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The expression of human interferon alpha genes

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We have determined the levels of mRNAs for IFN-β, IFN-γ and various α-IFNs (IFN-α1, -α2, -α4, -α5, -α6, -α7, -α8 and -α14) in normal and leukaemic human blood leucocytes and several cell lines induced in different fashions. The ratio of α to β IFN transcripts varied greatly, depending on the cell type. The levels of the individual IFN-α RNAs were very different: IFN-α1, -α2 and -α4 RNAs constituted the major fraction of the IFN-α transcripts measured, while IFN-α6, -α7, -α8 and -α14 were minor components in normal, induced leucocytes. Moreover, there was a striking difference in the proportion of individual IFN-α mRNA species in different cell types, in particular between normal and leukaemic cells; for example all cases of myeloblastic leukaemia examined showed a high expression of IFN-α14. Use of different induction protocols did not significantly affect the proportion of IFN mRNAs.

Analysis of the human IFN-α1 gene by reversed genetics led to the identification of a segment of 5′ flanking sequence between positions 117 and 68 upstream of the cap site which is required for inducibility by virus.

Introduction

Human interferons (IFNs) are classified into three antigenically distinct groups, designated IFN-α, IFN-β and IFN-γ (Stewart 1979). The α-IFNs are encoded by a multigene family consisting of at least 13 non-allelic and 8 allelic members (Nagata *et al.* 1981; Goeddel *et al.* 1981; Weissmann *et al.* 1982); β- and γ-IFNs are each encoded by a single gene (Ohno & Taniguchi 1981; Tavernier *et al.* 1981; Gray & Goeddel 1982; Taya *et al.* 1982).

None of the IFN genes are expressed at detectable levels under normal conditions; induction of appropriate cells with virus or double-stranded RNA leads to the transient synthesis of α- or β-IFN, or both mRNAs and IFNs (Stewart 1979). IFN-γ is synthesized by T lymphocytes following exposure to immune stimuli or to mitogens (Wheelock 1965, Wietzerbin *et al.* 1977).

(a) *Expression of IFN genes in vivo*

Peripheral blood leucocytes (Cantel *et al.* 1981), permanent lymphoblastoid cell lines (Finter & Bridgen 1977) and human fibroblasts (Billiau *et al.* 1977; Havell & Vilček 1972) have been important sources of IFN for research and clinical studies. In response to induction, leucocytes and lymphoblastoid cells produce a heterogeneous mixture of IFNs, as judged by various analytical methods (Allen & Fantes 1980; Levy *et al.* 1981). While some of this heterogeneity may be due to post-translational modification of IFNs (Levy *et al.* 1981; Knight 1976; Bridgen *et al.* 1977), it is clear that several different gene products are represented in preparations derived from leucocytes and lymphoblastoid cell lines (Nagata *et al.* 1981; Goeddel *et al.* 1981; Allen & Fantes 1980; Levy *et al.* 1981). The ratio of IFN-α to IFN-β depends on the cell type

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used for induction and at least in one case may vary with the nature of the inducer (Havell *et al.* 1978). We undertook to determine whether the different species of IFN-α are always expressed in the same ratio, or whether this too may depend on the nature of the cell and its induction.

The levels of mRNAs for various human α-IFNs, IFN-β and IFN-γ in normal and leukaemic human peripheral blood leucocytes, lymphoblastoid (Namalwa), HeLa and fibroblastic cells, induced in different fashions were determined by an S_1 assay under conditions that gave specific and quantitative results (Hiscott *et al.* 1984).

