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Transient Expression of the Beta Interferon Promoter in Human Cells

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A human transient expression assay was used to examine the inducible transcriptional activation of beta interferon (IFN-β) and IFN-α1 promoters in a homologous cellular environment. Use of 293 cells, an adenovirus DNA-transformed human embryonic kidney cell line, permitted Sendai virus-inducible expression of IFN-β–*CAT* hybrid genes. Introduction of the simian virus 40 (SV40) enhancer 5′ or 3′ to the IFN-*CAT* gene increased basal (uninduced) levels of chloramphenicol acetyltransferase (CAT) activity; in one construct the SV40 enhancer – IFN-β regulatory region combination increased the induced CAT activity 50- to 100-fold, suggesting that this may be a generally useful inducible enhancer-promoter combination. No expression from the IFN-α–*CAT* hybrid gene was detected in 293 cells, indicating that human epithelioid cells lack a factor required for expression of the IFN-α promoter. However, when the IFN-α regulatory region was combined with the SV40 enhancer, a low level of inducible CAT activity was detected in the human transient system.

Human interferons (IFNs) represent a unique class of inducible cellular proteins with a wide range of antiviral, antiproliferative, and immune regulatory activities (18, 29). IFNs are classified into three antigenically distinct groups, designated IFN-α, IFN-β, and IFN-γ. The IFN-α group is encoded by two multigene families, IFN-α_I, consisting of at least 20 distinct genes or pseudogenes, and IFN-α_{II}, encoded by at least 6 genes (4, 18). IFN-β is encoded by a single gene (25), although a related, intron-containing IFN-β_2 has been described (16); IFN-γ is encoded by a single, intron-containing gene (11). Induction of many cell types with viruses, synthetic polyribonucleotides, or bacterial antigens leads to the secretion of IFNs (29); in general, fibroblastic and epithelial cells synthesize IFN-β, while peripheral blood leukocytes and lymphoid and myeloid cell lines produce a heterogenous mixture of IFN-α and IFN-β as a consequence of differential gene transcription (14).

Stable introduction of the human IFN-α1 or IFN-β gene into murine fibroblastic cell lines by permanent transformation has defined the *cis*-acting elements involved in IFN inducibility. A 46-nucleotide IFN-α1 promoter fragment from positions −109 to −64 conferred virus inducibility upon a heterologous rabbit β-globin gene (20, 26, 28). The sequences involved in poly(rI-rC) activation of the human IFN-β gene in mouse C127 cells are located −37 to −77 relative to the mRNA cap site and display the characteristics of an inducible enhancer element (8, 33); a study involving the use of mouse L929 cells identified a different IFN-β promoter fragment, spanning positions −117 to −40 relative to the mRNA cap site, required for virus inducibility (6). Recently, an elegant study has demonstrated that the IFN-β enhancer element is negatively regulated and has identified regions of the IFN-β promoter to which putative regulatory factors bind (7, 34). In addition to sequences required for transcriptional activation at the 5′ flanking end of the gene, sequences at the 3′ untranslated end of the gene and mRNA are involved in RNA stability and turnover (23, 24, 27).

Studies on the characterization of *cis*-acting sequences involved in human IFN gene regulation have been carried out almost exclusively with murine fibroblastic cells cotransformed with human IFN genes and a selectable marker (3, 6–8, 13, 20, 25, 28, 33, 35). The murine and human fibroblast cell systems, however, differ fundamentally in that after virus infection in mouse cells, IFN-α and IFN-β mRNAs and proteins are synthesized in an approximately equimolar mixture (15, 32), whereas in human fibroblasts, virus infection and double-stranded RNA treatment result in predominantly IFN-β_1 synthesis (14, 27). Therefore, with these differences in mind, we set out to develop a human transient expression system to examine the transcriptional activation of IFN-α and IFN-β genes in a homologous cellular environment and to ask whether different IFN genes are transcriptionally activated by common regulatory factors or whether expression of the transfected genes reflects the specificity of the human cell.

