Volume 11 Number 16 1983 Nucleic Acids Research

# The 5'-flanking sequence of human interferon-$\beta_1$ gene is responsible for viral induction of transcription

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Received 6 June 1983; Revised and Accepted 26 July 1983

ABSTRACT

The structural gene for Herpes simplex virus (HSV) thymidine kinase (Tk) was fused downstream of the 5'-flanking sequence (from -284 to +20;numbering relative to the putative transcription initiation site) of the cloned human interferon-$\beta_1$ (IFN-$\beta_1$) gene. The fusion gene was linked to the vector pSV2-Ecogpt and the recombinant plasmid was used to transform mouse FM3A cells. All cloned transformants in which the fusion gene was integrated in an intact form produced the Tk specific transcript with the distinct 5' terminus corresponding to that of the authentic IFN-$\beta_1$ mRNA when they were exposed to Newcastle disease virus (NDV). Thus, the results reported here provide evidence for the presence of specific DNA sequences in the 5'-flanking region of the IFN-$\beta_1$ gene required for the virus mediated activation of transcription.

## INTRODUCTION

Among three types ($\alpha,\beta,\gamma$) of interferon (IFN), IFN-$\beta_1$ is the principal IFN produced by human fibroblasts upon exposure to viruses or double-stranded RNA such as Poly(I):Poly(C). Induction of the IFN production depends on the accumulation of IFN-$\beta_1$ specific mRNA which is not detectable in uninduced cells(1). Earlier studies suggested that induction of IFN-$\beta_1$ production in human fibroblasts is controlled primarily at the level of transcription(1). On the other hand, since the level of IFN mRNA accumulation is increased and continued for a prolonged time following addition of metabolic inhibitors such as cycloheximide, there also seems to be another mechanism by which the level of the mRNA is controlled(1).

We have previously introduced the 1.8 kb human DNA segment containing the human IFN-$\beta_1$ gene and its flanking sequences (284 bp and 684 bp at 5' and 3',respectively) into cultured mouse FM3A cells and studied the inducibility of the gene. The mouse

cells produced human IFN-$\beta_1$ mRNA and IFN-$\beta_1$, in parallel with mouse IFN, upon treatment with NDV or with Poly(I):Poly(C) (2). Thus, this system permits identification of the DNA sequence responsible for the induced expression of the human IFN-$\beta_1$ gene. Similar results were also reported for human IFN-$\beta_1$ (3,4,5) and human IFN-$\alpha_1$ (6).

One of the intriguing questions concerning the expression of the IFN gene is whether the induced expression is due to transcriptional activation (or derepression) of the gene or to the other events such as stabilization of the mRNA, or to a combination of both.

In the present study, we constructed a fusion gene in which the 5'-flanking sequence of the human IFN-$\beta_1$ gene (7) is linked to the coding sequence of HSV Tk gene (8,9) and asked whether the fusion gene responds to the regulatory signal in an assay system similar to that described above. We show that transcription of the fusion gene is specifically induced under the control of 5'-flanking sequences of human IFN-$\beta_1$ gene. The results provide evidence that the 5'-flanking region of the IFN gene mediates the virus-mediated induction of the human IFN-$\beta_1$ gene expression which appears to be controlled at the level of transcription.

## MATERIALS AND METHODS

### Construction of hybrid plasmids

The 0.3 kb EcoRI-TaqI fragment (from -284 to +20) from the human IFN-$\beta_1$ gene (7), in which the TaqI site was rendered blunt by Klenow fragment of DNA polymerase I, was inserted into EcoRI and BamHI (rendered blunt similarly) sites of vector pBR322. The 0.3 kb EcoRI-BamHI fragment isolated from the resultant plasmid and the 2.8 kb BglII-BamHI fragment (downstream from +53 with respect to the Tk gene) of the Tk gene (8,9) was inserted into EcoRI and BamHI sites of pSV2-Ecogpt (10). The recombinant in which the Tk gene is inserted in the same orientation with the IFN gene was isolated. The nucleotide sequence of the junction between DNA from genes for human IFN-$\beta_1$ and for HSV Tk was confirmed by sequencing (11).

