Volume 12 Number 9 1984 Nucleic Acids Research

Differential expression of human interferon genes

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Received 6 April 1984; Accepted 17 April 1984

ABSTRACT

We developed a method for quantitating closely related mRNAs by S_1 mapping and used it to determine the levels of mRNAs for IFN-β, IFN-γ and various alpha IFNs (IFN-α1, -α2, -α4, -α5, -α6, -α7, -α8 and -α14) in human peripheral blood leukocytes, lymphoblastoid (Namalwa), HeLa and human fibroblastic cells, induced in different fashions. The ratio of alpha to beta 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. Moreover, there was a striking difference in the proportion of individual IFN-α mRNA species in different cell types. Use of different induction protocols did not significantly affect the proportion of IFN mRNAs. IFN production was not proportional to mRNA level in all cases, as lymphoblastoid cells induced by incubation at high density and virus-induced HeLa cells contained high levels of IFN-β but produced little antiviral activity.

INTRODUCTION

Human interferons (IFNs) are classified into three antigenically distinct groups, designated IFN-α, IFN-β and IFN-γ (1). The alpha IFNs are encoded by a multigene family consisting of at least 13 non-allelic and 8 allelic members (2-4); Todokoro and Weissmann, unpublished results). Beta and gamma IFNs are each encoded by a single gene (5-8).

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 alpha and/or beta IFN mRNAs and IFNs (1). IFN-γ is synthesized by T lymphocytes following exposure to immune stimuli or to mitogens (9,10). Expression at the molecular level has recently been studied using recombinant DNA technology. Transcription of the cloned human IFN-α1 gene in mouse cells was observed only

after induction by virus and was shown to be due to activation of the promoter rather than to stabilization of rapidly turning-over RNA (11,12). Induction was mediated by a segment of 117 5' flanking nucleotides of the IFN-α1 gene (13). Similar conclusions were reached for the IFN-β gene (14-18).

Peripheral blood leukocytes (19), permanent lymphoblastoid cell lines (20) and human fibroblasts (21,22) have been important sources of IFN for research and clinical studies. In response to induction, these cells produce a heterogeneous mixture of IFNs, as judged by various analytical methods (23,24). While some of this heterogeneity may be due to post-translational modification of IFNs (24-26), it is clear that several different gene products are represented in preparations derived from leukocytes and lymphoblastoid cell lines (2,3,23,24). It has been shown that the ratio of IFN-α to IFN-β depends on the cell type used for induction and at least in one case may vary with the nature of the inducer (27). It is however not known 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.

In this paper we report the levels of mRNAs for various human alpha IFNs, IFN-β and IFN-γ in human peripheral blood leukocytes, lymphoblastoid (Namalwa), HeLa and fibroblastic cells, induced in different fashions. The ratio of alpha to beta IFN transcripts varied greatly, depending on the cell type. The proportion of the different IFN-α RNA species, in particular IFN-α1 and -α2 also varied between different cell types. IFN production is likely not proportional to mRNA level in all cases, as virus-induced Hela cells as well as lymphoblastoid cells induced by incubation at high density (28,29) contained high levels of IFN-β mRNA but produced relatively little antiviral activity.

MATERIALS AND METHODS

Cell culture

Lymphoblastoid (Namalwa) cells (from Dr. G. Klein, Stockholm) were grown in suspension in RPMI 1640 medium (Gibco) supplemented with 5% fetal calf serum (FCS). GM2504 cells (Human

Genetic Mutant Cell Repository, Cambden, N.J.) were grown in McCoy's 5A (modified) medium (Gibco) supplemented with 10% FCS.

RNA from induced eukaryotic cells

Namalwa cells grown with or without stimulating compounds were suspended in RPMI 1640 without serum at 5 to 15 X 10^6 cells/ml. The cells were shaken with 500-1000 hemagglutinating units (HAU)/ml Sendai virus for 90 min at 37°, washed and resuspended at 2 X 10^6 cells/ml in serum free medium, and incubated at 37^{0}. Infection with vesicular stomatitis virus (VSV), or Mengo virus was at 20 plaque forming units (pfus) per cell. RNA was isolated (30) at the times indicated.

Confluent monolayers of GM2504 cells were induced with polyinosinic-polycytidylic acid (poly(I)-poly(C); P-L Biochemicals) under conditions of superinduction as described (22) or with Newcastle Disease Virus (NDV). Cells were rinsed with phosphate-buffered saline (PBS) and infected with 30 HAU/plate of NDV. After 90 min, the cells were washed as above and incubated at 37° in McCoy's 5A (modified) medium containing 0.5% FCS. RNA was isolated 9 h later (30). Sendai virus-induced leukocyte RNA was prepared as described (31); poly(A^+) RNA was isolated by oligo(dT) cellulose chromatography (32).