(i) *IFN mRNA in peripheral blood leucocytes*

(a) *Unfractionated buffy coat leucocytes.* IFN mRNAs were measured in pooled buffy coat leucocytes at various times after Sendai virus infection. Figure 1 shows that IFN-α and IFN-β mRNA reached a maximum steady state level of about 6000 copies per cell (average) at 6 h
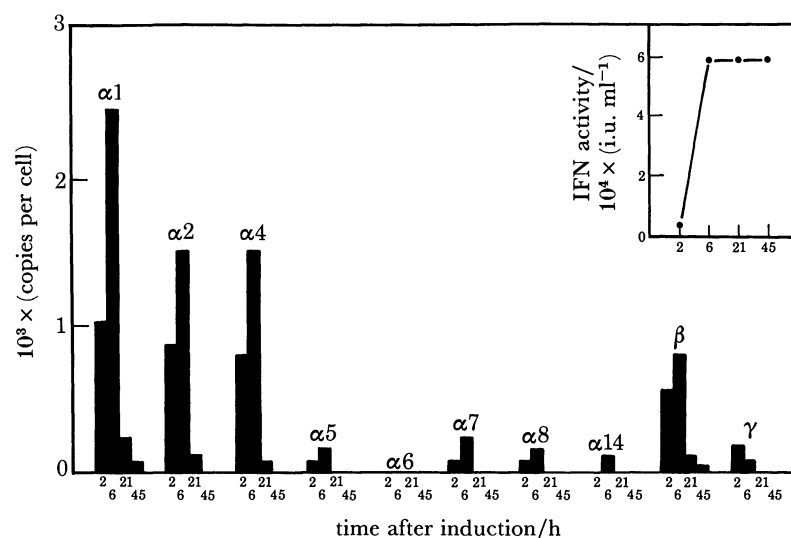

FIGURE 1. IFN transcripts in Sendai virus-induced buffy coat leucocytes. Buffy coat leucocytes were infected with Sendai virus (Cantell *et al.* 1981), samples were taken at 2, 6, 21 and 45 h, the antiviral activity of the supernatants was determined and poly(A)$^+$ RNA was isolated (all by K. Cantell in Helsinki) and subjected to quantitative S_1 mapping (Hiscott *et al.* 1984). Inset: antiviral activity as a function of time. From Hiscott *et al.* (1984).

post-infection (p.i.), and declined to 300 copies by 21 h p.i. IFN-α1, -α2 and -α4 mRNAs represented the major species, accounting for 35, 20 and 20%, respectively, of the IFN RNAs measured, while the transcripts of the IFN-α5, -α7, -α8, -α14 and IFN-β genes accounted for 2–15% of the total each. No IFN-α6 transcripts were detected at any time. IFN-γ mRNA was present at 30 and 15 copies per cell (average) at 2 and 6 h p.i., respectively (figure 1), and is likely the consequence of a mixed leucocyte reaction (Katz & Benacerraf (1974)). As most if not all of the IFN-α and IFN-β is produced by adherent mononuclear cells (see below), which constitute about 5–15% of the leucocyte preparation, the number of cognate mRNAs per producing cell may be as much as 20 times that indicated as average value.

