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A human IFN-β_1 gene deleted of promoter sequences upstream from the TATA box is controlled post-transcriptionally by dsRNA

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ABSTRACT

Induction of IFN-β_1 RNA was studied in the mouse cell line SR117-21E transformed by a BPV episome containing the human IFN-β_1 gene deleted of promoter sequences upstream from position -40. Nuclei isolated from these cells synthesize constitutively IFN-β_1 RNA from the partially deleted promoter. The IFN-β_1 RNA synthesized by nuclei of uninduced SR117-21E cells is similar to that made by nuclei of poly(rI):(rC)-induced cells, but does not accumulate and hence no IFN is produced unless the cells have been treated either by ds RNA or by cycloheximide. We conclude that the IFN-β_1 gene has, in addition to the transcription control due to upstream promoter sequences, an additional post-transcriptional control acting on mRNA accumulation and linked to sequences close to the TATA box and RNA start site. Both controls are relieved by ds RNA.

INTRODUCTION

Expression of the human fibroblast interferon gene IFN-β_1 (1-5) is induced when cells are virus infected or exposed to double-stranded (ds) RNA (6,7). Accumulation of IFN-β_1 mRNA is increased, and more IFN is produced, if the cells are superinduced by treatment with cycloheximide or other metabolic inhibitors in addition to ds RNA (7-12). The IFN-β_1 gene retains its inducibility by poly(rI):(rC) when introduced into heterologous cells by co-transformation (13-16), by Bovine Papilloma virus vectors (17,18) or by SV40 vectors (19,20) and IFN-β_1 mRNA with authentic 5' ends accumulates in these cells only after induction (14-19). The requirement for induction is lost if the IFN-β_1 gene is fused to another promoter as mouse β-globin (19,21) or the SV40 early promoter (22). Nucleotide sequences around position -75 and further upstream in the 5'-flanking region of the IFN-β_1 promoter appear to be involved in the induction of transcription by poly(rI):(rC)(20,23,24). However, we have observed that the IFN-β_1 gene deleted from all sequences upstream from position -40 is still regulated since, under conditions of transient expression in an enhancer-containing SV40 vector, no IFN-β_1 transcripts with authentic 5' ends accumulate unless the cells have been induced by

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poly(rI):(rC)-cycloheximide treatment (19).

In this work we further characterize the induction process of IFN-β_1 gene with promoter deletion at -40, by using the mouse cell line SR117-21E which contains such a gene in a stably propagated BPV episome. In these cells, human IFN-β_1 synthesis is induced by poly(rI):(rC) but, surprisingly, cycloheximide alone also leads to accumulation of IFN-β_1 RNA and secretion of IFN-β_1 activity (25). Induction by cycloheximide alone is not seen in human fibroblasts or in mouse cells transformed by BPV-IFN-β_1 recombinants with an intact IFN-β_1 promoter. By measuring in vitro RNA synthesis in isolated nuclei, we show that the regulation of the -40 deleted IFN-β_1 gene in SR117-21E cells is altered and is no more at the transcriptional level as it is in human fibroblasts. Poly(rI):(rC) as well as cycloheximide act in this case post-transcriptionally and induce the accumulation of newly formed IFN-β_1 transcripts. This post-transcriptional control appears linked to the nucleotide sequence close to the TATA box and RNA start site of the IFN-β_1 gene.

MATERIALS AND METHODS

Cell cultures: BPV-IFN-β_1 transformed SR117-21E cells were obtained (18) by transformation of mouse C127 fibroblasts with plasmid 117-21 (Fig. 1A). This DNA consists of the BPV-1 69% transforming fragment, linked in opposite orientation to a 1.6 kb Hind III human genomic fragment containing the IFN-β_1 gene, and cloned in E. coli via a 3.7 kb modified Sal I fragment of pBR322 (28). Before transfection, the 117-21 DNA was linearized by Sal I which removes the pBR sequences. Among the transformants inducible for human IFN production, the SR117-21E clone was characterized by a more efficient induction of IFN-β by poly(rI):(rC)-cycloheximide than by NDV (18) and by the ability of cycloheximide alone to induce the human IFN (25). In the present work, we used subclone A1 of SR117-21E cells (25). The other BPV DNA transformants (18) and human FS11 diploid foreskin fibroblasts (29) have been described. Cell culture conditions and Interferon titration with Vesicular Stomatitis virus (VSV) on FS11 cells were as detailed before (25). Units were calculated with NIH IFN-β standard G023-902-527.