Cell culture and DNA transfection

Mouse FM3A cells (strain FStk$^-$/Oua$^r$-1) (provided by Dr. H. Koyama) were maintained as suspension culture in ES medium supplemented with 2% fetal calf serum (FCS) (normal medium) (12). One hour before transfection, cells (2 x $10^6$ cell/3 ml) in ES medium containing 0.5% FCS were seeded in 10 cm culture dish and incubated at 37°C. DNA precipitate (15 µg DNA in 0.5 ml) was prepared (13,14) and added to the culture dish in which the cells had become attached to the bottom. Four hours later, cells were treated with 20% glycerol for one min and cultured in normal medium for 40 hours. Cells were then spread onto agar plates (15) with normal medium supplemented with xanthine (250 µg/ml) and mycophenolic acid (2.5 µg/ml) (selection medium). Colonies were picked up 7 to 10 days after transfection and expanded in the selection medium.

Interferon induction

Mouse FM3A cells (5 x $10^7$ cells/ml) in normal medium were treated by NDV at m.o.i. of 10 for one hour at room temperature. Cells were then diluted ten fold with normal medium and cultured for 11 hours before RNA extraction.

Nucleic acids extraction and blot hybridization

High molecular weight DNA was prepared as described (16) and blotting analysis was carried out by the method of Southern (17). Poly(A) RNA was prepared essentially as described (18) and the RNA blotting analysis was performed as described (19,20).

S1 nuclease mapping

Total cellular poly(A) RNA (5 µg) from cell transformant t5 treated or mock-treated with NDV was annealed with an excess of the end-labeled DNA probe (5000 cpm, 5 fmol) in 50% formamide solution containing 400 mM NaCl, 40 mM Pipes (pH, 6.4) and 1 mM EDTA at 42°C for 18 hours. After the annealing, 10 µl hybridization mixture was diluted into 200 µl of S1 nuclease digestion solution (0.25 M NaCl; 30 mM Na-acetate, pH 4.6; 1 mM $ZnCl_2$) and incubated with 100 U of S1 nuclease for 60 min at 30°C. Nucleic acids were purified and analysed by electrophoresis on 5 % polyacrylamide gel under denaturing condition. The $P^{32}$-end-labeled single stranded probe was prepared as follows.

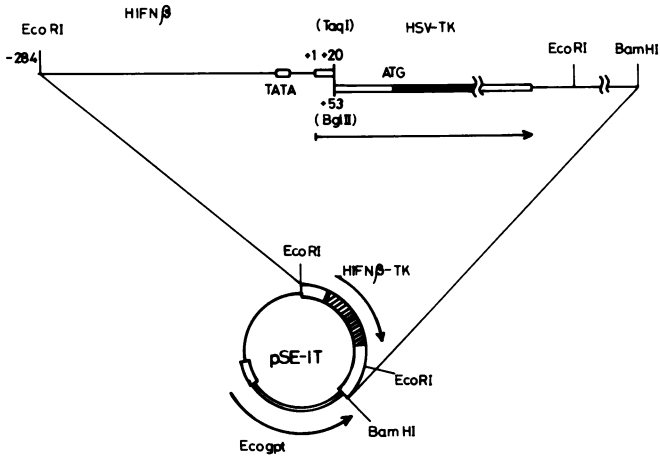


Figure 1. Structure of the hybrid plasmid pSE-IT. Plasmid pSE-IT contains the human IFN-$\beta_1$-HSV Tk fusion gene in an opposite orientation to the SV40-Ecogpt gene in the vector pSV2-Ecogpt (10).

The 260 bp AluI-TaqI fragment (from -137 with respect to human IFN-$\beta_1$ gene to +157 with respect to Tk gene) was isolated from plasmid pSE-IT and labelled at 5'-ends. The 260 bp anti-coding strand DNA was isolated by polyacrylamide gel.

## RESULTS AND DISCUSSION

A human IFN-$\beta_1$-HSV Tk fusion gene in which the 5'-flanking sequence (from -284 to +20) of the human IFN-$\beta_1$ gene was linked to the coding sequence (downstream of the BglII site at +53) of HSV Tk gene was linked to a vector pSV2-Ecogpt (10) in the opposite orientation to the SV40-Ecogpt gene (Fig. 1.). The resulting plasmid DNA was introduced into cultured mouse FM3A cells.

