Difference in the Production of Human Interferon-α and -β in Mouse Cells

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SUMMARY

Human interferon-α, and interferon-β genes with their flanking regions were introduced into mouse LMTK− cells. Although transfected cells contained the interferon genes with a similar copy number and produced a similar amount of interferon-specific mRNA, cells containing the human interferon-β gene secreted about 10 times more human interferon than cells transfected with the human interferon-α gene. When the coding region of the interferon-β gene was replaced by that of the interferon-α1 gene (hybrid interferon β/α gene), the human interferon production of transfected cells fell by approx. one order of magnitude. These results show that in the case of exogenous interferon genes a translational or post-translational mechanism might significantly affect the final level of human interferons, resulting in higher titres of interferon-β than of interferon-α.

INTRODUCTION

Human interferon-β1 (HuIFN-β1) and murine (Mu) IFN-β are each encoded by a single gene (Ohno & Taniguchi, 1981; Higashi et al., 1983). Human IFN-α genes have been shown to represent a family with at least 15 functional members (Nagata et al., 1980; Goeddel et al., 1981; Allen & Fantaz, 1980; Henco et al., 1985), and similarly several MuIFN-α genes have been discovered (Zwarthoff et al., 1985). IFN-β and IFN-α genes can be activated mainly in fibroblasts and in leukocytes, respectively, and therefore the production of IFNs is tissue-specific (Burke, 1981). The IFN genes are regulated transcriptionally (Raj & Pitha, 1983; Kelley & Pitha, 1985); however, Hiscott et al. (1984) found that IFN-α and IFN-β production was not always proportional to the level of IFN mRNA in human peripheral blood leukocytes, lymphoblastoid, HeLa and human fibroblastoid cells. This result suggests that IFN synthesis might also be regulated at the post-transcriptional level.

Here we have studied the differential expression of IFN genes in mouse fibroblasts by using transfected DNA clones encoding HuIFN-α1, HuIFN-β and hybrids of them, and have shown that in virus-induced cells, the levels of IFN-α and IFN-β mRNAs are comparable, but that the production of IFN-β is significantly higher than that of IFN-α. This phenomenon is not linked to the promoter of the IFN-β gene; rather, the structural gene seems to be important.

METHODS

Construction of molecular clones. All plasmids were constructed by using standard recombinant DNA techniques (Maniatis et al., 1982). Plasmid IFN-α (gift of K. Nielsen, Interferon Research Laboratory, Hjørring, Denmark) contained the chromosomal gene for HuIFN-α1 in a 3.4 kb HindIII–BamHI fragment at those sites of pBR322 (Fig. 1a). Plasmid IFN-β was derived from pBlIFN210 (gift of T. Maniatis, MIT, Cambridge, Mass., U.S.A.; Zinn et al., 1983) by excision of the HuIFN-β gene in a 1.9 kb HindIII–EcoRI fragment, and its insertion into the HindIII and EcoRI sites of pUC18 (Fig. 1a).

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The hybrid IFN genes were constructed from pIFN-α1 and pIFN-β (Fig. 3). First, the promoter sequence of the IFN-β gene was ligated to the coding region of the IFN-α gene to yield pIFN-β/α. In detail, a HindIII linker was ligated to the NcoI site of pIFN-β resulting in pIFN-β. This procedure removed the NcoI–HindIII fragment of pIFN-β containing the coding region of the IFN-β gene. Then, the HindIII–EcoRI fragment of the IFN-β promoter from pIFN-β-pro was inserted into the identical sites of pAT153 to yield pATIFN-β-pro. The Stul site of the IFN-α gene was first converted to a HindIII site by addition of a linker, then the HindIII–BamHI fragment of this plasmid, containing the coding region of the IFN-α gene was inserted between the HindIII and BamHI sites of pATIFN-β-pro to yield pIFN-β/α.

