing the coding region destabilizer are only slightly affected by either drug (34). In order to test the effect of ActD on the two IFN-β destabilizers, cells containing IRE/hGH, IRE/hGH(IFN−10/638), or IRE/hGH(IFN638/794) were induced with virus for 10 h. Half of each cell type was then treated with 5 μg of ActD per ml, and RNA was isolated every 2 h for RNase protection analysis. ActD stabilized hGH transcripts which contained either mRNA destabilizer (Fig. 8). Therefore, we cannot distinguish between the two destabilizers on the basis of their responses to ActD. ActD also slightly increased the stability of the hGH transcript, but only after 6 h of ActD treatment. We note that the endogenous mouse IFN-β gene transcript was not stabilized by ActD. This difference could be species specific, or the intact IFN-β mRNA may contain an ActD-independent destabilizer that is missing in the partial IFN-β mRNA sequences present in the fusion gene mRNA.

DISCUSSION

We have shown that the transient accumulation of IFN-β mRNA after viral induction is a consequence of transcriptional activation followed by the repression and rapid decay of IFN-β mRNA. Previous in vitro nuclear transcription studies demonstrated that viral induction results in an increase in the rate of IFN-β gene transcription (26; Zinn, Ph.D. thesis). We confirmed this result and showed that the postinduction decrease in the level of IFN-β mRNA coincides with a decrease in the rate of transcription. This observation is inconsistent with a previous report that the IFN-β gene continues to be transcribed at a time during induction when IFN-β mRNA levels have decreased (26).

Treatment of cells with metabolic inhibitors during induction is known to prevent the postinduction decrease of IFN-β mRNA (4, 25, 30–32), but previous studies did not directly distinguish between the effects on mRNA stability

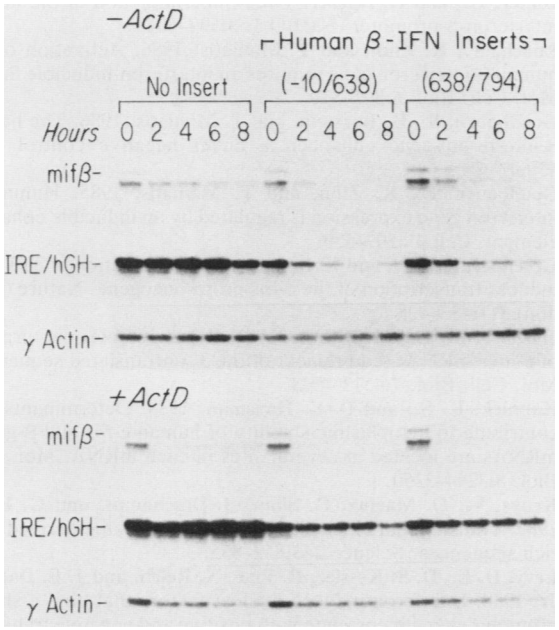

FIG. 8. ActD stabilizes transcripts containing an IFN-β mRNA destabilizer. Cells containing IRE/hGH, IRE/hGH(IFN−10/638), or IRE/hGH(IFN638/794) were induced for 10 h with virus, and half of each cell type was then treated with 5 μg of ActD per ml. RNA was isolated at various times following ActD addition as indicated above the figure. Probes used for RNase protection are described in the legends to Fig. 4 and 6. −ActD, RNase protection for virus-induced cells not treated with ActD; +ActD, RNase protection for virus-induced cells treated with ActD. The autoradiograms for both panels were exposed for the same amount of time.

and IFN-β gene transcription. We find that the postinduction decrease in IFN-β mRNA can be blocked by CHX. In addition, our results definitively demonstrate that the increased level of IFN-β mRNA observed in the presence of CHX results from continued transcription of the IFN-β gene. This conclusion is consistent with studies of human IFN-β promoter fusion genes which show that the IFN-β promoter is superinducible by CHX (7, 20).

The requirement of protein synthesis for transcriptional repression suggests that a virus-inducible repressor is involved in turning off the IFN-β gene. Transcription of both endogenous (28, 35) and transfected (24) IFN-β genes can be induced by treating some cell types with CHX in the absence of virus or double-stranded RNA. These studies, analysis of IFN-β promoter mutants (9), and genomic footprinting experiments (42) are consistent with a model of negative control in which the inactivation or displacement of a labile transcriptional repressor is required for IFN-β gene expression. Whether the same repressor molecules are involved both in maintenance of the gene in the off state prior to viral induction and in postinduction turnoff of the gene remains to be established.

The postinduction decrease of IFN-β mRNA is a consequence of transcriptional repression (Fig. 1) and the relative instability of IFN-β mRNA (25; Fig. 4). At present, there is no evidence that mRNA stability is regulated during viral induction, since the kinetics of induction for transcription and mRNA accumulation are similar. Rather, our data are consistent with a model in which IFN-β mRNA decays at a constant rate throughout induction and the postinduction decrease is due primarily to a decrease in the rate of transcription.