Production of endogenous IFN mRNA in human cells. To assess the expression of endogenous IFN genes in human cell lines after induction by Sendai virus, total RNA was isolated from various human cell lines at 5.5 h after induction and hybridized with ^{32}P-end-labeled IFN-β and IFN-α1 probes (Fig. 1). Analysis of RNA from Sendai virus-induced MG63, HeLa, and 293 fibroblasts (Fig. 1; see also Fig. 4) and two human myeloid cell lines, U937 and KG-1, identified IFN-β transcripts in all cells examined, but at widely variant levels; in addition, IFN-α1 was synthesized in U937 and KG-1 cells. No IFN-α1 was detected in HeLa or 293 cells, and the amount of IFN-α1 in MG63 cells was just above the level of detectability (about 5 copies per cell). Thus although fibroblastic or epitheloid cells produced variable amounts of IFN-β in response to Sendai virus infection, IFN-α_1 RNA either was not detectable (HeLa, 293) or was present at very low levels (MG63) in these cell types. The myeloid cells, on the other hand, synthesized both IFN-α1 and IFN-β RNA after virus induction. Introduction of the IFN-β and IFN-α1 promoters into 293 cells will therefore permit a comparison of the expression of genes with fibroblastic-epithelial (IFN-

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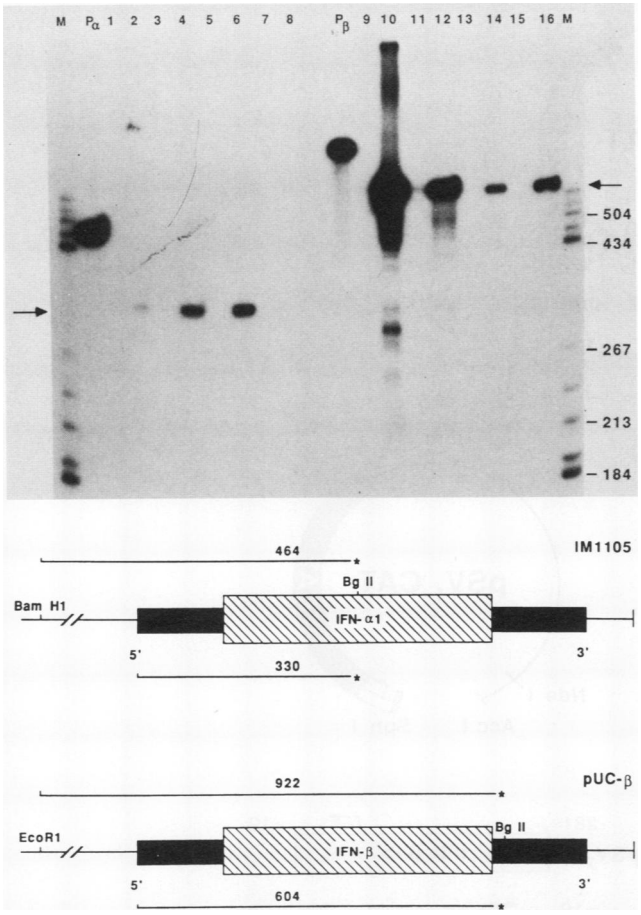

FIG. 1. Production of endogenous IFN-α1/IFN-β mRNA in human fibroblastic and myeloid cells after Sendai virus induction. The probes for mapping IFN-αl and IFN-β transcripts are indicated in the diagrams of the human IFN genes. Symbols ▨, protein-coding sequences; ▬, nontranslated 5′ and 3′ regions; ——, bacterial sequences. The distance (in nucleotides) between the ^{32}P-labeled 5′ end of the probe (marked with an asterisk) and the 3′ end is indicated above the IFN gene. The distance in nucleotides between the 5′ end of the probe and the cap site is indicated below the IFN gene. Total cellular RNA was isolated from uninduced (odd-numbered lanes) and induced (even-numbered lanes) cells at 5.5 h after initial Sendai virus infection and analyzed for IFN-αl mRNA (lanes 1 to 8) or IFN-β mRNA (lanes 9 to 16) by S1 mapping (23). Lanes: P_α, IFN-αl 464-bp probe alone; 1 and 2, MG63 cell RNA; 3 and 4, KG-1 cell RNA; 5 and 6, U937 cell RNA; 7 and 8, HeLa cell RNA; Pβ, IFN-β 922-bp probe alone; 9 and 10, MG63 cell RNA; 11 and 12, KG-1 cell RNA; 13 and 14, U937 cell RNA; 15 and 16, HeLa cell RNA; M, marker of pBR322 digested with *Hae*III and labeled with [γ-^{32}P]ATP.