A similar approach was used to construct pSVIFN-α1. The Stul–BamHI fragment of pIFN-α1 was inserted using a synthetic HindIII linker into the HindIII–BamHI sites of pSV2Neo (Southern & Berg, 1982), resulting in pSVIFN-α1. In this plasmid the IFN-α gene is under the control of the simian virus 40 (SV40) promoter.

The IFN-α/β hybrid gene was constructed by ligation of the HindIII–Stul fragment of pIFN-α1 and the NcoI–HindIII fragment of pIFN-β (Fig. 3).

**Cell cultures and transformations.** Human HEp-2, bovine MDBK, mouse L929 and LMTK- cells were grown in monolayer cultures in Dulbecco’s MEM (DMEM) supplemented with 10% foetal calf serum, 50 international units (IU)/ml penicillin and 50 µg/ml streptomycin. Approximately 5 x 10^4 LMTK- cells were seeded per 60 mm Petri dish, 1 day before transformation. Introduction of plasmids into the cells was carried out by the standard calcium phosphate precipitation technique (Graham & van der Eb, 1973), using 10 µg plasmid DNA per Petri dish. Plasmids containing the human IFN-β, IFN-α or hybrid genes (5 µg) were cotransfected with plasmid pAG60 (5 µg) containing the neo gene, which encodes a bacterial phosphotransferase (provided by F. Colbere-Garapin, Institut Pasteur, Paris, France; Colbere-Garapin et al., 1981). Cells were incubated with the DNA precipitate for 4 h, then the monolayers were treated with 10% DMSO for 2 min, washed twice and further incubated in DMEM.

Selection for transformants was started 48 h after transfection, when the cells were transferred to 170 mm Petri dishes. Transformants were selected in medium supplemented with 500 µg/ml of the antibiotic G418 (Gibco). After 3 weeks, clones were grown into mass cultures in the presence of 125 µg/ml G418. Clones containing pIFN-α1 were designated as clone IFN-α1, clone IFN-α2, etc. A similar designation was used for clones containing pIFN-β (clones IFN-β1), pIFN-β/α (clones IFN-β/α1), pIFN-α1/β (clones IFN-α1/β) and pSVIFN-α1 (clone pSVIFN-α1). Cells were passaged with trypsinization by dilution at a 1:4 ratio.

**IFN induction and assay.** Approximately 5 x 10^5 cells were seeded in 35 mm Petri dishes and the cells were induced with 25 to 200 haemagglutination units per ml Sendai virus, and the following day culture medium was collected. A c.p.e. reduction assay, using vesicular stomatitis virus as a challenge virus was employed to determine the IFN titres.

Human IFN-α1 was titrated on MDBK cells. Its specific antiviral activity is 5 x 10^8 IU/mg protein on these cells (Tymms et al., 1986; M. I. Toth et al., unpublished results). Human IFN-β was titrated on human foreskin fibroblast or HEP-2 cells by using the human IFN-β reference reagent (National Institutes of Health, Bethesda, Md., U.S.A.). The specific activity of pure human IFN-β is 5 x 10^8 IU/mg protein (Novick et al., 1983). Since the specific activities of IFN-α1 and IFN-β were essentially the same in our cell systems, we could directly compare the amounts of human IFN-α1 and IFN-β synthesized in mouse cells. Nevertheless in this study we used IFN protein concentrations (pg/ml) calculated from the specific activities and the antiviral titres of human IFNs.

Mouse IFN was titrated on L929 cells and titres were expressed in IU/ml by using mouse IFN reference reagent (National Institutes of Health).

In neutralization tests, IFN samples were incubated with antiserum to human leukocyte IFN (EGIS Pharmaceutical Company, Hungary), HuIFN-β, MuIFN-β (Lee Biomolecular, San Diego, Ca., U.S.A.) or MuIFN-α/β (National Institutes of Health) at 37 °C for 30 min, then titrated on the appropriate cell line.