Preparation of nuclei from poly(rI):(rC)-induced cells: Cultures of SR117-21E-A1 cells (5×10^6 cells) were seeded in 480 cm^2 tissue culture trays (Nunc) and grown 4 days in Dulbecco modified Eagle's medium with 10% fetal calf serum (FCS), 100 units/ml penicillin G and 0.1mg/ml streptomycin, at 37°C in 8% CO_2. For induction, 8 trays (2×10^8 cells) were washed and exposed to medium containing 25 μg/ml polyriboinosinic acid-polyribocytidylic acid

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(Poly(rI):(rC), PL-Biochemicals) and 600 μg/ml DEAE-dextran ($0.5x10^6$ Mr, Pharmacia Fine Chemicals). After 1 hour, medium was replaced by new medium with poly(rI):(rC) only, to minimize the toxic effects of DEAE-dextran; in this way, the total RNA synthesis in nuclei from induced and non-induced cells was the same. At 3 hours after beginning of induction, the cells were washed twice with 10ml/tray of 10mM Tris-Cl pH 7.6, 150mM NaCl and swelled to 10 minutes with 5ml/tray of 10mM Tris-Cl pH 7.6, 5mM $MgCl_2$, 0.1mM EDTA, 1mM dithiothreitol at 4°C. Cells were scraped and disrupted by 100 strokes of a Dounce homogenizer type B with a tight-fitting pestle. The 40ml lysate was centrifuged for 5 min at 4,000xg, the pellet was resuspended in 5ml of 10mM Hepes buffer pH 7.6, 25mM KCl, 5mM $MgCl_2$, 0.1mM EDTA, 1mM dithiothreitol (Buffer S) and centrifuged for 15 min at 3,000xg through 10ml of 25% glycerol in Buffer S. The nuclei were resuspended in 25% glycerol-Buffer S at 10^8 nuclei/ml and in most cases frozen in liquid air before use. Interferon assays were done on parallel cultures, refed with culture medium for 20 hours after the end of induction. For human FS11 cells, superinduction was done for 3 hrs with 50 μg/ml poly(rI):(rC) and 50 μg/ml cycloheximide, and when indicated with priming by an additional 16 hours pretreatment of the cells by 100 U/ml IFN-β. FS11 nuclei were prepared as above but with only 10 strokes of the homogenizer.

In vitro RNA synthesis and hybridization to IFN-β_1 DNA: About $2x10^7$ nuclei were incubated 40 min at 30°C in 0.3ml of 30mM Hepes buffer pH 7.9, 165mM KCl, 1mM $MnCl_2$, 9mM $MgCl_2$, 0.5mM K_2HPO_4, 0.1mM EDTA, 5mM dithiothreitol, 0.4mM GTP and CTP, 1mM ATP, 25 μM UTP, 250 μCi [^{32}P]-α-UTP (400 Ci/mmol), 5mM NaF and 12% glycerol. The nuclei were then treated 30 min at 20°C with 30 μg/ml DNase I (Worthington, RNase free). Two volumes of 3M urea, 1% dodecyl sulfate, 20mM Tris-Cl pH 8, 10mM NaCl, 0.5mM EDTA were added, and RNA was extracted by phenol-chloroform, alcohol precipitated, DNase treated, reextracted by phenol chloroform and passed through Sephadex G50, as described (30).

The two strands of the 1.86 kb EcoR1 genomic DNA fragment containing the IFN-β_1 gene were separated by cloning in phage M13 mp7 (31) and DNAs from phage clones containing either the C or NC strand of IFN-β_1 (see Fig. 1) were electrophoresed on 0.7% agarose gels and blotted onto nitrocellulose (32). Blots (1 μg M13 mp7-IFN-β_1 DNA, 1 cm wide) were incubated 12 hours at 42°C in 50% formamide, 0.5% dodecyl sulfate, 0.1 M Hepes-KOH pH 7.5, 0.75M NaCl, 1mM EDTA and [^{32}P]-labeled nuclear RNA heated 5 min to 68°C was added at $5x10^6$ cpm/ml and hybridization carried out 48 hours at 42°C. Blots were washed twice in 2xSSC, treated 30 min at 22°C with 20 μg/ml RNase A, washed 1 hour at

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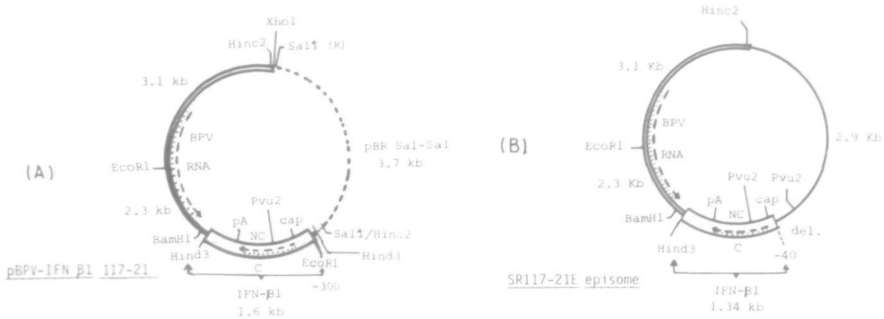