β) or hematopoietic (IFN-α1) cell specificity in a human epithelial environment.

Enhancer-mediated expression of IFN-CAT plasmids in human fibroblasts. The parental chloramphenicol acetyltransferase (CAT) plasmids were constructed by removing either the entire simian virus 40 (SV40) promoter-enhancer combination (SV_0CAT) or most of the SV40 72-base-pair (bp) repeats (SV_1CAT) with the exception of 22 bp of one repeat; as described previously (9), expression of CAT activity in SV_1CAT-transfected 293 cells was only about 4% of the level observed in SV_2CAT-transfected cells (see Fig. 5); conversion of [^{14}C]chloramphenicol with lysates from SV_0CAT-transfected cells was only 2% of the control level. Promoter fragments from IFN-β and IFN-α_1 were subcloned into SV_1CAT and SV_0CAT vectors (Fig. 2), and in some plasmids the *Nco*I-*Pvu*II 233-bp SV40 enhancer-containing fragment (2, 12, 22) was placed 5′ or 3′ to the SV_0β-*CAT* hybrid gene in either the *Nde*I or the *Bam*HI site, respectively (Fig. 3). The transcriptional activity of the SV40–IFN-β enhancer-promoter combinations was analyzed by using 293 cells after they had been transfected with hybrid genes, induced with Sendai virus at 20 h after transfection, and harvested for CAT assay 20 h later. In this experiment SV_2CAT- and SV_0β-transfected lysates (170 μg) produced a relative induction of 0.96 and 41.7, respectively, although the absolute conversion of [^{14}C]chloramphenicol by induced lysates differed by a factor of 5.6 (28 versus 5%, respectively). The insertion of the SV40 enhancer either 5′ or 3′ to the IFN-β–*CAT* hybrid gene increased basal *CAT* expression 2.5- to 7.5-fold. After virus induction, relative CAT enzyme activity was increased 95-fold in $SV5^+$β-transfected lysates; insertion of the SV40 enhancer either in the opposite (−) orientation at the 5′ site or in its normal (+) orientation at the 3′ site produced only a 12-fold relative induction. Absolute conversion values for $SV5^+$β and SV_2CAT lysates (66.5 and 28.1%, respectively) suggested that transcription from the $SV5^+$β promoter was occurring at 2.4 times the rate of transcription from the SV40 promoter. Taking into consideration the cumulative synthesis over 62 h of CAT enzyme in SV_2CAT-transfected cells and the induced CAT synthesis over 24 h in $SV5^+$β cells, it appeared that the highly inducible $SV5^+$β enhancer-promoter combination functioned about five times better in human 293 cells than did the SV40 enhancer-promoter alone.

Kinetics of SV5β inducibility. The kinetics of the IFN-β promoter linked to the SV40 enhancer in 293 cells was examined relative to the endogenous IFN-β promoter. Cells were transfected with $SV5^+$β hybrid plasmid, infected with Sendai virus 20 h later, and assessed for accumulation of CAT activity at different times after induction (Fig. 4). By 6 h after virus infection CAT enzyme began accumulating, and the amount steadily increased during the next 14 h. The drop at 44 h may be due to degradation of accumulated CAT enzyme as a result of virus infection. Endogenous IFN-β mRNA was not measurable at 2 h after induction, but was identified by S1 mapping at 6 and 9 h (Fig. 4, inset). Analysis of transcripts synthesized from the SV5β plasmid demonstrated that before virus induction, mRNA was initiated downstream of the SV40 promoter fragment, which includes the enhancer- and Sp1-binding sites; after induction, however, transcripts initiated at the authentic IFN-β cap site were also identified (S. Xanthoudakis and D. Alper, unpublished data). CAT activity in SV_2CAT-transfected cells was first detectable at 8 h after transfection, accumulated in a linear manner, and was not affected by Sendai virus induction (data not shown), as expected for a constitutive enhancer-promoter combination. Therefore, the IFN-β–*CAT* construct was expressed with kinetics similar to that of the endogenous human IFN-β gene in 293 cells, and once the promoter was activated, CAT activity accumulated for about 14 h.