IFN RNA from E. coli

The individual IFN-α RNAs to be used as standards were isolated from E.coli harboring the appropriate expression plasmid. In these plasmids the 5' untranslated and signal sequences of each IFN gene were precisely replaced by a prokaryotic control region (see for example, Weissmann et al. (4)). The β-lactamase control region was joined to the mature coding sequence of IFN-α1, IFN-α2, IFN-α4, IFN-α5, and IFN-α6 (in plasmids M11, M21, M41, M51 and M61, respectively, prepared by W. Boll and M. Mishina); expression was constitutive. The heat inducible P_L control region of phage λ was joined to IFN-α7 and IFN-α8 coding sequences (p8α7 and pYH2α8, prepared by T. Kovacic, Biogen, Inc.) and induced by temperature shift in E. coli C600/ts cI857 as described (33). The tryptophan control region (3) was used for the IFN-β and IFN-γ expression plasmids (Fi99 and IIF41, prepared by B. Allet, Biogen Inc.). Total RNA was isolated from the E. coli strains as described (34).

The proportion of IFN-specific mRNA in total E. coli RNA was estimated by hybridizing the appropriate 5'-^{32}P-labelled probe of known specific activity (usually 0.5-2.0 X 10^6 cpm/pmole) to increasing amounts of bacterial RNA in 80% formamide, and analyzing the fraction of radioactivity in DNA:RNA hybrids by hydroxyapatite (HAP) column chromatography (35) as well as by S_1 mapping (36). The proportion of IFN specific RNA was determined from the slope of the linear part of the hybridization curve.

Preparation of IFN-specific probes and S_1 mapping

Plasmids containing chromosomal IFN genes were cleaved at an appropriate restriction site (see Fig. 1) within the coding or 3' untranslated sequence and ^{32}P-labelled at the 5' terminus (37). Kinased fragments were cleaved with a second appropriate restriction enzyme at a position upstream of the CAP site and the probe was isolated from low melting temperature (LMT) agarose. Only in the case of IFN-α2 and IFN-γ was a cDNA clone used which did not comprise the CAP site. Unlabelled IFN-α probes were isolated in the same fashion, omitting the kinasing step. Total RNA was analyzed by S_1 mapping (36).

Quantitation of IFN mRNA in induced cells

The number (N) of IFN mRNA strands/induced cell was estimated from the amount (M) of total cell RNA analyzed, the amount of RNA per cell (2.10^{-11}g), the molecular weight of IFN mRNA (330,000) and the radioactivity (R_S) of the protected DNA probe relative to the radioactivity (R_{ST}) given in a parallel hybridization by the homologous E. coli derived RNA, containing the amount M_S of IFN RNA (determined as described above):

$$N = \frac{R_S \times M_S}{R_{ST} \times M} \times 3.61 \times 10^7$$

Determination of antiviral activity

Antiviral activity of IFN-α and IFN-β was determined by the cytopathic effect reduction assay using HEp-2 or MDBK cells (ATCC CCL-23 and -22, respectively) challenged with Mengo virus (31). IFN-α2 from E. coli which had been titered against an international standard was used as reference.

RESULTS

The assay system: specificity of IFN probes

Since IFN-α and IFN-β genes are only about 50% homologous (38) and IFN-γ has no significant homology to either IFN-α or IFN-β (39), cross hybridization between members of the three IFN classes does not occur under the usual conditions. However, as the members of the IFN-α family are about 85-95% homologous, the identification of individual IFN-α mRNA species depends on the ability to distinguish between perfect hybrids formed between a particular DNA probe and the cognate IFN mRNA, and the imperfect hybrids formed with closely related RNA species.