FIGURE 1: A) Structure of the BPV-IFN-β_1 117-21 recombinant DNA cloned in E. coli (18). The box shows the EcoR1-HindIII fragment of human DNA containing the IFN-β_1 gene. The IFN-β_1 mRNA (arrow) start site (cap) and polyadenylation site (pA) are shown. The DNA strand which hybridizes to IFN-β_1 RNA is designated C-strand (dots); the other is NC. The double line shows the 69% transforming region of BPV-1 DNA, the arrow indicating the direction of BPV RNA transcription. The dotted line is the pBR322 fragment. B) Structure of the BPV-IFN-β_1 episome from the SR117-21E A1 mouse cells (18). The deletion of the IFN-β_1 promoter at 40 nucleotides upstream from the RNA start site (cap) is shown (del). The continuous line represents foreign DNA insertion.

42°C with 2xSSC, 0.5% dodecyl sulfate and three times for 30 min at 50°C with 0.5xSSC, 0.5% dodecyl sulfate. The blots were dried, exposed to XR-5 Kodak films for 1-7 days and the bands intensities were measured by scanning in a Beckman DU-8 spectrophotometer. In some experiments, 1 µg DNA was spotted on nitrocellulose and the [^{32}P]-nuclear RNA hybridized was counted. Analysis of RNA-DNA hybrids by S_1 nuclease was carried out as described (19). DNA sequencing was done according to Maxam and Gilbert (33).

RESULTS

1) Structure of the BPV-IFN-β_1 episome in SR117-21E cells.

The SR117-21E subclone A1 cells, were isolated from the transformants of mouse C127 fibroblasts with pBPV-IFN-β_1 117-21 DNA (Fig. 1A) which had been cut with Sal I, thereby leaving the IFN-β_1 promoter at one end of the linearized DNA (18). Restriction mapping of the Hirt-extracted BPV episome from SR117-21E cells (Fig. 1B) revealed a deletion of the 5' end of the IFN-β_1 gene and the insertion of 2.9 kb of foreign DNA with a Pvu II site, which probably occurred during recircularization of the DNA. The 400 bp PVU II fragment containing IFN-β_1 DNA sequences was subcloned in the Pvu II site of pBR322 and sequenced. Comparison with the human IFN-β_1 gene indicates that all sequences upstream from nucleotide -40 with respect to the RNA start site, were deleted and

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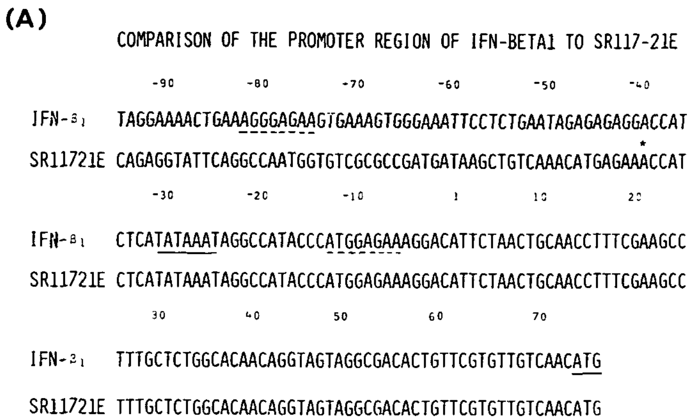
(A)



(B)

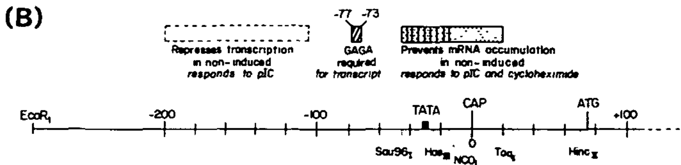


FIGURE 2: A) Sequence of the human IFN-β_1 gene promoter region compared to that of the deleted gene in the episome of SR117-21E A1 cells. The star at -40 indicates the limit of the deletion-insertion. The TATA box and ATG codons are underlined. Numbering is from the RNA start site. B) The 5'flanking regions of the IFN-β_1 gene is shown with the position of the transcription signals defined by Zinn et al. (24). The box on the right indicates the sequences which maybe involved in the post-transcriptional control. For details, see text.

replaced by unrelated DNA (Fig. 2A). The IFN-β_1 sequence downstream from -40 was unchanged (not shown). The deletion in the IFN-β_1 promoter in SR117-21E cells, removed therefore important transcriptional signals which were localized by Zinn et al. (24) around position -75 (Fig. 2B). The deleted IFN-β_1 promoter in this BPV episome is similar to the Sau96 I-deleted IFN-β_1 promoter that we studied before in transiently replicating SV40 vectors (19).