Transfected IFN-α1 promoter is not expressed in the transient assay. No detectable CAT enzyme activity was observed in SV_0α-transfected 293 lysates, either before or after virus induction (Fig. 3). The influence of the SV40 enhancer upon the inducibility of the IFN-α1 regulatory element was examined relative to similar IFN-β–*CAT* constructs in 293 cells by subcloning the SV40 enhancer adjacent to the SV_0α-*CAT* and SV_1α-*CAT* hybrid genes (Fig. 5). After

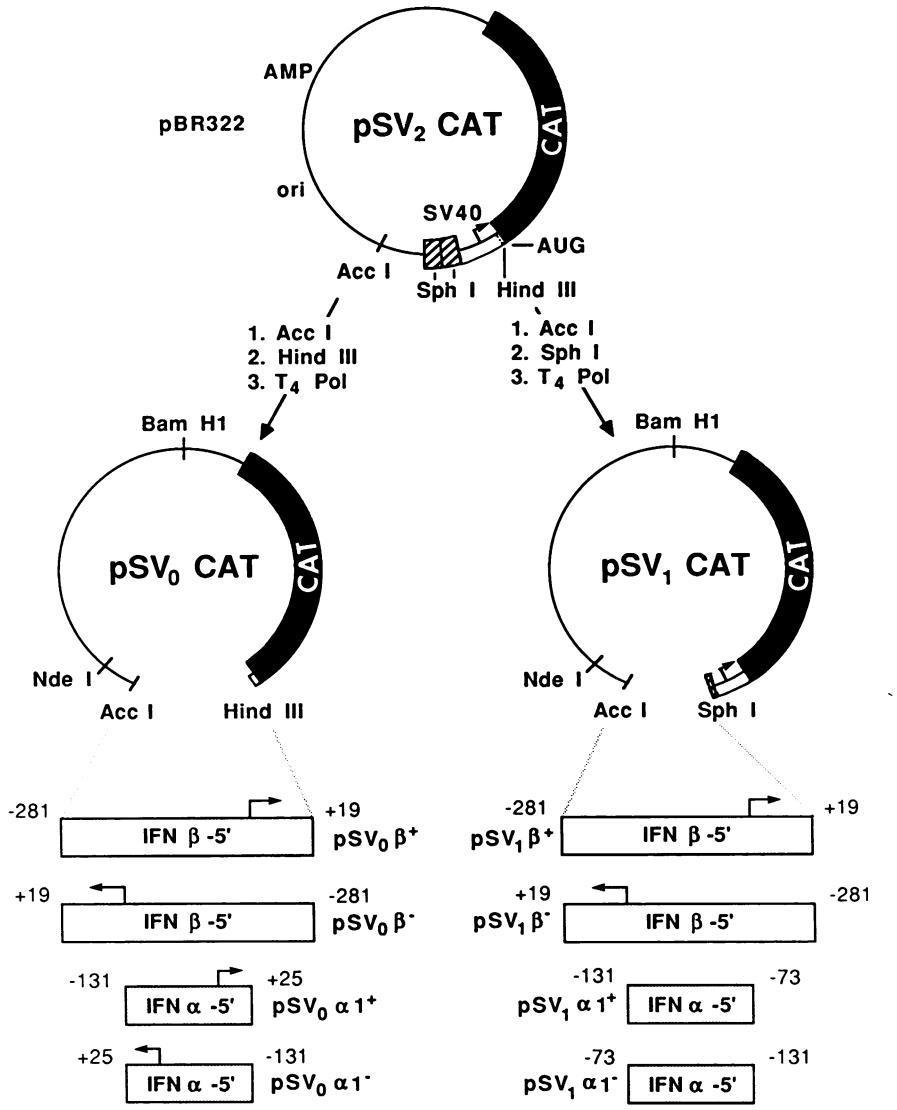

FIG 2. Construction of IFN-*CAT* plasmids. The structures of pSV_2CAT, pSV_1CAT, and pSV_0CAT (15) are illustrated; IFN-β and IFN-α_1 promoter fragments (illustrated below the SVCAT plasmids) were inserted into SV_1CAT and SV_0CAT vectors by blunt-end ligation. The IFN-β *Eco*RI-*Taq*I fragment and the IFN-α1 *Bam*HI-*Hin*fI fragment from IM1105 were cloned into SV_0CAT in both orientations; the same IFN-β fragment and the IFN-αI *Bam-Sau* 961 fragment were cloned into SV_1CAT in both orientations. The *Nde*I and *Bam*HI sites were used to insert the SV40 *Nco*I-*Pvu*II 233-bp fragment at the 5′ and 3′ ends of the IFN-*CAT* hybrid genes, respectively. Symbols: ▬, CAT enzyme coding sequences; ▭, SV40 promoter; ▨, 72-bp repeats. Transcription initiation sites and direction of transcription are indicated by the arrows on the SV40 or IFN promoter. Bacterial sequences including origin of replication and location of β-lactamase gene are shown by solid lines, ori, and AMP respectively. The translation initiation codon for the CAT protein (AUG) is the first initiation codon in the IFN-*CAT* hybrid genes.