(b) *Fractionated, normal blood leucocytes.* Leucocytes from normal donors were fractionated as indicated in figure 2. The fraction enriched for adherent cells (comprising the monocytes) produced the highest level of IFN-α mRNA after induction, while the granulocyte- and
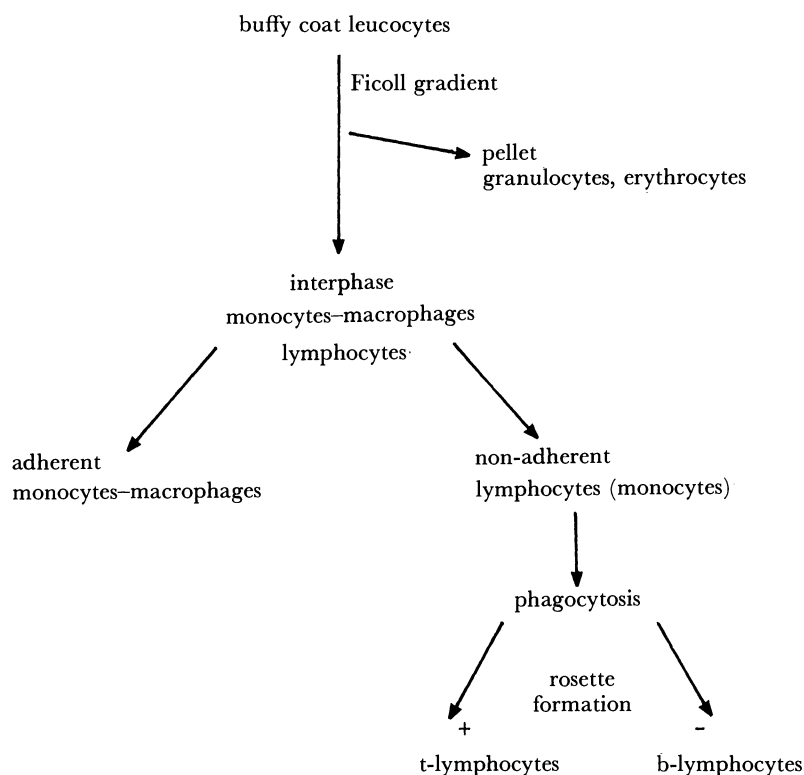
FIGURE 2. Fractionation of buffy coat leucocytes. Buffy coats were formed from heparinized blood for 1 h at 37 °C and isolated as described (Cantell *et al.* 1981) with some minor differences. Leucocytes were diluted 1:1 with PBS, layered over Ficoll and centrifuged 20 min at 2000 rev min^{-1} (Boyum 1968). Cells from the interphase and pellet were washed twice with PBS and interphase cells were incubated for 1 h at 37 °C in RPMI plus 10 % FCS in 10 cm plastic Petri dishes. Non-adherent cells were rinsed off with PBS and incubated in RPMI containing 1 µg ml^{-1} carbonyl iron at 37 °C for 1 h with shaking. Phagocytic cells were repeatedly swept to the edge of the dish with a magnet and removed (Yam *et al.* 1971). Non-adherent, non-phagocytic cells were incubated at 10^7 cells per millilitre in RPMI containing 0.01 volumes sheep erythrocytes for 4 h at 4 °C (Kaplan & Clark 1974). Rosetting cells were separated from non-rosetting cells or Ficoll as described above, erythrocytes were lysed with 0.83 % ammonium chloride and non-rosetting (interphase) cells were rinsed in PBS.

lymphocyte-enriched fractions gave little IFN-α transcripts (table 1). The expression pattern of the monocyte-enriched fraction (figure 3*a*) was similar to that of the buffy coat (figure 1); the major transcripts were α1 and α2, in a ratio of about 2.5. However, in contrast to the results with the buffy coat leucocytes, there was little IFN-α4 mRNA. It is not yet clear how the lack of IFN-α4 transcripts is to be accounted for. The buffy coats used in the experiment of figure 1 had been prepared and induced (Hiscott *et al.* 1984) by K. Cantell in Helsinki, while all other leucocytes were obtained and processed in Zürich by a somewhat different protocol. Subsequent experiments suggest that the absence of IFN-α4 mRNA was not due to fractionation of the buffy coat or to variations in the induction protocol. It seems that the critical parameter is the source of leucocytes or the method of preparing them. PHA treatment of different fractions did not induce IFN-α or IFN-β mRNA, but induced IFN-γ and IFN-γ mRNA in non-adherent, E-rosetting lymphocytes (Table 1).

(*c*) *Fractionated, leukaemic blood leucocytes.* Mononuclear cells were isolated from a variety of leukaemias by Ficoll gradient centrifugation, and the IFN-α transcripts determined with or without viral induction. As shown in figure 3*b*, mononuclear cells from patients with acute myeloblastic leukaemia showed drastic differences in their RNA patterns compared with

TABLE 1. IFN PRODUCTION BY FRACTIONATED, INDUCED BUFFY COAT LEUCOCYTES

fraction	differential analysis (percentage of cell population)		antiviral activity (i.u. per 10^7 cells) IFN α/β	IFN γ
leukophoresis	lymphocytes	57	10000	300
	monocytes	32		
	granulocytes	11		
pellet	granulocytes	81	200	0
	lymphocytes	13		
	monocytes	6		
interphase	lymphocytes	57	30000	1000
	monocytes	43		
non-adherent	lymphocytes	81	15000	3000
	monocytes	16		
	granulocytes	3		
non-phagocytic	lymphocytes	95	1500	5000
	monocytes	3		
	granulocytes	2		
adherent	monocytes	89	50000	0
	lymphocytes	11		
	(esterase+)	85		