**Southern and Northern blotting.** High M<sub>c</sub> DNA was isolated and digested with restriction endonucleases by standard methods (Maniatis et al., 1982). Cytoplasmic RNA was isolated from induced and uninduced cells 6 h after induction by Sendai virus as described (Toth et al., 1987). DNA and RNA were transferred onto nitrocellulose membranes by the method of Southern from 0.7-1.5% agarose gels, respectively (Southern, 1975).

Hybridization was carried out at 42 °C in a hybridization solution containing 50% formamide and 10% dextran sulphate and in the case of Northern blots in the presence of 0.1% SDS (Wahl et al., 1979). Washing of filters was carried out under stringent conditions (0.1 x SSC, 0.1% SDS at 68 °C). Agarose gel-purified plasmid fragments were labelled with 32P by nick translation.

**RESULTS**

Plasmids IFN-α1 and IFN-β, containing the human chromosomal IFN-α1 and IFN-β genes (Fig. 1a), were introduced into LMTK- cells with the pAG60 marker plasmid by the standard calcium phosphate precipitation method. Transformation frequencies in five independent
Fig. 1. (a) Restriction map of plasmids pIFN-α1 and pIFN-β. Open boxes indicate flanking regions, hatched boxes represent IFN sequences and wavy lines show plasmid sequences. (b) Southern blot analysis of DNAs of transfected mouse cells. High M, DNA was extracted from each cell line, 10 μg DNA was cleaved with MspI for IFN-α clones (lanes 1 to 3) or EcoRI and HindIII for IFN-β clones (lanes 4 and 5) and electrophoresed on agarose gels. Nitrocellulose filters were hybridized with the 1.3 kb EcoRI fragment from pIFN-α1 and the 1.9 kb EcoRI–HindIII fragment from pIFN-β. Lane 1, clone IFN-α6; lane 2, IFN-α1; lane 3, IFN-α13; lane 4, IFN-β1; lane 5, IFN-β2. (c) Northern blot analysis of IFN-specific RNA of clone IFN-α1 (lanes 1 and 2) and IFN-β1 (lanes 3 and 4). The 707 bp IFN-α1 probe (StuI–EcoRI fragment of pIFN-α1) and the 624 bp IFN-β probe (Neol–BglII fragment of pIFN-β) were mixed in equimolar ratio and labelled by nick translation. Arrows show the 865 bp IFN-α1 and the 837 bp IFN-β mRNA transcripts. Cells represented in lanes 2 and 4 were induced with Sendai virus; those in lanes 1 and 3 were not induced.

experiments ranged from $2 \times 10^{-3}$ to $4 \times 10^{-3}$. Several clones were isolated, and cells were maintained in the presence of 125 μg/ml G418.

Approximately 50% of both IFN-α and IFN-β clones at early (five to eight) passage levels responded to virus induction by producing 50 to 1000 pg/ml HuIFN. In our assay systems 1 pg/ml concentration of both HuIFN-α1 and HuIFN-β corresponded to 0.5 IU/ml antiviral activity (see Methods). The MuIFN in the culture supernatants did not interfere with the HuIFN assays, since the MDBK and HEp-2 cells used for HuIFN titration are insensitive to MuIFN (Table 1).

We proved by using anti-HuIFN-α and anti-HuIFN-β sera that the antiviral activity of culture supernatants of Sendai virus-induced cells containing IFN-α and IFN-β clones were indeed due to the presence of HuIFN-α and HuIFN-β (data not shown). Uninduced cells produced neither HuIFN nor MuIFN in detectable quantities.
Fig. 2. Human (—) and mouse (---) IFN production of transformed mouse cell lines. Transformed cells containing (a) the human IFN-α gene (○, clone IFN-α1; □, clone IFN-α4; ●, clone IFN-α6; △, clone IFN-α13; ■, clone IFN-α21; ○, clone IFN-α21; ▽, LMTK cells) or (b) the IFN-β gene (●, clone IFN-β1; ○, clone IFN-β2; ▲, clone IFN-β9; △, clone IFN-β10B; ■, clone IFN-β10G; ◐, clone IFN-β11; ▽, clone IFN-β12) were induced with 0 to 200 haemagglutinating units (HAU)/ml Sendai virus, and 24 h later HuIFN concentrations were determined on MDBK (HuIFN-α) and HEp-2 (HuIFN-β) cells, and expressed in pg/ml. In these assays 1 pg HuIFN corresponded to 0.5 IU antiviral activity (Methods). MulIFN titres were determined on L929 cells.