induction, the $SV5^+\beta$ plasmid converted about five times more chloramphenicol than SV_2CAT did and produced a 54-fold relative induction; $SV_0\beta$-*CAT* displayed a 57-fold relative inducibility. Curiously, the construct with the IFN-β promoter in the opposite orientation was also 16-fold inducible, although the absolute conversion was about 4-fold lower than that by SV_2CAT. It has not been determined what makes up the transcriptional start site of this plasmid.

The juxtaposition of the SV40 enhancer fragment and the IFN-α regulatory element produced some inducible CAT activity with the $SV5^+\alpha^+$ and $SV5^-\alpha^+$ plasmids (4.5 and 7.0, respectively), but the percent conversions were four to five times lower than for SV_2CAT. Despite the low level of inducible IFN-α gene expression, these results indicate that the SV40 enhancer can complement the IFN-α element to overcome the block to transcriptional activation in 293 cells.

The choice of 293 cells in the development of a human transient system to analyze the inducible expression of the IFN promoter provides the advantage that transfected DNA is unusually stable in these cells (1) and that IFN-β is the predominant species synthesized after virus induction. Analysis of IFN-β–*CAT* promoter activation in 293 cells indicates that the transfected gene is expressed with kinetics similar to those for the endogenous IFN-β gene. Nonetheless, since 293 cells are transformed by adenoviral DNA and produce E1a proteins, it is necessary to consider interference with transfected gene expression by E1a; transfection studies, for example, have demonstrated that transcription from the

