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Priming Affects the Activity of a Specific Region of the Promoter of the Human Beta Interferon Gene

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Treatment of Daudi or HeLa cells with human interferon (IFN) α8 before induction with either poly(I)-poly(C) or Sendai virus resulted in an 8- to 100-fold increase in IFN production. The extent of priming in Daudi cells paralleled the increase in the intracellular content of IFN-β mRNA. IFN-α mRNA remained undetectable in poly(I)-poly(C)-treated Daudi cells either before or after priming. An IFN-resistant clone of Daudi cells was found to produce 4- to 20-fold more IFN after priming, indicating that priming was unrelated to the phenotype of IFN sensitivity. IFN treatment of either Daudi or HeLa cells transfected with the human IFN-β promoter (−282 to −37) linked to the chloramphenicol acetyltransferase (CAT) gene resulted in an increase in CAT activity after induction with poly(I)-poly(C) or Sendai virus. A synthetic double-stranded oligonucleotide corresponding to an authentic 30-base-pair (bp) region of the human IFN-β promoter between positions −91 and −62 was found to confer virus inducibility upon the reporter CAT gene in HeLa cells. IFN treatment of HeLa cells transfected with this 30-bp region of the IFN-β promoter in either the correct or reversed orientation also increased CAT activity upon subsequent induction. IFN treatment alone had no detectable effect on the activity of either the 30-bp region or the complete human IFN promoter.

Interferons (IFNs) are an important group of secreted polypeptides which exhibit pleiotropic biological activities. Treatment of cells with low doses of IFN before induction has been reported to increase markedly the amount of IFN and IFN mRNA produced in response to a variety of inducers (8, 11, 24, 27, 29, 35). Although this phenomenon known as priming may represent an important means of amplifying the IFN response in vivo, its mechanism remains unclear.

Deletion analysis has defined the presence of *cis*-acting DNA sequences required for induction within the 5′-flanking regions of both the human IFN-α1 and IFN-β1 genes (3, 12, 17, 28). This region of the human β promoter contains seven copies of repetitive hexanucleotide sequences, certain copies of which have been shown to confer virus inducibility upon a reporter gene when present as a four- to eight-copy tandem repeat (13). Two positive regulatory domains have been defined within this region, PRD-I between positions −77 and −64 and PRD-II between positions −66 and −55 (16). Part of this region between positions −91 and −64 shows considerable homology with elements, particularly with the IFN-responsive sequence present within the flanking region of a number of unrelated genes, the expression of which is regulated by IFN (18, 20, 21, 26, 34). In view of the sequence homology between these sequences and regions within the IFN-β promoter, it was of interest to determine whether the priming effect of IFN resulted from an action of IFN on the IFN-β promoter.

Priming in IFN-sensitive and -resistant Daudi cells. Parental Daudi cells were found to produce low levels of IFN in response to induction with poly(I)-poly(C) (Table 1). Treatment of Daudi cells with recombinant human IFN-α8 for 4 h or more resulted in a 10- to 100-fold increase in the production of IFN upon subsequent induction with poly(I)-poly(C) (Fig. 1A and B).

Three clones of Daudi cells, resistant to both the antiviral and anticellular effects of human IFN (5, 6) were found to produce significantly higher levels of IFN in response to induction with poly(I)-poly(C) than IFN-sensitive parental Daudi cells did (Table 1). Clone DIF3 REV5, which had reverted to almost full sensitivity to both the antiviral and