Mycophenolic acid-resistant transformants were selected and DNA was extracted from each cell clone. Southern blot analysis of the DNA revealed the presence of the extraneous Tk specific DNA in 6 out of 7 transformants as shown in Fig. 2. When the high molecular weight DNA was digested by EcoRI and analysed by the blotting method, the presence of a 2.3 kb EcoRI fragment which should contain the intact fusion gene was detectable in

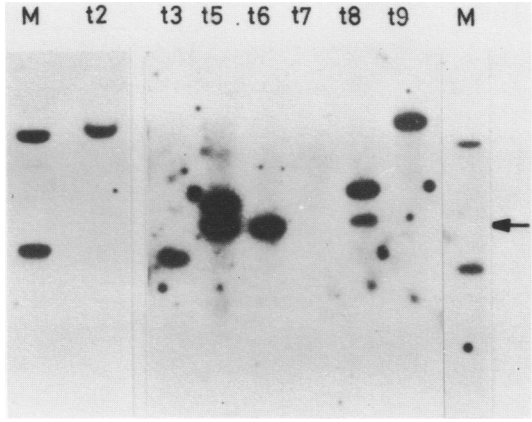


Figure 2. Human IFN-$\beta_1$-Tk fusion gene in transformed mouse cells. High molecular weight DNA from mouse cell transformants were digested with EcoRI and subjected to electrophoresis on a 0.8 % agarose gel. The DNA was denatured in situ, transferred to nitrocellulose and hybridized with the $P^{32}$-labeled probe (17). Probe used is 2.1 kb EcoRI fragment containing Tk gene (8,9). Lane M represents size marker, 8.6 kb and 1.8 kb. Arrow indicate the 2.3 kb band indicating the presence of the intact fusion gene.

the cell clones t5, t6 and t8. On the other hand, rearrangements of the 2.3 kb EcoRI fragment were observed in the clones t1, t2, t3, and t9. Further restriction endonuclease analysis of the DNA from each cell clone indicated that in all transformants the fusion gene was integrated in 1 to 2 copies per cell (data not shown).

The transformed cell clones were each treated by NDV and, 12 hours later, poly(A) RNA was isolated from them as well as from the mock-treated cells. The RNA was subjected to RNA blotting analysis using the 2.1 kb EcoRI fragment containing the Tk gene as probe. As presented in Fig. 3, a positive band corresponding to 15S, diagnostic for the Tk mRNA transcript appeared, when clones t2, t5, t6 and t8 were induced by NDV but not without induction. All other cell clones tested either produced no detectable Tk specific transcripts (clones t3 and t7) or transcripts of larger size ($\sim$ 23S) which appears independently of NDV treatment (clone t9) or upon NDV treatment (clones t2, t5 and t6). In addition, clones t2, t5 and t6

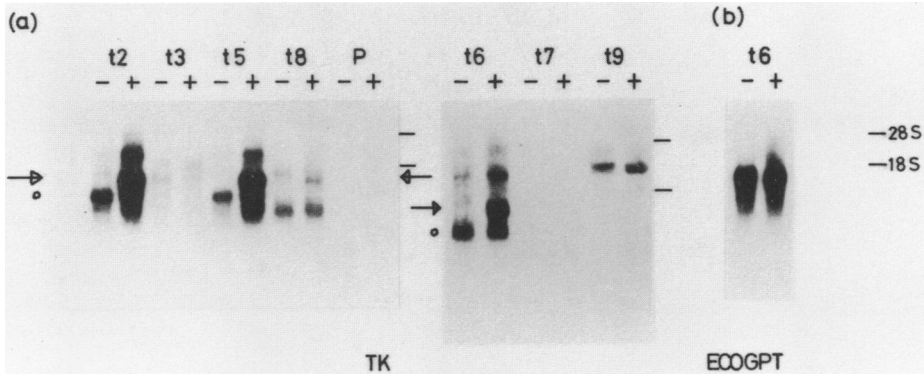


Figure 3. Induction of Tk RNA in mouse cells transformed with human IFN-$\beta_1$-Tk fusion gene. Poly(A) RNA (10 µg) from mouse cell transformants treated or mock-treated with NDV was denatured, fractionated on a 1.3 % agarose gel and analysed by blot hybridization method. Probes used are 2.1 kb EcoRI fragment containing Tk gene (a) or 2.3 kb PvuII-BamHI fragment containing Ecogpt gene (b). (→), 15S transcript; ( O ) 12S transcript.

produced a 12S transcript in the absence of induction. This may correspond to the 1.1 kb or 0.9 kb Tk mRNA, reported by Roberts and Axel, which is produced when the 5'-flanking region (upstream of +53) is deleted from HSV Tk gene and whose synthesis appeares to be initiated from an internal site of Tk-structural gene (21).