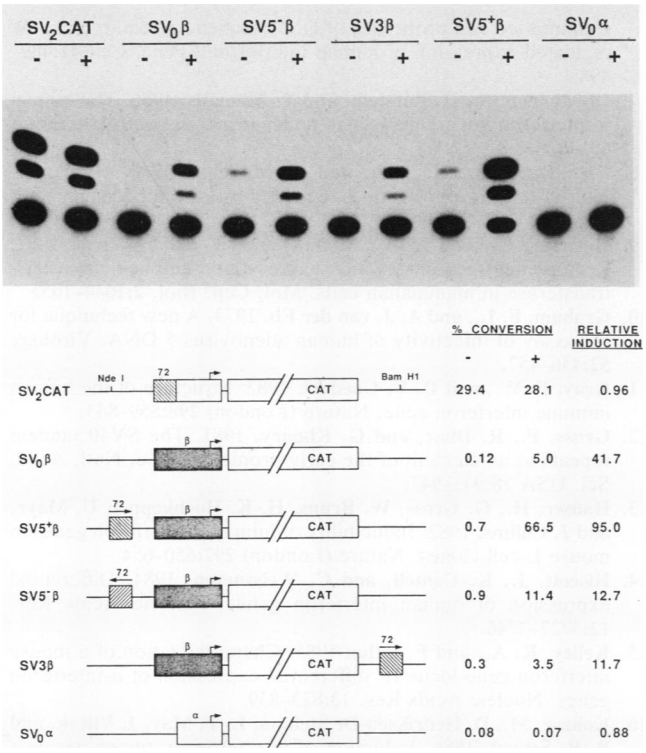

FIG. 3. Inducible expression of IFN-*CAT* hybrid genes in 293 cells and the influence of the SV40 enhancer. The illustrated IFN-*CAT* hybrid genes were transfected into subconfluent 293 cells by the calcium phosphate method (16) and, 20 h after transfection, either mock induced or infected with Sendai virus for 90 min. Cell lysates were prepared 20 h later, and 170 μg of protein (Bio-Rad protein assay) was analyzed in a 60-min CAT enzyme assay (15). The acetylated products of chloramphenicol were separated by thin-layer chromatography and visualized by autoradiography. The percent conversion was measured by counting the total radioactivity in 1- and 3-acetylchloramphenicol and dividing by the total radioactivity in the lane. Relative induction was determined by dividing percent conversion in the induced sample by that in the mock sample. Samples were analyzed in duplicate in two to four separate experiments. The schematic diagram illustrates the plasmids used in the experiment, and the adjacent table summarizes the percent conversion and relative induction. Symbols: −, mock-induced samples; +, Sendai virus-induced samples; ▨, SV40 233-bp enhancer. Arrows indicate the relative orientation of the 233-bp enhancer. The IFN-β and IFN-αl promoter fragments are identical to those in Fig. 2.

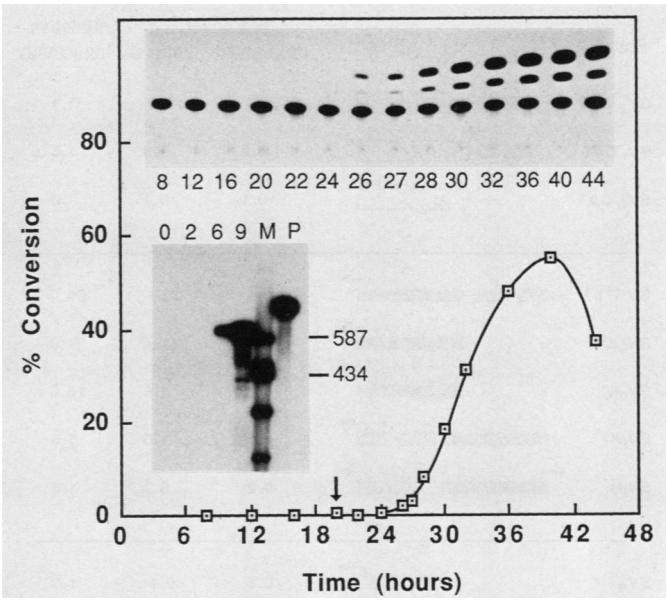

FIG. 4. Kinetics of activation of the IFN-β–*CAT* hybrid gene. The SV5⁺β plasmid was transfected into 293 cells and analyzed for the accumulation of CAT activity at various times after transfection and following virus infection. The induction of the endogenous IFN-β gene (shown in the inset) was analyzed by S1 mapping of total RNA (100 μg) by using the ^{32}P-end-labeled probe described in Fig. 1. The arrow indicates the time of Sendai virus infection (hours after transfection).

SV40 early promoter is repressed by adenovirus E1a proteins (17, 19, 30). In contrast, with this strain of 293 cells it has been found that SV40 T antigen is efficiently expressed as determined by indirect immunofluorescence, indicating that repression of the SV40 early promoter may not be absolute (J. Hiscott, unpublished data). The combined negative regulation imposed by the IFN-β promoter (7), together with the repression of the SV40 enhancer in the uninduced state, may contribute to the low background activity and high inducibility of the SV40–IFN-β enhancer-promoter combination.