2) Constitutive synthesis of IFN-β_1 RNA in nuclei of SR117-21E cells in the absence of RNA accumulation.

No accumulation of IFN-β_1 mRNA was found in uninduced SR117-21E cells by Northern blot or by S_1 analysis (18,25) indicating that the gene is still regulated. The deletion of transcription signals in the IFN-β_1 gene and the induction of IFN-β_1 mRNA accumulation by cycloheximide alone found in these cells

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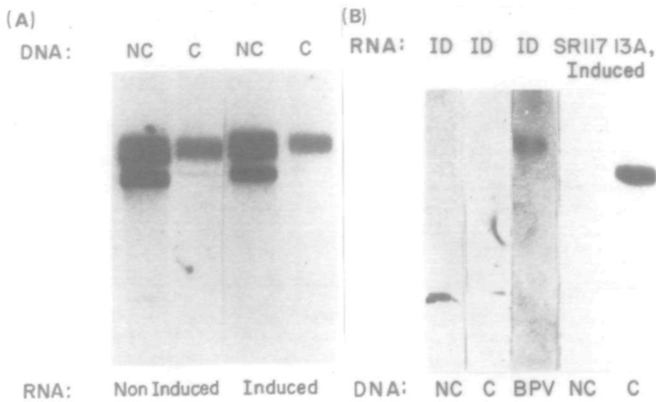

FIGURE 3: In vitro transcription of the IFN-β_1 gene in SR117-21E A1 nuclei. A) Phage M13mp7 DNAs containing either the C strand or the NC strand of IFN-β_1 were electrophoresed on 0.7% agarose gels, blotted onto nitrocellulose and hybridized to ^{32}P-RNA synthesized in nuclei freshly prepared from non-induced or poly(rI):(rC)-induced SR117-21E A1 cells. The double band represents two forms of the M13 DNA. B) Controls carried out as in A but ^{32}P-RNA was synthesized in nuclei from ID mouse cells, which contain a BPV episome without the IFN-β_1 gene; no hybridization is seen to M13 IFN-β_1 DNA (left two lanes) but hybridization is seen to BPV DNA (third lane). SR117-13A cells (18) contain the IFN-β_1 gene in the same orientation as the BPV RNA: no hybridization is seen to the IFN-β_1 NC strand showing that in SR117-21E cells, the RNA hybridizing to NC is due to the BPV promoter.

(25) prompted us to examine if the -40 deleted IFN-β_1 promoter was still controlled at the transcriptional level. This was investigated by measuring in vitro IFN-β_1 RNA synthesis in nuclei isolated from the permanently transformed SR117-21E cells.

Nuclei were incubated with [^{32}P]-α-UTP for 40 minutes and the radioactive RNAs were extracted. To detect newly synthesized IFN-β_1 RNA, hybridization was carried out with single-stranded DNA from phage M13-mp7 in which the complementary (C) strand of the IFN-β_1 1.8 kb EcoR1 genomic fragment, had been cloned. In this way, transcript of the opposite NC strand (Fig. 1B), which may be produced by read-through from the BPV early gene promoter, would not interfere with the assay of IFN-β_1 RNA synthesis. The amount of IFN-β_1 RNA synthesized by nuclei from SR117-21E cells, was compared by hybridization under DNA excess (Fig. 3A). Surprisingly, IFN-β_1 RNA was as efficiently synthesized by the nuclei of uninduced SR117-21E cells as by those from poly(rI):(rC)-induced cells (Table 1). In contrast, poly(rI):(rC), gave a 20-fold induction of the IFN-β_1 protein in these SR117-21E cells (Table 1). The discrepancy

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TABLE 1: CONSTITUTIVE SYNTHESIS OF IFN-β_1 RNA IN SR117-21E NUCLEI

	IN VITRO SYNTHESIZED NUCLEAR IFN-β_1 RNA				IFN ACTIVITY
	SR117-21E Nuclei	RNA Input for hybridization	IFN-β_1 RNA Hybridized to C-strand	NC-strand	PRODUCED BY CELLS
Expt.1		cpm	scan units*		U/ml
	Non-Ind.	$2x10^6$	4.3	8.6	96
		$4x10^6$	5.9	17.9	
	Induced	$2x10^6$	1.4	1.5	1900
		$4x10^6$	4.6	9.0	
Expt.2		cpm	cpm**		U/ml
	Non-Ind.	$2x10^6$	121	198	256
	Induced	$2x10^6$	130	179	6000

*from gel in Fig. 3. IFN-β_1 mRNA hybridizes to C-strand.
**counted 10 minutes.

between induction of human IFN activity and the absence of change in IFN-β_1 RNA synthesis in SR117-21E cells, was observed in various experiments using Southern electrophoretic blots or dot-blots (Table 1) and various preparations of nuclei, freshly prepared or stored frozen.