Minus strand probes for eight distinct IFN-α mRNAs, as well as for IFN-β and IFN-γ mRNAs were prepared from the cognate cloned DNA (Fig. 1). In most cases the probes spanned the CAP site, and the ^{32}P-labelled 5′ terminus mapped to the mature IFN coding sequence. The specificity of the assay was ensured by the following factors: (1) annealing was carried out under stringent conditions; (2) each hybridization reaction contained a ^{32}P-labelled probe corresponding to one of the IFN-α mRNAs and an excess of all other unlabelled IFN-α probes, so that each mRNA preferentially hybridized to the homologous probe and the ^{32}P-labelled probe did not score heterologous mRNAs; (3) in most cases the 5′ terminus of the probe was at a restriction cleavage site unique to the gene whose transcript was to be measured; thus, the ^{32}P-labelled end of a mismatched probe was susceptible to S_1 nuclease digestion; (4) only probe fragments protected by the mRNA from the 5′ end to the CAP site were scored; this discriminated against probe fragments derived from mismatched hybrids and mRNA fragments.

The specificity of the reaction was tested by hybridizing each ^{32}P-labelled probe against a panel of the individual IFN-RNA species. These IFN RNAs, which were obtained from E. coli harboring the appropriate IFN expression plasmids, contained the IFN coding sequence only up to the triplet encoding the first amino acid of the mature IFN sequence (4) and therefore protected a shorter probe segment than the natural mRNA. All IFN-α probes were completely specific for the cognate RNAs (Fig. 2); a more than twentyfold higher level of RNA derived from

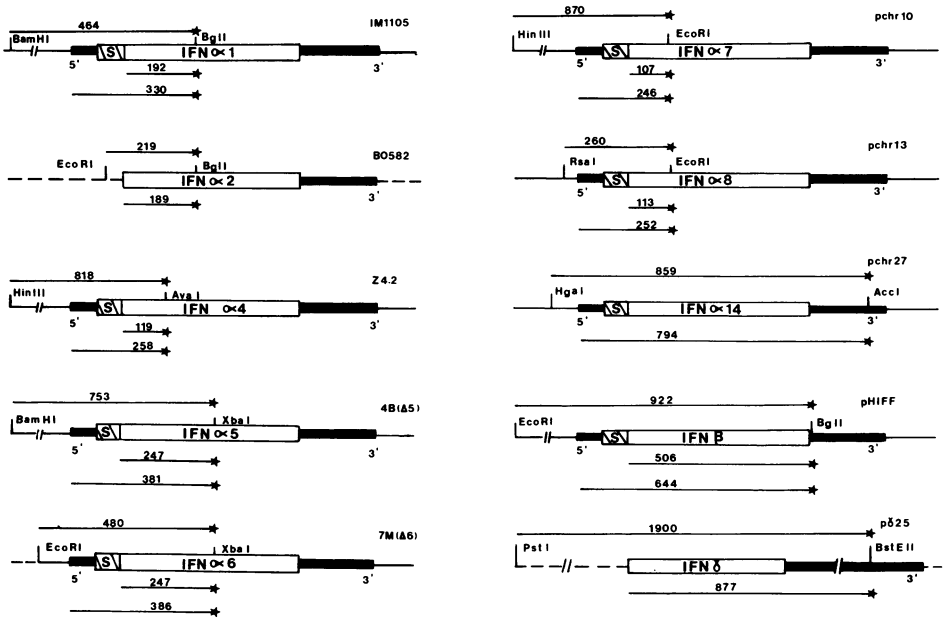


Figure 1. IFN-specific probes utilized for S_1 mapping.

The probes for mapping IFN-α, IFN-β and IFN-γ transcripts are indicated on the diagrams of the human IFN genes. The regions encoding the mature protein and signal peptide (S) are represented by open and hatched boxes, respectively; the non-translated 5′ and 3′ regions are indicated by solid boxes, chromosomal sequences by solid lines and bacterial sequences by dashed lines. The distance (in nucleotides) between the ^{32}P-labelled 5′ end of the probe, marked with an asterisk, and the (upstream) 3′ end is indicated above the IFN gene. The distance (in nucleotides) between the 5′ end of the probe and the CAP site or the beginning of the mature coding sequence is indicated below the IFN gene. IFN RNA from E.coli protects the probe up to the beginning of the mature coding sequence while IFN RNA from eukaryotic cells protects the probe to the CAP site, with the exception of IFN-α2 and IFN-γ probes which lack 5′ non-coding and signal sequences. An expression plasmid for IFN-α14 was not available.

The structure of the genes are from Ohno and Taniguchi (5) (IFN-β); Gray and Goeddel (7) (IFN-γ); Nagata et al. (2) (IFN-α1); Streuli et al. (50) (IFN-α2); and from unpublished work from K.Henco (IFN-α4); J.Fujisawa (IFN-α5); J.Schmid (IFN-α6); T.Kovacic (IFN-α7); M.Pasek (IFN-α8) (quoted in Weissmann et al. (4); and M.Wälchli and K. Todokoro (IFN-α14) (unpublished results).

other IFN-α expression plasmids did not yield any signal (data not shown).