Fractionated peripheral blood leucocytes, isolated as described in figure 2, were incubated at 2×10^6 cells per millilitre in the presence of Sendai virus (200 HAU ml^{-1}) or PHA (5 µg ml^{-1}) for 8 h and 36 h, respectively. Supernatants were analysed for antiviral activity as described and the results expressed relative to a IFN-α2 standard that had been titred against an international standard. The cell composition of the fractions was determined by differential analysis after May-Grünwald Giemsa staining.
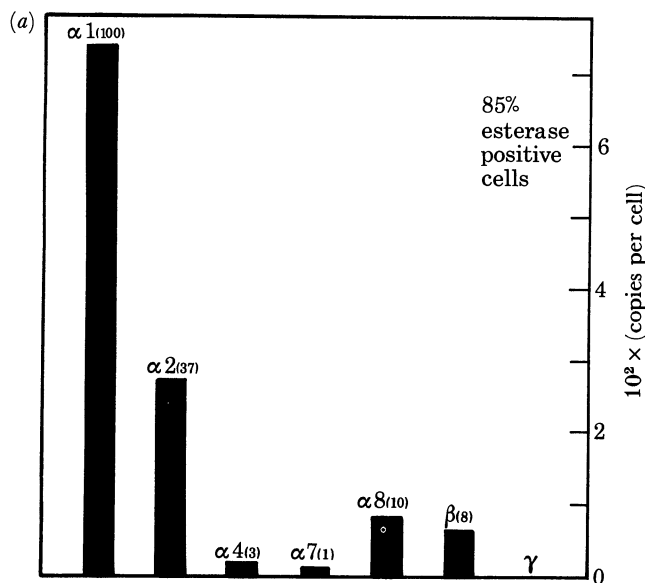

FIGURE 3. IFN mRNA expression in normal and leukaemic mononuclear cells. Non-adherent mononuclear cells from (a) a normal volunteer, (b) a patient with non-B, non-T lymphoblastic leukaemia and (c) a patient with acute myeloid leukaemia (M2) were induced with Sendai virus for 8 h and total RNA was prepared, analysed and quantified as described (Hiscott et al. 1984). May-Grünwald Giemsa differential analysis as well as the results of peroxidase and esterase histochemical staining are shown. The ratio of the individual IFN-α RNAs relative to IFN-α1 (= 100) is indicated in parentheses. In (a) the values for IFN-α5, IFN-α6 and IFN-α14 were not determined (but see figure 1).

(b)

myeloblasts 76%
promyelocytes 18%
lymphocytes 6%
peroxidase 93%
esterase 0%

$10^2 \times$ (IFN mRNA copies per cell)

$\alpha 2_{(300)}$

$\alpha 14_{(200)}$

$\alpha 1_{(100)}$

$\alpha 4_{(10)}$ $\alpha 5_{(10)}$ $\alpha 6$ $\alpha 7$ $\alpha 8_{(10)}$ $\beta_{(10)}$

(c)

$10^2 \times$ (IFN mRNA copies per cell)