The synthesis of IFN-β_1 RNA in nuclei from uninduced SR117-21E cells did not lead to the accumulation of IFN-β_1 RNA in these cells prior to poly(rI):(rC)-induction. When total unlabeled RNA was extracted from nuclei or from whole cells, and hybridized to a labeled IFN-β_1 DNA probe, RNA whose 5' end maps at the IFN-β_1 promoter was found to accumulate only if the SR117-21E cells had been induced 3 hours with poly(rI):(rC)(Fig. 4). We have previously shown that cycloheximide at 50 µg/ml for 3 hours, has the same effect on inducing IFN-β_1 RNA accumulation in these cells (25).

To verify that the _in vitro_ synthesis of IFN-β_1 RNA by nuclei of uninduced SR117-21E cells is due to the presence of the -40 deleted IFN-β_1 gene, we measured IFN-β_1 RNA synthesis in nuclei isolated from cells containing the intact gene. Figure 5A shows that nuclei from human diploid fibroblasts do not synthesise IFN-β_1 RNA unless the cells have been induced, in line with previous reports (12). In these cells, it can also be shown that priming in

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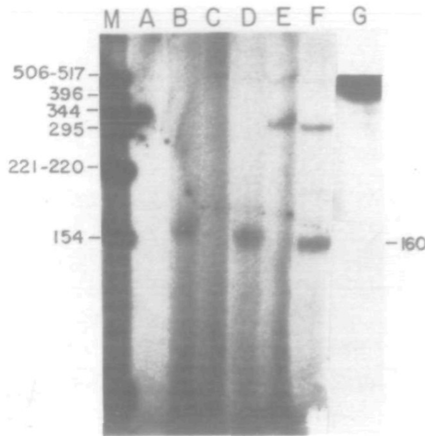

FIGURE 4: Measure of IFN-β_1 RNA accumulation in SR117-21E cells by S_1 nuclease analysis. Unlabeled RNA (50 µg) extracted from SR117-21E A1 nuclei was hybridized to a 5'-end labeled DNA probe (19) which is complementary to the first 160 nucleotides of IFN-β_1 RNA and to the entire promoter region. After S_1 nuclease digestion, the protected fragments were separated on a 6% acrylamide-urea gel. Lane A: nuclear RNA from non-inducing cells; B: nuclear RNA from cells induced 3 hours by poly(rI):(rC); C and D: same as A and B, but nuclei were incubated 40 min in a transcription reaction (without ^{32}P-UTP); E: Total cell RNA from uninduced cells; F: Total cell RNA from induced cells; M: DNA size markers; G: undigested DNA probe.

addition to superinduction, produces a large increase in IFN-β_1 RNA synthesis in the isolated nuclei (Fig. 5A). We also examined nuclei from monkey CV-1 cells in which the intact human IFN-β_1 gene was transfected as part of an SV40 replicating episome. Very little synthesis of IFN-β_1 RNA was seen unless these cells were induced (Fig. 5B). We conclude, therefore, that the constitutive synthesis of IFN-β_1 RNA in the SR117-21E nuclei reflects a true difference in the regulation of the -40 deleted IFN-β_1 gene, and indicates that this gene is no more controlled at the transcriptional level.

3) Characterization of the IFN-β_1 RNA synthesized by nuclei of the SR117-21E cells.

Since the IFN-β_1 RNA synthesized in nuclei of uninduced SR117-21E cells does not accumulate unless the cells have been induced, suggesting a post-transcriptional instability, we examined whether the uninduced transcripts are different from those made after poly(rI):(rC)-induction. We first compared the size of the transcripts. The ^{32}P-labeled RNAs synthesized by the isolated nuclei were sedimented on a sucrose-gradient and fractions were hybridized to the C-strand of the IFN-β_1 gene. The size of the nascent IFN-β_1 RNA chains

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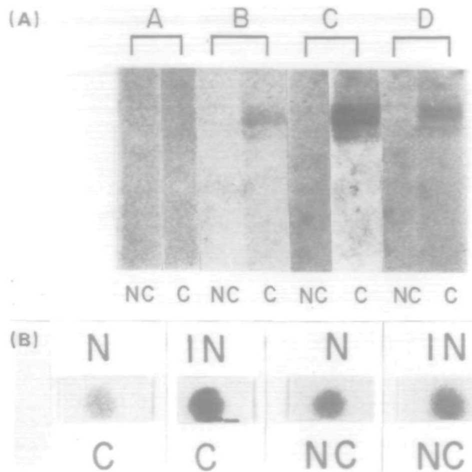