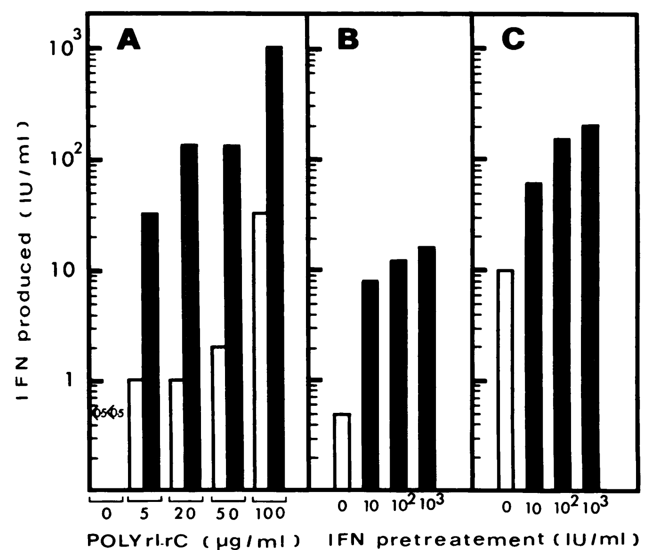

FIG. 1. Priming of IFN-sensitive and IFN-resistant Daudi cells. Daudi cells were treated for 16 h with either RPMI 1640 medium with 15% fetal calf serum alone or 10^3 IU of human recombinant IFN-α8 (Ciba-Geigy, S.A., Basel, Switzerland) per ml, washed once, and induced for 90 min with the concentration of poly(I)-poly(C) (Pharmacia, Uppsala, Sweden) indicated in the figure (A). Cells were then washed twice with phosphate-buffered saline and 5% fetal calf serum and incubated overnight in culture medium. IFN-sensitive (B) and a clone of IFN-resistant Daudi cells, DIF3 (5, 6) (C) were treated for 16 h with IFN-α8 with the concentration indicated in the figure before induction with 20 μg of poly(I)-poly(C) per ml.

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TABLE 1. IFN production by IFN-sensitive and -resistant clones of Daudi cells[a]

Cell line	IFN sensitivity[b]	IFN Titer (IU/ml)				
		Expt 1	Expt 2	Expt 3	Expt 4	Expt 5
Daudi	++++	1	2	2	10	<10
DIF8	±	40	20	16	160	190
DIF3	±	10	40	60	320	380
DIF3-17	±		320	80	320	380
DIF3 REV5	+++			10	40	760

[a] IFN-sensitive or -resistant clones of Daudi cells were treated with either 20 μg (experiments 1 to 3) or 100 μg (experiments 4 and 5) of poly(I)-poly(C) per ml for 1.5 h. IFN titers (31) were then determined 18 to 20 h after the start of induction.

[b] IFN sensitivity is expressed on an arbitrary scale ranging from ± for minimal sensitivity (<5% reduction in cell number after 72 h of treatment with 10 IU of IFN-α per ml) to ++++ for full sensitivity (>75% reduction in cell number under the above conditions.

anticellular effects of IFN but which remained resistant to the effect of IFN on c-*myc* expression (4), produced high levels of IFN (Table 1). These results suggest that the association between increased IFN production and the phenotype of IFN resistance is complex and does not reflect a simple causal relationship.

The response of Daudi cells to the priming action of IFN-α was also found to be unrelated to the phenotype of IFN sensitivity. Thus, an IFN-resistant clone, DIF3, was found to produce 4- to 20-fold more IFN in response to priming with IFN-α than untreated cells (Fig. 1C). Although this

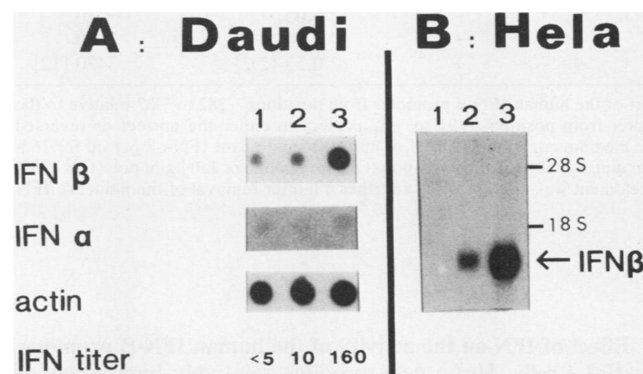

FIG. 2. Expression of human IFN-α and IFN-β mRNA in primed Daudi and HeLa cells. (A) Daudi cells were treated with either the medium alone (lanes 1 and 2) or with medium containing 10^3 IU of human recombinant IFN-α8 per ml (lane 3) for 16 h before induction with 100 μg of poly(I)-poly(C) per ml (lanes 2 and 3). Polyadenylated RNA (2.5 μg), isolated by a modification of the guanidinium thiocyanate procedure of Chirgwin et al. (2) and chromatography on oligo(dT)-cellulose, was hybridized with either a cDNA probe of the human IFN-β gene (1.8-kilobase *Eco*RI fragment) (30), DNA probe of the coding region of the human IFN-α1 gene (1), or β actin (4) labeled by random priming to a specific activity of 3×10^9 cpm/μg of DNA. Autoradiograms were exposed for 1 or 24 h after hybridizing of dot blots with actin or IFN-β and IFN-α probes, respectively. (B) HeLa cells were treated for 16 h with either the medium (lanes 1 and 2) alone or with medium containing 250 IU of human recombinant IFN-α8 per ml (lane 3) before induction with 45 hemagglutin units of Sendai virus (lanes 2 and 3). Polyadenylated RNA (5 μg) was then electrophoresed in a denaturing agarose gel and transferred to a nitrocellulose filter as described previously (4). The blot was hybridized with the same human IFN-β probe as described above and exposed for 24 h at −80°C.