Furthermore, when the poly(A) RNA from each of all the cell clones described above was analysed for the presence of SV40-Ecogpt sequence (using PvuII-BamHI fragment of pSV2-Ecogpt as probe) which is linked to the IFN-$\beta_1$-Tk fusion gene or for the presence of endogenous adenine phosphoribosyltransferase (APRT) gene sequence (using 1.8 kb PvuII fragment of chinese hamster APRT genomic DNA as probe, 22), the specific transcript (17S or 12S, respectively) was shown to be expressed independently of viral induction (Fig. 3 b; data not shown). On the other hand, 12S transcript specific for endogenous mouse IFN-$\beta_1$ gene was also shown (using 0.8 kb cDNA of mouse IFN-$\beta_1$, Higashi et al., submitted for publication.) to be expressed upon induction for all cell clones (data not shown). Thus, the results clearly indicate that the 15S Tk-specific transcript is

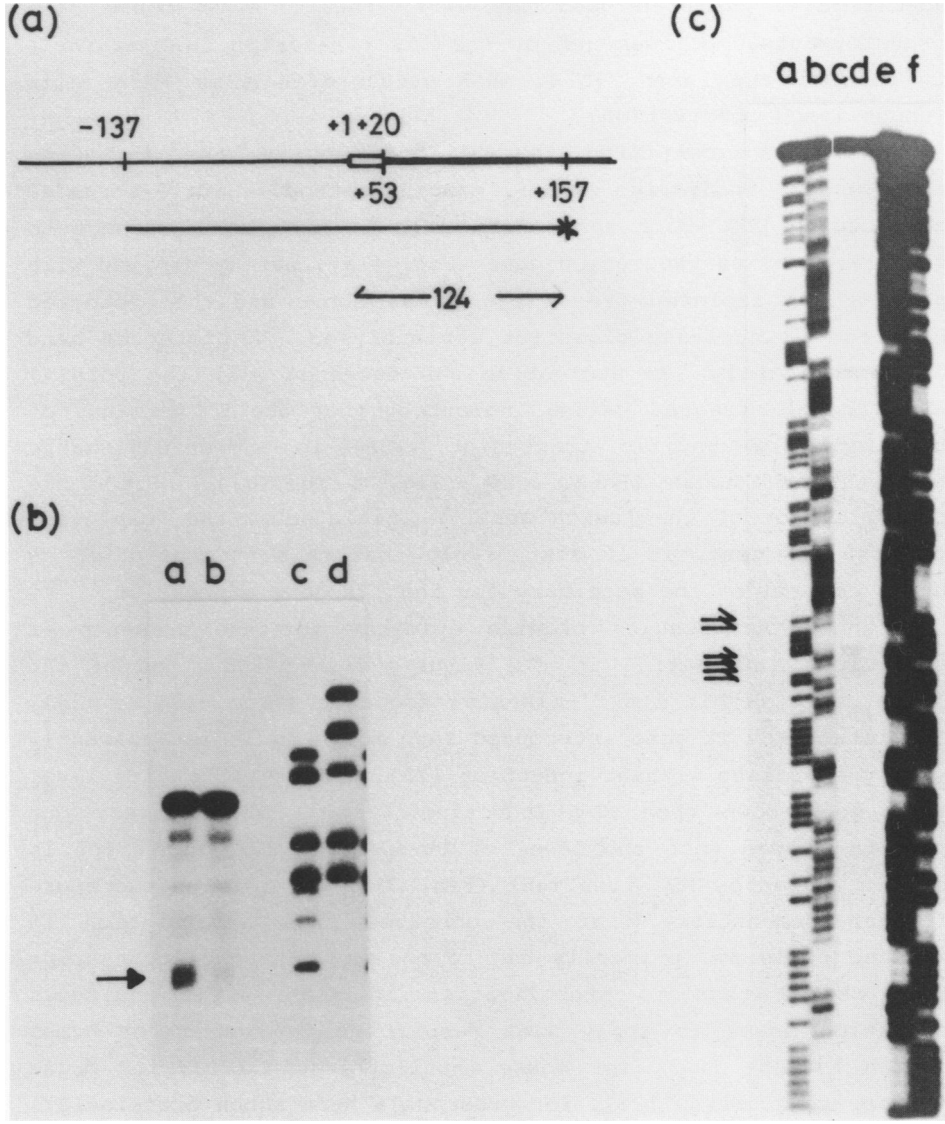


Figure 4. S1 nuclease mapping of the Tk RNA specific for the viral induction. (a) The $P^{32}$-end-labeled single stranded probe is indicated. (b),(c) Lanes (b)a and (c)c; poly(A) RNA from induced t5 cells. Lanes (b)b and (c)d, poly(A) RNA from mock-induced t5 cells. Lanes (b)c and d; size markers (320,290,197,167,150,140 and 120 for c; 612,409,290,197 and 167 for d) Lanes (c)a,f or (c)b,e; the relevant DNA fragments modified for A+G or T+C, respectively, by the Maxam-Gilbert procedure (11).

specifically produced upon the viral induction of mouse cell transformants. The amount of the 15S transcript is comparable to that of the human IFN-$\beta_1$ mRNA produced in mouse FM3A cells (unpublished observation).

Next we identified the 5' terminus of the induction specific 15S transcript by a S1 mapping method. Single-stranded anti-coding DNA which spans from -137 to +124 (with respect to the IFN gene) of the fusion gene (Fig. 4(a)) was hybridized with poly(A) RNA isolated from clone t5 as above and the protected DNA from S1 nuclease digestion was analysed. A protected band of approximately 120 nucleotide was detected with the poly(A) RNA from NDV-treated cells, indicating that the 5' terminus of the induction-specific transcript (15S) is indistinguishable from that of human IFN-$\beta_1$ mRNA (Fig. 4 (b),(c)). Since the transcription of the fusion gene is initiated at the "cap" site for the IFN gene, it is most likely that this transcription is under control of the machinary for the IFN gene expression.