FIGURE 5: In vitro transcription of the IFN-β_1 gene with an intact promoter. A) ^{32}P-RNA synthesized in nuclei of human fibroblast FS11 was hybridized to electrophoretic blots of phage M13 DNA containing the C or NC strand of IFN-β_1 DNA. Lane A: nuclei from uninduced FS11 cells were used; lane B: nuclei from FS11 cells treated 4 hours with 50 µg/ml poly(rI):(rC) and 50 µg/ml cycloheximide; lane C: same as lane B, but cells were also primed with 100 U/ml IFN-β_1 for 16 hours prior to induction; lane D: same but nuclei were prepared from FS11 cells 20 hours after removal of inducer. At this time cells do not accumulate any more measurable amounts of IFN-β_1 mRNA (not shown). B) ^{32}P-RNA synthesized in nuclei from monkey CV-1 cells transfected by pSVIF-R1 DNA (19) containing the IFN-β_1 gene with the intact 300 bp flanking region. The RNA was hybridized to dot-blots of M13 DNA containing either the C or the NC strand of IFN-β_1 DNA. Lanes N: without induction; lanes IN: after 4 hours of induction with poly(rI):(rC) and cycloheximide 50 µg/ml, and 600 µg/ml DEAE-dextran.

was the same with or without induction (Fig. 6) with an average size of 9S. In another experiment (not shown), we measured the ratio of 5'-proximal to 3'-proximal sequences transcribed, by hybridization to an electrophoretic blot of the IFN-β_1 C-strand cut with Hae III, which cuts this ss DNA (34) in two fragments: from -20 to 540 and from 540 to 876, i.e. 115 nucleotides after the polyadenylation site (2). The first fragment contains 64% of the IFN-β_1 RNA sequence, the second contains 36%. The ratio of hybridization to the two fragments was the same with or without poly(rI):(rC)-induction, showing that the IFN-β_1 RNA made in nuclei of uninduced cells represent transcripts of the entire gene.

An important question is whether the uninduced in vitro transcripts start from the -40 deleted IFN-β_1 promoter or from some unknown upstream element (Fig. 1B). We, therefore, prepared a Pvu II-Taq I fragment of the episomal

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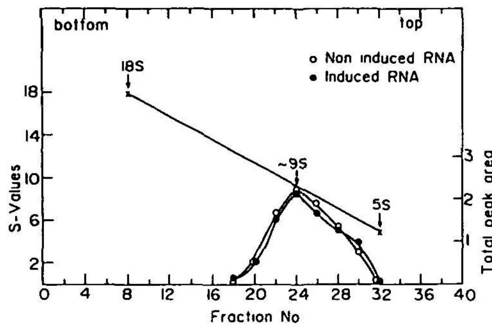

FIGURE 6: Size of ^{32}P-IFN-β_1 RNA synthesized in nuclei of SR117-21E A1 cells. ^{32}P-RNA (10^7 cpm) were fractionated on a 15-30% (w/w) sucrose gradient at 220,000xg for 19 hours, and fractions were hybridized to blots of M13 DNA containing the C-strand of IFN-β_1 DNA. The amount of ^{32}P-IFN-β_1 RNA was measured by scanning the autoradiography. rRNA markers were used for size calibration. Nuclei from non-induced (o) or poly(rI):(rC)-induced (•) SR117-21E A1 cells were used.

DNA present in SR117-21E cells containing 150 bp of 5'-flanking sequences upstream from the IFN-β_1 start site (fragment A, Fig. 7). The C and NC strands of this fragment were separated by gel electrophoresis (35) and ^{32}P-labeled RNA synthesized in nuclei from uninduced SR117-21 cells was hybridized to