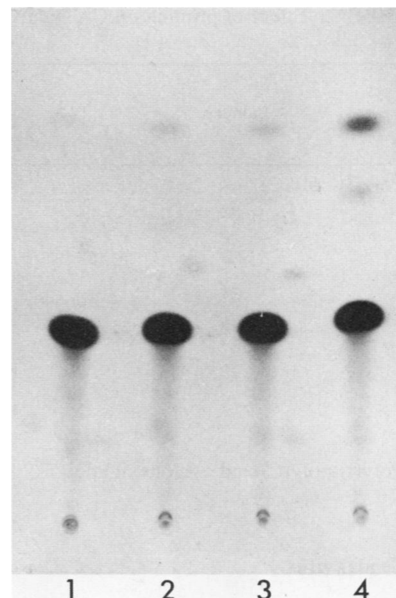

FIG. 3. Effect of priming on CAT activity in Daudi cells transfected with the pHPβ245CAT plasmid. IFN-sensitive Daudi cells were transfected with 20 μg of plasmid pHPβ245CAT (see text) by using DEAE-dextran (14). Transfected cells were treated with either the medium alone (lanes 1 and 2) or with 10^3 IU of IFN-α8 per ml for either 1 (lane 3) or 4 h (lane 4) before induction with 100 μg of poly(I)-poly(C) (lanes 2 to 4) per ml. CAT activity was then determined 20 h later. The cell pellet was washed once with phosphate-buffered saline at 4°C and once with TNE (40 mM Tris hydrochloride [pH 7.4], 150 mM NaCl, 1 mM EDTA), frozen, and thawed four times in 200 μl of extraction buffer (250 mM Tris hydrochloride [pH 8.0]). The supernatant was heated to 56°C for 10 min. A constant amount of cellular protein was used in each assay (Bio-Rad protein assay). The CAT reaction was performed with [^{14}C]chloramphenicol (0.2 μCi; Amersham, United Kingdom) and 25 mM acetyl coenzyme A in 250 mM Tris hydrochloride (pH 8.0) for 2 h at 37°C, and the products were separated by chromatography and subjected to autoradiography. Enzyme activity was determined as the percentage of acetylation of incorporated radioactivity.

clone binds IFN-α8 in a manner identical to that of the parental cells (7), only certain proteins that are induced by IFN treatment in IFN-sensitive parental Daudi cells are induced in these cells (5, 32). The extent of the priming effect observed in parental Daudi cells, as determined by increased production of IFN upon subsequent induction with poly(I)-poly(C), was found to reflect a corresponding increase in the intracellular content of IFN-β mRNA (Fig. 2A) in the absence of a detectable effect on the intracellular concentration of actin mRNA. IFN-α mRNA remained undetectable in poly(I)-poly(C)-treated Daudi cells either before or after priming (Fig. 2A).

Effect of priming on the activity of the human IFN-β promoter in Daudi cells. To determine whether priming resulted in enhanced activity of the human IFN-β promoter, parental Daudi cells were transiently transfected with a plasmid pHPβ245CAT containing the 5′-flanking region of the human IFN-β gene (25) (positions −282 [*Eco*RI] to −37 [*Ava*II] relative to the cap site [36]) cloned into the *Sma*I site of the plasmid pH34ΔE4CAT. The region of the IFN-β promoter studied has been shown to contain those sequences necessary to confer inducibility upon the IFN-β gene (3, 12, 17). Plasmid pHP34ΔE4CAT contains the bacterial chloramphenicol acetyltransferase (CAT) gene under

TABLE 2. Effect of priming on CAT activity and IFN production in cells transfected with plasmids containing part of the human IFN-β promoter[a]