The proportion of IFN specific mRNA in total RNA from E. coli was measured by the hydroxyapatite method (see Methods

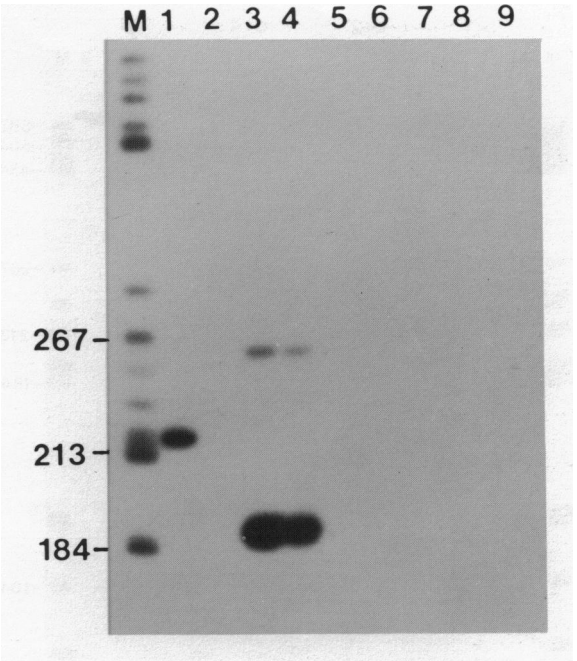


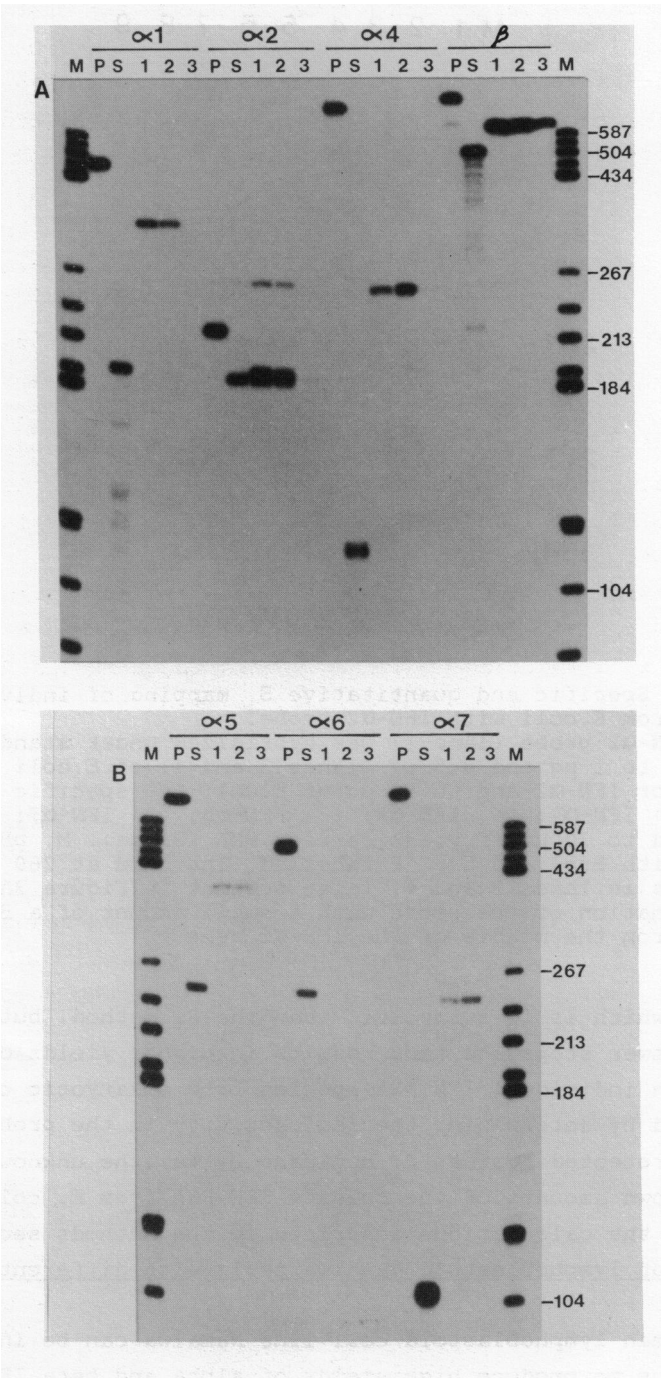
Figure 2. Specific and quantitative S_1 mapping of individual IFN RNAs from E.coli with IFN-α2 probe.