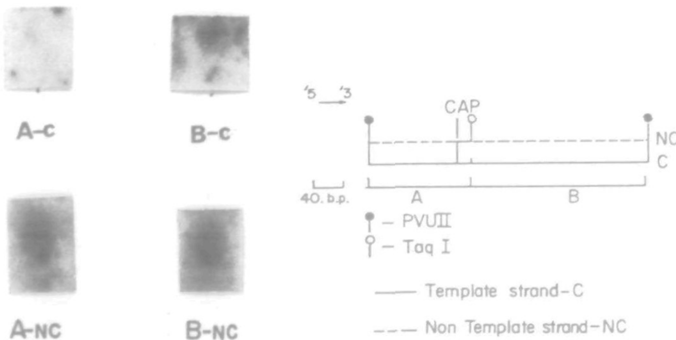

FIGURE 7: ^{32}P-IFN-β_1 RNA synthesized in nuclei of SR117-21E cells starts at the IFN-β_1 promoter. The PvuII fragment of the SR117-21E episome (Fig. 1B) was cut by TaqI to isolate the 5'-flanking region (fragment A) from the transcribed region (fragment B). The C and NC strands were separated (35)by electrophoresis on acrylamide gels after DMSO (fragment B) or NaOH (fragment A) treatment, applied to nitrocellulose and hybridized to 1.5x10^7 of ^{32}P-RNA synthesized in nuclei from non-induced SR117-21E A1 cells. Conditions as in Methods, except that after hybridization the blots were not RNase treated but washed three times in 0.5 SSC, 0.5% SDS at 68°C. No transcripts hybridize to the C-strand of the 5'-flanking fragment A.

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dot-blots of these ss DNA. Figure 7 shows that there was no hybridization to the C-strand of fragment A, while the RNA hybridized to the C-strand of a DNA fragment immediately downstream from the IFN RNA start site (fragment B of Fig. 7). With the NC-strand carrying upstream BPV promoter (Fig. 1) we saw, on the other hand, hybridization to both A and B fragment as expected (Fig.7). Thus, there is no evidence that the newly made IFN-β_1 transcripts in SR117-21E nuclei start from from an upstream promoter. The S_1 analysis done on unlabeled SR117-21E nuclear RNA (Fig. 4) also shows that all the IFN-β_1 RNA present starts at the correct cap nucleotide.

We also investigated the kinetics of IFN-β_1 RNA synthesis in nuclei of uninduced SR117-21E cells and tried to detect an eventual degradation _in vitro_. The amount of IFN-β_1 RNA synthesized increased from 10 min to 40 min. of nuclear incubation, with the same kinetics with or without poly(rI):(rC)-induction (not shown). After reaching a maximum between 40 to 80 minutes, there was some decrease in the amount of [^{32}P]-IFN-β_1 RNA from 120 to 200 min. No difference in the rate of synthesis or of degradation of the nascent IFN-β_1 RNA chains could be seen in the isolated nuclei between induced and uninduced states. We also verified that the synthesis of IFN-β_1 RNA (measured as in Fig. 3) was inhibited to the same extent (75%) by α-ammanitine (1 μg/ml) in nuclei from induced and uninduced cells, indicating that in both cases the transcripts are produced by RNA polymerase II.

DISCUSSION

Expression of a human IFN-β_1 gene which has lost all promoter sequences upstream from nucleotide -40, was studied in mouse SR117-21E cells where it is propagated as part of a BPV-1 episome. Despite the deletion, expression of the gene is still regulated since production of IFN and accumulation of the specific mRNA requires induction by ds RNA. This confirms our previous results obtained using transiently replicating SV40 vectors (19) and further shows that poly(rI):(rC) by itself is an inducer of the -40 deleted IFN-β_1 gene. _In vitro_ RNA synthesis experiments indicate, however, an alteration in the regulation of the -40 deleted gene, since nuclei isolated from uninduced SR117-21E cells actively produce IFN-β_1 transcripts. In nuclei of uninduced human fibroblasts, no IFN-β_1 RNA synthesis was seen prior to induction, as found also by Raj and Pitha (12). RNA synthesis in isolated nuclei probably reflects the transcriptional activity in the intact cell and is a function of the number of RNA polymerase molecules engaged on the gene prior to nuclei isolation (36). Thus, the -40 deleted IFN-β_1 gene seems to have lost a trans-

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criptional control operating in the wild type gene. The RNA made by uninduced SR117-21E nuclei appears identical in its rate of synthesis as well as in its size and structure to the IFN-β_1 RNA made after ds RNA induction, with the major difference that it does not accumulate in the cells unless ds RNA has been applied.

The deletion of promoter sequences upstream from nucleotide -40 can explain the alterations of IFN-β_1 regulation in SR117-21E cells. Zinn et al.(24) have proposed that a short sequence around -75 is required for the transcriptional effect of ds RNA (Fig. 2B). In addition further upstream sequences between -100 and -200 probably exert a negative effect on transcription: removal of these sequences leads to a small constitutive expression (24) and in SV40 vectors, transcription from external promoters was stimulated by a -40 deletion (19). A hairpin structure from -165 to -201 may be involved in this transcription block. Deletion of these two transcriptional signals (24) can explain the ds RNA-independent nuclear transcription of the IFN-β_1 gene in SR117-21E cells. The remaining regulation of the -40 deleted gene indicates that there is a third regulatory region which controls accumulation and expression of IFN-β_1 RNA (Fig. 2B). This third region would comprise sequences between -40 and +20 based 1) on the study of the -40 deleted gene and 2) on the fact that fusion of the mouse β-globin promoter to nucleotide +20 of IFN-β_1 produces a constitutive accumulation of IFN-β_1 RNA which is insensitive to inducers of IFN (19,21). Thus, downstream sequences have no regulatory functions. Inducers have also no effect if the IFN-β_1 coding region is fused to the SV40 early promoter (22,37).