$\alpha 2_{(770)}$

lymphoblasts 55%
lymphocytes 45%
peroxidase 0%
esterase 3%

$\alpha 4_{(135)}$

$\alpha 1_{(100)}$ $\alpha 5_{(20)}$ $\alpha 7_{(20)}$ $\alpha 8_{(20)}$ $\alpha 14_{(20)}$ $\beta_{(20)}$
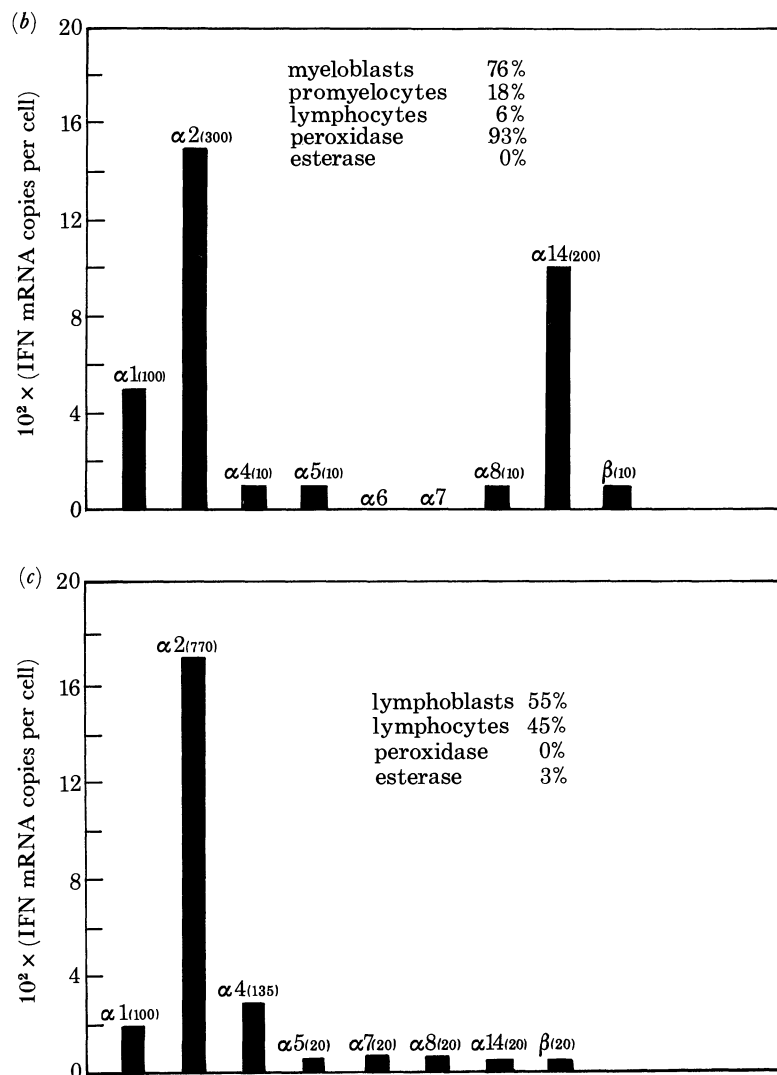
FIGURE 3(b,c). For legend see facing page.

normal monocytes or buffy coat leucocytes. In the case of acute myeloid leukaemias, the ratios of IFN-α1 to IFN-α2 mRNA were low, but there was as much IFN-α14 as IFN-α2 mRNA, a pattern never found in any other cells. Mononuclear cells from cases of acute lymphoblastic leukaemia expressed high levels of IFN-α2 transcripts, relative to IFN-α1 (figure 3c). The only case of acute monoblastic leukaemia examined so far was unusual in that there was a high *constitutive* level of IFN in the serum; the RNA pattern was similar to that of normal monocytes (see figure 3a).

(ii) *IFN mRNA in lymphoblastoid cells*

The human lymphoblastoid cell line Namalwa can be induced by Sendai virus to produce high yields of α- and β-IFNs (Havell *et al.* 1976). Of the IFN-α-specific RNAs examined, IFN-α2, -α1 and -α4 were the predominant species, with 100–500 copies per cell each, while IFN-α5, -α7 and -α8 were each around 25–50 transcripts per cell. No transcripts corresponding to

IFN-α6, IFN-α14 or IFN-γ were detected in this or other Namalwa induction experiments (Hiscott *et al.* 1984). Induction with other viruses or use of different protocols did not give rise to a different pattern of IFN mRNAs.

(iii) *IFN transcripts in human fibroblasts*

Human fibroblasts induced by viruses or poly(I):poly(C) synthesize predominantly IFN-β (23), although IFN-α activity has been detected after induction of GM258 cells with Newcastle disease virus (NDV) (Havell *et al.* 1978).

We analysed the IFN-specific transcripts in GM2504 (the new designation for GM258) cells after either superinduction with poly(I):poly(C) (Havell & Vilček 1972) or infection with NDV. In both cases medium obtained at the time of RNA isolation contained about 3000 i.u. ml^{-1} of antiviral activity and each cell had about 1000 copies of IFN-β RNA. After induction with NDV, but not with poly(I):poly(C), low levels (two to five copies per cell) of IFN-α1 and IFN-α4 were detected; other IFN-α specific transcripts were not observed (Hiscott *et al.* 1984). Thus the number of IFN-β transcripts is greater by a factor of 100 than the IFN-α RNA in virus-induced fibroblasts.