The IFN-α2 probe (lane 1) was hybridized under standard conditions to 1 μg and 0.5 μg (lanes 3 and 4) of E.coli RNA specific for IFN-α2 and to 20 μg of E.coli RNA specific for (2) IFN-α1; (5) IFN-α4; (6) IFN-α5; (7) IFN-α6; (8) IFN-α7; (9) IFN-α8, and to 20 μg of yeast carrier RNA (Sigma). M, pBR322 digested with BspI and 5'-^{32}P-labelled. The band at 260 nucleotides in lanes 3 and 4, (also present in Figure 3A) is due to contamination of the probe with a small amount of a BglII fragment from the middle of the IFN-α2 gene.

section), which is less specific than the S_1 method, but requires fewer steps and thus results in higher yields of hybrids. An individual IFN RNA species from eukaryotic cells was quantitated by determining the radioactivity in the probe fragment protected against S_1 nuclease by (a) the unknown sample and (b) known amounts of the cognate IFN RNA from E. coli, and performing the calculations described in the Methods section.

Induction of lymphoblastoid Namalwa cells with different viruses.

The human lymphoblastoid cell line Namalwa can be induced by Sendai virus to produce high yields of alpha and beta IFNs (40).


A
∝1
∝2
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β
M P S 1 2 3 P S 1 2 3 P S 1 2 3 P S 1 2 3 M
587
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434
267
213
184
104
B
∝5
∝6
∝7
M P S 1 2 3 P S 1 2 3 P S 1 2 3 M
587
504
434
267
213
184
104

The steady state level of IFN-α mRNA at 4 and 10 h post infection (p.i.) was about 600, and at 22 h p.i., about 200 copies/cell (Figures 3 and 4, and Table 2). Of the IFN-α specific RNAs examined, IFN-α2, -α1 and -α4 were the predominant species, with 100 to 500 copies/cell each between 4 and 10 h p.i., 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 (Table 2, Figure 3B and 4) were detected in this or other Namalwa induction experiments.

To determine whether different inducers preferentially stimulate certain IFN-α genes, Namalwa cells were induced with Newcastle Disease Virus (NDV, a paramyxovirus), Mengo virus (a picornavirus), vesicular stomatitis virus (VSV, a rhabdovirus), and with poly(I): poly(C); total RNA was isolated 8 h later. NDV stimulated IFN activity in Namalwa cells to a similar level as Sendai virus, to which it is closely related, and gave rise to a similar transcript profile (Table 2). VSV and Mengo virus also induced IFN transcripts and IFN antiviral activity in Namalwa cells, but at a 20 fold lower level; in both cases IFN-β, IFN-α1 and IFN-α2 were the major transcripts followed by IFN-α4 (Table 2). In repeated experiments this strain of Namalwa cells was not induced by poly(I): poly(C).

Induction of lymphoblastoid cells pretreated with stimulating compounds.

Treatment of Namalwa cells with n-butyrate, BUdR or dexamethasone does not *per se* induce IFN activity; however, subsequent induction by virus leads to a 10-20 fold higher level of IFN activity per cell at 24 h p.i. than when the pretreatment

Figure 3. S_1 analysis of IFN transcripts in Sendai virus-induced Namalwa cells.

Namalwa cells were induced with Sendai virus and total RNA was isolated at various times after infection. Namalwa RNA (40 µg) was analyzed by S_1 mapping, using the probes and IFN RNA from E. coli, all as described in the Methods section and Figure 1. The probes used for S_1 mapping are indicated above the tracks of the gel. P, ^{32}P-labelled full-length probe (3-10x10^6 cpm/µg); S, the IFN RNA from E.coli indicated above the lane (0.05 to 2 µg, depending on the IFN RNA content); 1, 2, 3, refers to Namalwa RNA isolated at 4 h, 10 h, and 22 h after induction, respectively.

M, ^{32}P-labelled BspI fragments of pBR322.