The third regulatory region contains the TATA box and RNA start site of IFN-β_1 and our data indicate that it determines the ds RNA-dependent accumulation of IFN-β_1 transcripts. It could be argued that the IFN-β_1 transcripts fail to accumulate in uninduced SR117-21E cells because they start at an external promoter which we could not detect as the result of a splicing mechanism operating in the _in vitro_ nuclear system. However, IFN-β_1 transcripts initiated at external promoters did accumulate in other systems without induction (19,21). Moreover, since the RNA accumulating after poly(rI):(rC) clearly starts at the authentic IFN-β_1 RNA start site, one would have to assume that poly(rI):(rC) stimulates the use of the IFN-β_1 TATA box even without the signals described by Zinn et al. (24) and, judged from the amount of IFN produced (25), as efficiently as in the intact gene. In the absence of any indication for an upstream RNA start, or for any change in the size or abundance of the RNA transcripts after induction, we favour a poly(rI):(rC)-

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dependent post-transcriptional effect on RNA stability for the -40 to +20 region. The mechanism by which ds RNA causes IFN-β_1 accumulation is unknown but the localisation of the third control region would be compatible with an effect on capping which is considered as important for mRNA stability (38). It is noteworthy that the purine sequence AGGGAGAA present in the -75 transcriptional control region (24) is almost exactly repeated at position -10 (ATGGAGAA). Thus, a ds RNA binding protein might interact with both regions, explaining how ds RNA could have both transcriptional and post-trancriptional effects. Whatever the mechanism, our results imply a new function for poly (rI):(rC) in alleviating a regulatory process linked to the -40 to +20 region and which prevents accumulation of the IFN-β_1 RNA made in uninduced cells. It is likely that ds RNA exerts such a post-transcriptional effect in human fibroblasts as well, in addition to its ability to stimulate transcription of the IFN-β_1 gene in these cells (12). Evidence for a post-transcriptional effect of poly(rI):(rC) was recently reported also in some hamster cells transformed by the IFN-β_1 gene (39).

A post-transcriptional control of the IFN-β_1 gene is seen in human fibroblasts at late time (12-16 hours) after poly(rI):(rC) treatment, since nuclei continue to synthesize IFN-β_1 RNA but these transcripts do no accumulate anymore in the cells and IFN secretion stops (Fig. 5A and ref. 12). Cycloheximide was shown to reduce the rate of degradation of IFN-β_1 RNA in these cells, when added with ds RNA in the superinduction regimen (10-12). Despite this stabilizing effect, cycloheximide by itself does not induce IFN-β_1 RNA and protein synthesis in normal fibroblasts (10-12,40). In contrast, we found that the -40 deleted gene in SR117-21E cells again differs from the intact gene by the ability of cycloheximide alone to induce accumulation of IFN-β_1 RNA with authentic 5' ends and IFN secretion to levels of 40-75% those produced by ds RNA (25). With the gene containing a 300 bp promoter as in BPV-transformants SR117-13C, the effect of cycloheximide was only 5% that of ds RNA (25). The same was observed in other BPV-IFN-β_1 transformed cells, one with a -40 deletion created by cutting with Sau96 I (19) showing 40% induction by cycloheximide, and two with intact promoters showing 0-6% induction by cycloheximide as compared to ds RNA (Mitrani-Rosenbaum and Howley, unpublished). The simplest interpretation would be that cycloheximide stabilizes the constitutive transcripts made from the -40 deleted gene, as it acts in human fibroblasts after ds RNA induction (12). However, an effect of cycloheximide on transcription was reported in another system (39). Indeed a more efficient transcription may compensate for the IFN-β_1 RNA instability

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since we observed accumulation of IFN-β_1 RNA in uninduced cells by bringing the strong SV40 transcriptional enhancer very close to the -40 deleted gene (19). The transcriptional and post-transcriptional controls operating in the IFN-β_1 gene may be difficult to differentiate in most cells, especially since inducers such as poly(rI):(rC) and cycloheximide, seem to act on both. Thus the -40 deleted gene carried in the BPV episome of SR117-21E, offers a convenient system to study by itself the control of IFN-β_1 RNA accumulation.

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