Hence our results provide evidence for the presence of sufficient information in the Virus-mediated induction of the IFN-$\beta_1$ gene within its 5'-flanking sequence (from -284 to +20). The intact HSV Tk gene introduced into mouse cells is apparently stably expressed without induction (23) and the expression level even goes down upon NDV induction (28). In addition, our results showed that the level of Tk-specific 12S transcript is not increased by NDV treatment (Fig. 3). The results therefore further demonstrate that the observed mRNA accumulation in induced cells are primarily due to the activation of transcription rather than to stabilization of mRNA. The analogous situation seems to apply with respect to the genes for human growth hormone (24), for mouse metallothionein (25), for mouse mammary tumor virus (26), for Drosophila heat shock protein (27) and for human IFN-$\alpha_1$ (28). On the other hand, Pitha and her colleagues have presented results suggesting that the 5'-flanking sequence is not required for the induction of human IFN-$\beta_1$ synthesis mediated by poly(I):poly(C) and cycloheximide (29). The obvious discrepancy between our results and theirs are very difficult to explain at present, partly because of the different assay systems used.

The 304 bp human DNA segment (from -284 to +20) which was shown to mediate the viral induction of the genes linked downstream of it harbors several interesting structural features. As reported previously, the region of the sequence between -96 and -61 is extremely purine rich in the coding strand and, moreover, this sequence shows significant homology (60 %) to the human IFN-$\alpha_1$ gene whose expression is under similar control (6) whereas the homology in the coding sequences between the two extends only to 45 % (30). This region also contains tandemly repeated sequence, with the consensus sequence being TGAPuAGAGGPuAA, as well as an inverted repeats which could constitute a stem-loop structure. The question as to how such sequence functions in cooperation with other cellular agents still remains open.

ACKNOWLEDGEMENT

This work was supported in part by a grant from Ministry of Education, Science and culture of Japan.

We thank Drs. P. Berg, R. Axel, C. Weissmann, H. Koyama and H. Taira for providing us with pSV2-Ecogpt, pHaprt1, pTkM2, FStk /Oua$^r$-1 and NDV. We are indebted to Drs. M. Muramatsu, H. Koyama and R. Kominami for valuable discussions.

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