(b) *Studies on the molecular requirements for induction*

Mantei & Weissmann (1982) introduced the human IFN-α1 gene into mouse L cells and found that correctly initiated transcripts in permanently transformed mouse cell lines occurred only after viral induction. To determine whether the appearance of IFN-α mRNA was due to onset of transcription or to a reduced rate of mRNA degradation, the inducibility of hybrid genes was determined. A hybrid gene in which the β-globin promoter was linked to the IFN-α1 transcription unit was expressed constitutively but showed no response to viral induction, whereas a gene consisting of the IFN-α promoter and the β-globin transcription unit showed the same behaviour as the IFN-α1 gene itself. As the β-globin gene showed constitutive expression, which was diminished rather than increased after virus infection, it was concluded that induction was not due to stabilization of the transcript, but to increased transcription caused by the 5′ flanking region of the IFN-α1 gene (Weidle & Weissmann 1983). Deletion of the 5′ flanking sequence downstream to position −117 did not prevent inducible transcription; deletions to position −74 and beyond completely abolished transcription (Ragg & Weissmann 1983). Thus, the upstream border of a region essential for induced transcription was localized between position −117 and −74.

It is not possible to deduce from this experiment whether the sequences removed by the deletion are responsible for induction or whether they are required for initiation of transcription *per se*. To resolve this question, a number of genes with *hybrid promoters* were prepared, consisting of upstream segments of the IFN-α1 promoter linked to the downstream part of the β-globin promoter at different points; in all cases the transcription unit was the rabbit β-globin sequence.

As shown in figure 4, a segment of the IFN-α1 gene, extending to position −68 or further downstream, could render the β-globin promoter inducible, whether the joint was at position −109, −78 or −56 of the β-globin segment. As shown by the analysis of the β-globin gene (Dierks *et al.* 1983), a β-globin promoter segment extending upstream to position −109 gives full constitutive transcription in mouse cells; none the less, the IFN-α1 segment increased the induced transcript level ninefold, giving about 14000 transcripts per cell. This is a very high level and may be compared to the β-globin RNA content of induced Friend cells, about

	TRANSCRIPTS PER CELL		
	MOCK	NDV	IND.
HP5	58	39	0.7
HP5A	36	233	6.7
HP5B	1164	3491	3.0
HP6	3	524	175
HP7	23	145	6.3
HP13	1454	13575	9.3
HP6B	4	4	1.0
HP9	24	12	0.5
HP15	400	200	0.5
ID1	4	4	1.0
ID2	36	18	0.5
ID3	2000	1200	0.6