Previous studies demonstrated that the coding or 3′ untranslated regions of IFN-β mRNA contribute to its instability (22). In this paper, we show that both regions of the gene are sufficient for normal turnover during induction. The destabilizing sequence within the 3′ untranslated region of IFN-β mRNA was localized to a region between +760 and +807 nt. This AU-rich sequence is similar to a family of 3′ untranslated sequences found in many inducible mRNAs (3). The mRNA destabilizing activity of this sequence family was first demonstrated by Shaw and Kamen (33) and subsequently shown to play a role in determining the half-life of c-*fos* mRNA (34, 39). In addition, the 3′ untranslated regions of c-*myc* (12), IFN-α (38), and interleukin-2 (27) mRNAs, which contain AU-rich sequences, are thought to contribute to mRNA destabilization. Although the 3′ untranslated AU-rich sequences of these genes are similar, their functions may not be identical. For example, conditions that lead to the stabilization of certain mRNAs containing this sequence do not stabilize all mRNAs of this class (16, 29). Differences in the stability of mRNAs containing the AU-rich sequence may reflect differences in the AU-rich sequences which allow them to interact with different regulatory factors. Alternatively, other destabilizing sequences within these mRNAs may account for the differences in stability.

We found that, in addition to the AU-rich sequence, sequences in the 5′ region (−10 to +638) of IFN-β mRNA can destabilize the fusion gene transcript. This result is consistent with the observation that deletion of the AU-rich region of IFN-β mRNA did not lead to an increase in mRNA stability (Fig. 3B). Examination of sequences in the 5′ region did not reveal the presence of an AU-rich sequence, suggesting that the mechanisms by which these destabilizers work may be different. However, we found that the destabilizing activities of both regions can be blocked by ActD, an inhibitor of transcription. Two distinct destabilizers have also been identified in c-*fos* mRNA, an AU-rich sequence and another destabilizer within the coding region of the gene (13, 34). In this case, the destabilizing activities of the two sequences were differentially sensitive to ActD, suggesting that the mechanisms for decay are different.

A number of mechanisms have been proposed for the role of the AU-rich sequence in mRNA turnover. On the basis of the observation that deadenylation precedes the degradation of mRNA destabilized by the AU-rich sequence, it has been proposed that the AU-rich sequence pairs with the poly(A) tail and the mismatched bases are recognized by a specific nuclease (39). However, direct evidence for this and other models implicating the AU-rich sequence in deadenylation has not been obtained. In the in vitro system described by Brewer and Ross (2), deadenylation of c-*myc* mRNA is followed by specific cleavage in the 3′ untranslated region and subsequent degradation of the message. This result suggests that an endonuclease clips at the AU-rich sequence to cause destabilization of the message. Further evidence for a factor recognizing the AU-rich sequence is provided by RNA band shift assays in which cytoplasmic factors have been shown to bind to the c-*myc* 3′ untranslated region (J. A. Alberta, personal communication) and to an in vitro-transcribed RNA containing four copies of the AUUUA motif (17). Cross-linking studies indicate that a 30-kilodalton nuclear protein binds to the 3′ untranslated region of GMCSF (E. Vakalopoulou, personal communication). However, it is not known whether these factors have a role in mRNA degradation. In *Xenopus* oocytes in which messages containing the AU-rich destabilizer are not degraded, it has been shown that the AU-rich sequences of c-*fos*, GMCSF, and

IFN-β block translation (14). It is not clear whether such a block occurs in somatic cells.

We observed a small increase in the rate of deadenylation associated with the AU-rich destabilizer of IFN-β mRNA. Although this result is consistent with results of earlier studies of c-*fos* and c-*myc* mRNA, there was also a similar increase in the rate of deadenylation of transcripts containing the destabilizer in the 5′ region of the IFN-β transcript. Thus, accelerated deadenylation is not specific for the AU-rich sequence. In the case of the AU-rich sequence, deadenylation precedes mRNA turnover (2, 39), but it is possible that the destabilizer in the 5′ region of the IFN-β transcript leads to mRNA degradation prior to or concurrent with deadenylation. We also found that CHX affects the poly(A) tail length distribution of transcripts whether or not they contain either IFN-β destabilizer, demonstrating that the posttranscriptional effect of CHX is not specific for the AU-rich destabilizer or IFN-β mRNA.

The mechanisms involved in the transient induction of IFN-β gene expression may be similar to those involved in regulating other highly inducible genes. For example, IFN-inducible genes are turned off at the level of transcription, and the treatment of induced cells with CHX prevents this repression (15). Thus, IFN-inducible genes may also be switched off by a labile transcriptional repressor. The c-*fos* and c-*myc* oncogenes are induced rapidly and transiently by various mitogenic stimuli. c-*myc* is regulated at both the transcriptional and posttranscriptional levels, and protein synthesis inhibitors are thought to prevent transcriptional repression and stabilize the c-*myc* transcript (for a review, see reference 19). As with viral induction of the IFN-β gene, the induction and subsequent turnoff of the c-*fos* gene are regulated primarily at the transcriptional level (11, 21). Furthermore, a constitutive level of transcript instability is necessary for the rapid decay kinetics of c-*fos* (34, 39) and IFN-β, and both transcripts contain 3′ AU-rich destabilizers as well as coding-region destabilizers. These similarities in strategy for the rapid turnoff of gene expression may reflect a common need for stringent control of inducible genes whose prolonged expression is detrimental.

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