Cell line	Inducer	Flanking sequence positions	Treatment		CAT activity (% of conversion [fold increase])	IFN titer (IU/ml [fold increase])
			IFN	Inducer		
Daudi	Poly(I)-poly(C)	−282 to −37	−	−	0.10	ND[b]
			−	+	0.39	
			+	+	2.32 [6]	
			−	−	0.21	<10
			−	+	0.49	40
			+	+	1.19 [2.4]	640 [16]
			−	−	0.49	<10
			−	+	1.66	32
			+	+	3.06 [1.8]	320 [10]
HeLa	Poly(I)-poly(C) and cycloheximide	−282 to −37	−	−	0.76	ND
			−	+	3.16	
			+	+	8.86 [2.8]	
	Sendai virus	−282 to −37	−	−	0.53	<10
			−	+	3.41	320
			+	+	9.41 [2.8]	2560 [8]
			−	−	0.50	<10
			−	+	1.35	40
			+	+	3.17 [2.3]	640 [16]
	Sendai virus	−91 to −62	−	−	0.28	<10
			−[c]	+[c]	1.83	100
			+	+	4.37 [2.4]	800 [8]
			−	−	0.05	<10
			−[d]	+[d]	0.24	40
			+	+	0.53 [2.2]	480 [12]

[a] Daudi or HeLa cells were transfected with plasmid pHPβ245CAT containing part of the human IFN-β promoter from positions −282 to −37 relative to the cap site or with plasmid pHPβ30CAT containing part of the human IFN-β promoter from positions −91 to −62 present in either the correct or reversed orientation. Transfected cells were then treated with either the medium alone or the medium containing 250 IU of human recombinant IFN-α8 per ml for 16 h before induction. Cells were induced for 1.5 h with Sendai virus (30 or 300 hemagglutin units) or 100 μg of poly(I)-poly(C) per ml alone or 150 μg of poly(I)-poly(C) per ml together with 50 μg of cycloheximide per ml (in which case cycloheximide treatment was continued for a further 4 h after removal of the inducer). IFN titers and CAT activity were determined 18 h after the start of induction.

[b] ND, Not determined.

[c] Correct orientation.

[d] Reverse orientation.

the control of part of the adenovirus type 2, E4 promoter (positions −89 to +32) (15). The pHPβ245CAT construction exhibited a low level of spontaneous CAT activity in Daudi cells, similar to that exhibited by pHP34ΔE4CAT. Treatment of Daudi cells transfected with pHPβ245CAT with human IFN-α8 for 4 h resulted in a significant increase in CAT activity upon subsequent induction with poly(I)-poly(C) relative to cells treated with poly(I)-poly(C) alone or cells treated with IFN for 1 h only (Fig. 3). IFN alone or poly(I)-poly(C) alone or IFN plus poly(I)-poly(C) had no significant effect on CAT activity in Daudi cells transfected with a plasmid pHP34ΔE4CAT without the human IFN-β sequence. The increase in CAT activity observed in primed, poly(I)-poly(C)-induced Daudi cells transfected with plasmid pHPβ245, CAT was accompanied by a concomitant increase in IFN production (Table 2), reflecting enhanced activity of the chromosomal genes. The extent of priming in IFN-treated Daudi cells, as reflected by increased IFN activity, was consistently greater than that indicated by the increase in CAT activity in the same cells (Table 2), raising the possibility that priming results from effects in addition to the increase in transcriptional activity which we have observed.

Effect of IFN on the activity of the human IFN-β promoter in HeLa cells. HeLa cells produce relatively high levels of IFN in response to induction with Sendai virus and can be readily primed as shown by both increased levels of IFN-β mRNA and IFN production (Fig. 2B and Table 2).

Treatment of pHPβ245CAT-transfected HeLa cells with human IFN-α8 resulted in an approximately twofold increase in CAT activity after induction with either poly(I)-poly(C) together with cycloheximide or Sendai virus alone (Table 2). IFN treatment had no significant effect on CAT activity in induced or uninduced HeLa cells transfected with the pHP34ΔE4CAT plasmid, which did not contain the human IFN-β promoter.

A synthetic oligonucleotide corresponding to an authentic 30-base-pair (bp) region of the human IFN-β promoter between positions −91 and −62 relative to the cap site was found to confer virus inducibility upon a reporter gene after transfection in HeLa cells. This region contains four repetitive hexanucleotide sequences, two of which have been shown to confer virus inducibility upon a reporter gene when present as a four to eight copy tandem repeat (13). The most efficient of these polymeric constructions $(AAGTGA)_4$ does