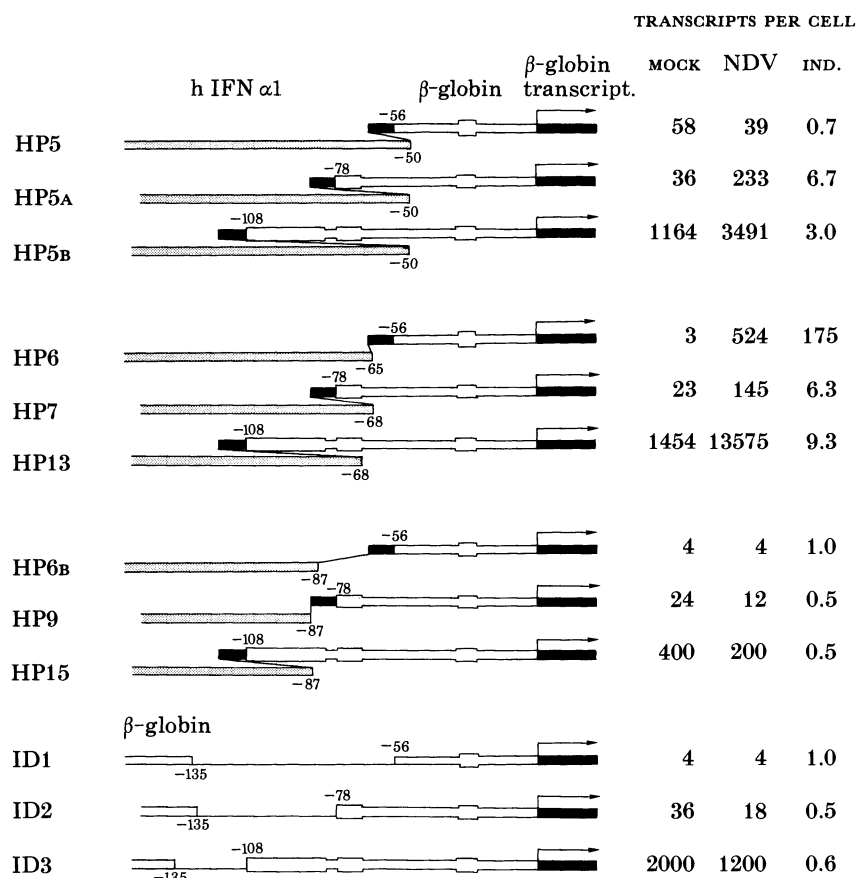FIGURE 4. Inducibility of hybrid interferon β-globin promoters. Hybrid promoters (HP) were constructed in which various lengths of the human interferon α1 5′-flanking sequence (stippled lines) from −675 to the 3′ position indicated (numbering relative to the cap site) were joined via *Hind*III linkers (black box) to the 5′ truncated promoters of β-globin genes (open boxes). These genes were cotransfected with the herpes simplex virus TK gene into mouse LMTK⁻ cells and TK⁺ colonies were selected by growth in HAT medium (Weidle & Weissmann 1983; Ragg & Weissmann 1983). After 14 days, 500–1000 colonies were pooled, infected with Newcastle disease virus or mock-infected and RNA was extracted 8–9 h thereafter. The steady state level of correctly initiated β-globin transcripts was determined by quantitative S_1 mapping (Dierks *et al.* 1983). As a control for the constitutive level of β-globin transcription, β-globin genes with internal deletions from −135 to −56(ID1), −78(ID2) or −109(ID3) were transfected in parallel. The open boxes within the β-globin promoter indicate the position of the three essential regions defined by Dierks *et al.* (1983). The start site for β-globin transcription is indicated by the arrow.

5000−10000 transcripts per cell. Shorter β-globin segments, extending upstream to position −78, that is, retaining only the CAAT box, gave a 50-fold lower constitutive transcript level; anteposition of the IFN-α1 sequence led to a sixfold higher transcript level after induction. Finally, when a β-globin sequence extending upstream only to position −56, that is, devoid of the CAAT box, was fused to an IFN sequence extending downstream to −65 the constitutive level of synthesis was reduced about 500-fold and a 175-fold increase in transcript level was found after induction. An important observation is that the β-globin promoter (extending to position −109) had the same constitutive expression level whether or not the IFN promoter segment was placed upstream of it. This suggests that the IFN promoter segment does not act by depressing the constitutive transcription level in the uninduced (negative control) state; rather, this segment mediates enhancement of transcription under conditions of induction.

DISCUSSION

Gene transcription in higher eukaryotes is thought to be subject to two kinds of control, developmental and environmental (see, for example, Puck 1983). Developmental control is directed by internal programmes and may involve structural changes at the chromatin and DNA level (see, for example, Davidson *et al.* 1983); transition from an inactive to an active state typically involves one or more cell divisions and is usually permanent. Environmental control is characterized by a rapid and transient transcriptional activation in response to external signals such as hormones (Kurtz 1981; Mulvihill *et al.* 1982; Knoll *et al.* 1983), metal ions (Pavlakis & Hamer 1983), heat shock (Pelham 1982) or viruses (Raj & Pitha 1983; Mantei & Weissmann 1982). In many cases sets of genes are converted into an inducible state in a tissue-specific manner during development and can only then be transcriptionally activated by an external signal (Laperche *et al.* 1983; McKnight *et al.* 1980; Tsai *et al.* 1980). Tissue-specific responses to environmental stimuli may thus involve both levels of control.

We have studied the transcription of β-, γ- and several α-IFN genes in different cells under a variety of induction conditions. We found that α-IFN genes as a group on the one hand and the IFN-β gene on the other were expressed in a cell-specific manner; the predominant species in induced fibroblasts and HeLa cells was IFN-β mRNA, while induced leucocytes and lymphoblastoid cells contained α- as well as β-IFN transcripts. The presence of IFN-β in lymphoblastoid cell IFN has been documented (Havell *et al.* 1976). As buffy coat IFN contains only a low level of IFN-β (Havell *et al.* 1975), it is possible that the IFN-β mRNA in leucocytes is not expressed efficiently, as in the case of HeLa cells (Hiscott *et al.* 1984).