To augment the expression of IFN-*CAT* hybrid genes, the SV40 enhancer was juxtaposed with the IFN-β promoter, and, in effect, the influence of a strong constitutive enhancer upon an inducible enhancer was assessed by these experiments. The evidence presented here supports the idea that negative regulation imposed by the IFN-β promoter in the uninduced state is dominant over the SV40 constitutive enhancing influence; nonetheless, the SV40 enhancer was able to modestly increase the basal uninduced level of expression from the IFN-β promoter (2- to 10-fold). After induction by virus, the presence of the SV40 enhancer increased the absolute and relative induction of *CAT* expression, but in an orientation- and position-dependent manner.

Human 293 cells contain the necessary *trans*-acting factors to produce inducer-specific activation of the IFN-β gene; however, the lack of expression of the IFN-α–*CAT* hybrid gene indicates that a distinct factor(s) not present in 293 cells is required for activation of a myeloid cell-specific IFN-αl promoter. The IFN-αl promoter contains all the sequence requirements for regulated expression in mouse fibroblasts (20, 26, 28) and yet is not sufficient for expression of the extrachromosomal IFN-αl promoter in 293 or HeLa cells. The endogenous IFN-αl promoter is also not activated by Sendai virus induction in these cells, reflecting a fundamental difference between the human and murine systems, in which both IFN-αl and IFN-β are transcriptionally activated by virus induction. A human transient expression system will provide a convenient means to examine in vivo interactions between common regulatory factors required for transcriptional selectivity, distinct IFN-binding proteins, and the DNA sequences involved in inducible IFN gene expression.

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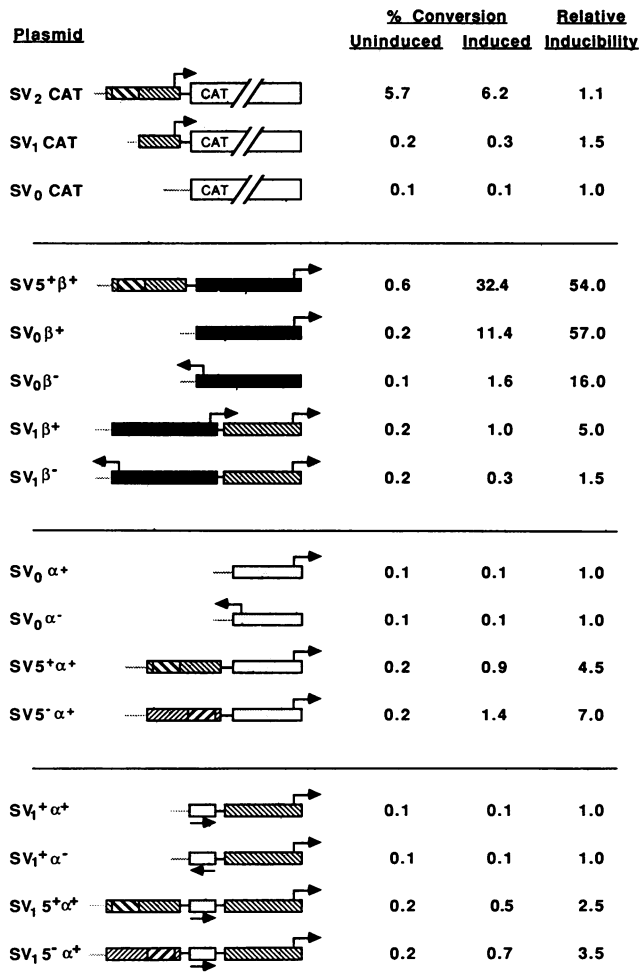

FIG. 5 Comparison of IFN-β and IFN-α_1 inducible expression in 293 cells. The plasmids illustrated on the left side of the figure were transfected into 293 cells, and Sendai virus-inducible CAT activity was analyzed as described in the legend to Fig. 3; the results are expressed as percent conversion and relative inducibility of induced and uninduced duplicate samples. The arrows above the boxes indicate transcription initiation sites. Symbols: ▬, IFN-β regulatory region; ▭, IFN-αl; ▨, SV40 *Nco*I-*Pvu*II 233-bp fragment containing the enhancer element (▭). The relative orientation of SV40 enhancer is indicated by the location of enhancer box; the IFN-α1 enhancer element (−131 to −73) and orientation are indicated by the stippled box and the arrow beneath the box.

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