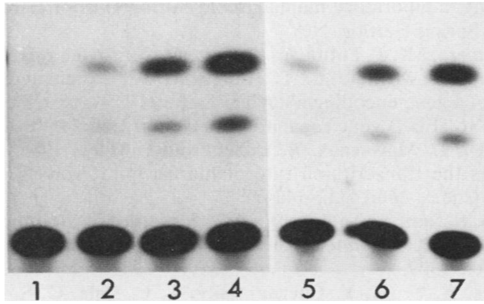

FIG. 4. Effect of priming on CAT activity in HeLa cells transfected with different regions of the human IFN-β promoter. Plasmid pHPβ30CAT was obtained by cloning (22) into the *Sma*I site of pHP34ΔE4CAT a synthetic double-stranded oligonucleotide (5'-gGGAAAACTGAAAGGGAGAAGTGAAAGTGGGccc-3') corresponding to the region of the human β promoter between positions −91 and −62 relative to the cap site. HeLa cells were transfected with 10 μg of plasmid DNA and 10 μg of bacteriophage γ carrier DNA by using calcium phosphate precipitation. Mock-transfected HeLa cells (lane 1) or HeLa cells transfected with either plasmid pHPβ245CAT (lanes 2 to 4) or plasmid pHPβ30CAT (lanes 5 to 7) were treated with either Dulbecco modified Eagle medium plus 10% fetal calf serum alone (lanes 1 to 3 and 5 to 6), or 250 IU of IFN-α8 per ml for 20 h (lanes 4 and 7) before induction with 300 hemagglutin units of Sendai virus (lanes 3, 4, 6, and 7). CAT activity was then determined 20 h later as described in the legend to Fig. 3.

in fact create a sequence similar to that found between positions −77 and −64 of the human IFN-β promoter, which has been defined as a positive regulatory domain (PRD-I) by Goodbourn et al. (16). PRD-I contains the binding site for the IFN regulatory factor 1 (IRF-1) which is thought to play an important role in transcriptional activation of the IFN-β gene

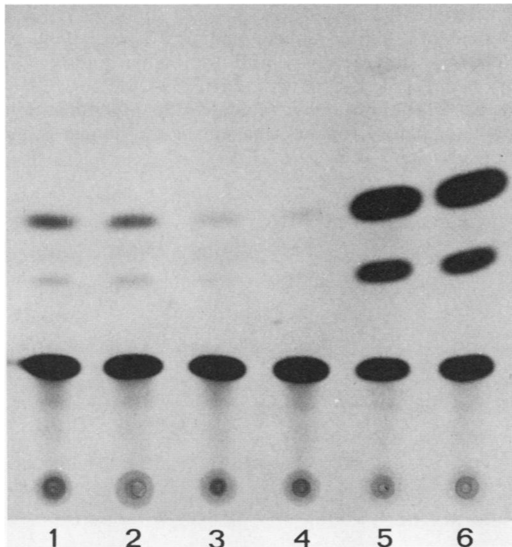

FIG. 5. Effect of interferon on CAT activity in HeLa cells transfected with different promoters. HeLa cells transfected with 10 μg of either plasmid pHPβ30CAT in the correct orientation (lanes 1 and 2) or reverse orientation (lanes 3 and 4) or with 1 μg of a plasmid pHP34SV40CAT, containing the simian virus 40 early promoter cloned in the *Sma*I site of plasmid pHP34ΔE4CAT (lanes 5 and 6) were treated with either the medium alone (lanes 1, 3, and 5) or with medium plus 250 IU of IFN-α8 per ml (lanes 2, 4, and 6) for 24 h. CAT activity was then determined 20 h later.

(10, 23). The 30-bp element which we have studied does not contain, however, either the positive regulatory domain (PRD-II) between positions −66 to −55 relative to the cap site or the negative regulatory domain (NRD-1) which is present downstream of this region (9, 33).

IFN treatment of HeLa cells transfected with the plasmid pHPβ30 CAT, which contains the 30-bp region of the human IFN-β promoter in either the correct or reversed orientation resulted in a twofold increase in CAT activity similar to that observed in cells transfected with the complete region of the IFN-β promoter between positions −282 to −37 (Fig. 4 and Table 2).

Our results show that IFN treatment alone has no detectable effect on the activity of the 30-bp region (−91 to −62) of the human IFN promoter which encompasses the PRD-I domain in either transfected Daudi or HeLa cells, even though IFN treatment potentiated the action of a virus inducer (Fig. 5). These results are in agreement with the observation that the PRD-I domain, when present as a single or even double copy of the sequence is insufficient to confer IFN inducibility upon a reporter gene (9).

It is of interest that the IRF-1 protein has also been shown in vitro to recognize elements within the IFN-response sequence of the mouse major histocompatibility antigen complex (23), the transcription of which is increased by IFN treatment (19). Enhanced production or activity of a transcriptional activator such as IRF-1 or an as yet unidentified regulatory protein in IFN-treated cells could explain the priming action of IFN on the expression of its own gene.

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