When expressed, the individual α-IFN mRNA species were present at widely different levels: in leucocytes from pooled human buffy coats (prepared in Helsinki by K. Cantell) and cultured lymphoblastoid (Namalwa) cells after induction *in vitro*, IFN-α1, IFN-α2 and IFN-α4 represented the major IFN-α RNA species, while IFN-α5, IFN-α7, IFN-α8 and IFN-α14 transcripts were present at 5- to 20-fold lower levels. For reasons that have not yet been clarified, induced buffy coat leucocytes obtained and prepared in Zürich, whether normal or leukaemic, showed only a low level of IFN-α4 transcripts after virus induction.

We have recently found that in buffy coat leucocytes IFN-α transcripts with a coding region indistinguishable from that of IFN-α1 transcripts are also produced by the IFN-α13 gene, which is non-allelic with the IFN-α1 gene (Todokoro *et al.* 1984). Thus, what we designate as IFN-α1 transcripts in this paper may be derived from both the IFN-α1 and -α13 genes. The IFN-α6 gene, which did not give rise to detectable transcript levels in any of our experiments, has a deletion of 12 nucleotides, from position -61 to -73, within the presumed promoter region (Ragg & Weissmann 1983). Although expression of the IFN-α6 coding sequence in *Escherichia coli* gave rise to biologically active IFN (M. Mishina and W. Boll, unpublished results), IFN-α6 may in fact be a pseudogene as regards the promoter.

There were significant differences in the ratios of some of the IFN-α RNA levels from one cell type to another. Thus, in Sendai virus-induced leucocytes, the ratio of IFN-α1 to IFN-α2 RNA was 1.7, while in Namalwa cells induced in a similar fashion the corresponding value was 0.4. Virus-induced leukaemic myeloblasts contained as much IFN-α14 as IFN-α2 RNA; transcripts of this gene have never been found at that level in any other type of cell. Diploid human fibroblasts (GM2504), after induction with NDV (but not with poly(I):poly(C)) contained low amounts of IFN-α1 and IFN-α4, but no detectable IFN-α2 RNA.

Cell-specific differences in the levels of α- and β-IFN mRNA could come about by different mechanisms: (i) there could be one induction mechanism, but the response of the individual genes could, as a consequence of developmental control, be different in different cell types; (ii) there could be a cell-specific difference in the degradation rate of the different IFN mRNAs; or (iii) there could be several pathways of induction each giving a different pattern of IFN gene expression, one of which would be prevalent in a certain cell type.

It seems unlikely that there would be independent induction mechanisms for the individual IFN-α genes, and we believe it is more likely that in different cell types the individual genes would be more or less susceptible to induction, as a consequence of developmental control. In this connection the finding that leukaemic myeloblasts express IFN-α14, which is not found in normal leucocytes, is of particular relevance.

Progress has been made in identifying the elements involved in the environmental control of the IFN-α and -β genes. It was found that the 5′ flanking region of these genes is responsible for the response to induction (Weidle & Weissmann 1983; Zinn *et al.* 1983; Ohno & Tanaguchi 1982), and it has been formally shown in the case of the IFN-α1 gene that the increase in IFN transcript level is due to increased transcription rather than to reduced IFN RNA turnover. The region essential for induced transcription was located between positions −117 (determined by external deletions (Ragg & Weissmann 1983)) and −68, as deduced from promoter hybrids. The fact that a IFN-α promoter fragment extending downstream to position −68 could impart inducibility upon a globin promoter, whether placed next to the β-globin TATA box or upstream of the complete promoter is reminiscent of the behaviour of an enhancer (Banerji *et al.* 1981; Fromm & Berg 1982); in this case we would be dealing with an inducible enhancer. Experiments currently in progress will show whether the DNA sequence in question will impart inducibility to a promoter even when placed downstream of it, and regardless of orientation, as expected of a respectable enhancer.

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