Table 1. Activities of human IFN-α, IFN-β and mouse IFN-α/β on different cell lines

<table>
<thead>
<tr>
<th>IFN</th>
<th>Origin of IFN</th>
<th>IFN concentration pg/ml (%)*</th>
<th>Antiviral activity (IU/ml) on L929 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>HuIFN-α</td>
<td>Clone SVIFN-α†</td>
<td>11640 (100)</td>
<td>&lt;8</td>
</tr>
<tr>
<td></td>
<td>Clone IFN-α21</td>
<td>136 (100)</td>
<td>&lt;8</td>
</tr>
<tr>
<td>HuIFN-β</td>
<td>Fibroblast‡</td>
<td>6720 (2)</td>
<td>332000 (100)</td>
</tr>
<tr>
<td></td>
<td>Clone IFN-β1</td>
<td>&lt;10</td>
<td>880 (100)</td>
</tr>
<tr>
<td>MulIFN-α/β</td>
<td>L929 cell¶</td>
<td>&lt;10</td>
<td>12800 (100)</td>
</tr>
<tr>
<td></td>
<td>LMTK cells§</td>
<td>&lt;10</td>
<td>23200 (100)</td>
</tr>
</tbody>
</table>

* One pg corresponds to 0.5 IU; the highest value for each sample is taken to be 100%.
† The HuIFN-α gene is under the control of the SV40 early promoter.
‡ Human fibroblast cells were induced by poly(rI):poly(rC) and IFN was purified to a specific activity of $10^7$ IU/mg protein.
§ ND, Not done.
¶ Cells were stimulated by Sendai virus and IFN was purified to a specific activity of $10^7$ IU/mg protein.
§§ IFN was produced by Sendai virus-stimulated cells.

The presence of HuIFN gene sequences was identified in all IFN producer clones by dot blot analysis, and in some cases by Southern blotting (Fig. 1b). No difference in the copy number of transfected IFN-α1, and IFN-β genes was found. Similarly, no difference was found in the steady state level of IFN mRNA in clones containing the IFN-α1, or IFN-β genes (see clones IFN-α21.
Regulation of IFN genes

Fig. 3. Structure of hybrid IFN genes ( ). IFN-α and IFN-β gene sequences. Asterisks at the HindIII linker show homology with the appropriate part of the IFN-α gene.

Fig. 4. HuIFN ( ) and MuIFN ( ) production of transformed mouse cells containing the hybrid IFN-β/α gene ( , clone IFN-β/α4; O, clone IFN-β/α8; A, clone IFN-β/α9E; Δ, clone IFN-β/α101; ◻, clone IFN-β/α10II; □, clone IFN-β/α10D). Cells were induced with 0 to 200 HAU/ml Sendai virus, and 24 h later IFN concentrations were determined as described in the legend of Fig. 2.

and IFN-β; Fig. 1c). However IFN-β producer clones secreted consistently about 10 times more IFN than IFN-α producers (Fig. 2). These observations raised the possibility that HuIFN-α and HuIFN-β synthesis is regulated differently at the post-transcriptional level, at least in our transformed mouse cells. To test this assumption by another means, we constructed hybrid genes in which the structural gene or the promoter of the HuIFN-α gene was replaced by the appropriate part of the HuIFN-β gene (Fig. 3). Plasmid IFN-β/α contains a fusion gene comprising IFN-β sequences from -210 to -5 joined via a synthetic HindIII linker to IFN-α.
sequences from \(-2\). Plasmid IFN-\(\alpha/\beta\) contains a fusion gene comprising IFN-\(\alpha\) sequences from \(-675\) to \(-3\) joined to IFN-\(\beta\) sequences from \(-8\).

We analysed the IFN secretion of 10 clones containing the IFN-\(\alpha/\beta\) gene (identified by dot blot hybridization), but no IFN producer clone was found. At the same time four clones, out of 11 transformed by pIFN-\(\beta/\alpha\), produced IFN-\(\alpha_1\). These clones, similarly to the IFN-\(\alpha\) clones, showed a low level of IFN-\(\alpha_1\) secretion (Fig. 4).

**DISCUSSION**

The IFN system is an attractive model to study the tissue-specific regulation of gene expression. For example, lymphocytes and lymphoblasts produce predominantly IFN-\(\alpha\), which is in contrast to fibroblast cells which secrete mainly IFN-\(\beta\). In many cases the preferential production of IFN-\(\alpha\) or IFN-\(\beta\) was the consequence of the selective expression of the corresponding IFN gene demonstrated both by the analysis of the relative mRNA levels and by the rate of transcription (Raj & Pitha, 1983; Kelley & Pitha, 1985). However, Hiscott et al. (1984) reported that in some cases IFN production was not proportional to IFN-\(\alpha\) and IFN-\(\beta\) mRNA levels in human peripheral blood lymphoblasts, HeLa and human fibroblast cells. Here we compared the expression of transfected HuIFN-\(\alpha_1\), HuIFN-\(\beta\) and hybrids of their genes in mouse cells.

Human IFN genes have been introduced into mouse cells by several research groups. In cells transfected with the HuIFN-\(\beta\) gene, both IFN-specific mRNA and IFN were synthesized (Canaani & Berg, 1982; Hauser et al., 1982; Maroteaux et al., 1983; Tavernier et al., 1983); however, in cells transfected with the HuIFN-\(\alpha_1\) gene only IFN-specific mRNA, but not the IFN itself, could be detected upon induction by Sendai virus (Mantei & Weissmann, 1982). The low sensitivity of the IFN detection system might explain this observation, since the relatively insensitive human WISH cells were employed for assaying HuIFN-\(\alpha_1\) (Weck et al., 1981).

We used both HuIFN-\(\alpha_1\) and HuIFN-\(\beta\) genes in the same system and found both IFN-\(\alpha\) and IFN-\(\beta\) production, but clones containing the HuIFN-\(\beta\) gene produced consistently about 10 times more IFN than cells transformed with HuIFN-\(\alpha_1\) gene (Fig. 2). The difference in the IFN-\(\alpha\) and IFN-\(\beta\) titres was not the consequence of a difference in the copy number of the genes or different mRNA levels (Fig. 1).

In another type of experiment, we replaced the structural gene of IFN-\(\beta\) for that of IFN-\(\alpha_1\), which resulted in a drop in the IFN titres. Taking these results together, it seems that the coding region of the HuIFN-\(\beta\) gene is responsible for the relatively high IFN production in transformed mouse cells. We assume that in the case of exogenous IFN genes, translational or post-translational mechanisms (efficiency of translation, processing and secretion of IFN) significantly affect the final level of HuIFNs, resulting in a higher IFN-\(\beta\) titre in mouse cells. In contrast, endogenous IFN genes are regulated mainly transcriptionally, since preferential production of HuIFN-\(\beta\) and MuIFN-\(\beta\) in human and mouse fibroblast cells seems to be the consequence of the preferential expression of the IFN-\(\beta\) gene (Raj & Pitha, 1983; Kelley & Pitha, 1985).

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