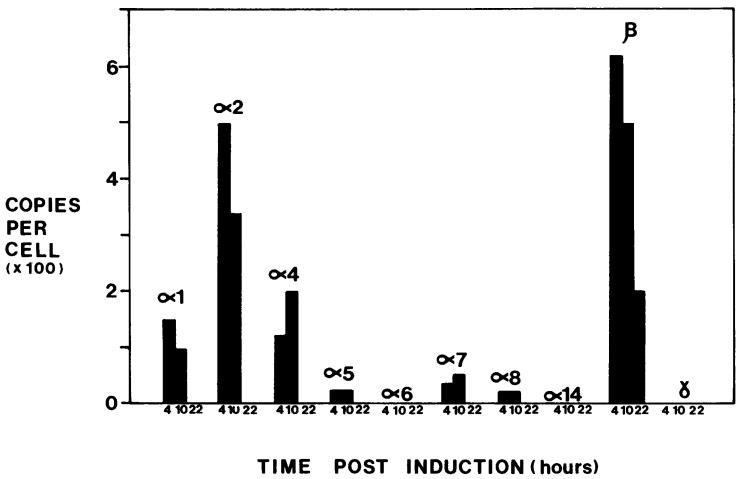


Figure 4. Quantitation of IFN mRNA in Sendai virus-induced Namalwa cells.
^{32}P-labelled DNA fragments corresponding to correctly initiated IFN mRNA species were cut from the gel and counted along with DNA fragments derived from DNA:RNA hybrids between IFN probes and E.coli RNA specific for individual IFN genes. The number of RNA strands per cell was determined as described in the Methods section.

is omitted (Table 1) (41,42). To examine the effects of such activators on IFN gene transcription, Namalwa cells were treated with BUdR (80uM), n-butyrate (1mM) or dexamethasone (10uM) for 48 h before Sendai virus infection. At 8 h p.i. total cellular RNA was extracted and analyzed as usual. IFN activity in the medium at 8 h p.i. was 3-5 fold higher in pretreated and infected cultures than in the infected control cultures.

In general, there was a 3-5 fold proportional increase of all IFN-α RNAs but not of the IFN-β RNA (Table 2). This agrees with the finding that IFN-α but not IFN-β antiviral activity is increased by pretreatment with n-butyrate or BUdR (43).

High density induction of lymphoblastoid cells.

Namalwa cells, incubated at 10^7 cells/ml spontaneously produce a low level of IFN activity (28). To determine which IFN genes were activated by high density incubation, Namalwa cells were incubated at 10^7 cells/ml for 24 h; total RNA was extracted and IFN activity in the culture medium was assayed. IFN-β transcripts, about 100 copies per cell, but no IFN-α

Table 1

Pretreatment (48 hr)	Cells/Culture ($x10^6$)	Interferon Titer ($IU/10^6$ cells)	Factor of Enhancement
None	6.4	170	-
Dexamethasone (μM)			
1	3.6	850	4.9
10	3.2	950	5.5
100	1.7	1800	10.3
5' Bromodeoxyuridine (μM)			
80	4.4	1800	10.7
320	2.2	1350	8.0
640	2.1	1400	8.4
n-Butyrate (mM)			
0.1	4.8	600	3.7
1.0	3.7	2800	16.3
10.0	1.7	180	1.0

Legend to Table 1. Effect of different enhancing compounds on Namalwa cell growth and interferon production.

Namalwa cells (5 X 10^5 cells/ml) in RPMI 1640 supplemented with 5% FCS were treated with n-butyrate, BUdR or dexamethasone at the concentrations indicated for 24 h (data not shown) or 48 h and the cell number was determined. The cells were collected by centrifugation, resuspended in 1 ml of the same medium and infected with Sendai virus (250 HAU per 10^7 cells) as described in the Methods section. Culture medium was removed 20 hours later and assayed for IFN activity on HEp-2.

transcripts (<2-5 per cell) were identified. It is remarkable that no IFN activity (<10 U/ml) was detectable in the culture medium; for comparison, fibroblasts containing about 1000 IFN-β mRNA molecules produced 3 X 10^3 IU/ml antiviral activity.

IFN transcripts in human fibroblasts induced with poly(I):poly(C) or NDV

Human fibroblasts induced by viruses or poly(I): poly(C) synthesize predominantly IFN-β (44), although IFN-α activity has been detected after induction of GM258 cells with NDV (27).

We analyzed the IFN specific transcripts in GM2504 (the new designation for GM258) cells after either superinduction with poly(I): poly(C) (22) or infection with NDV. In both cases medium obtained at the time of RNA isolation contained about 3000 IU/ml of antiviral activity and each cell had about 1000 copies of IFN-β RNA (Table 2). After induction with NDV, but not with poly(I): poly(C), low levels (2 to 5 copies per cell) of IFN-α1 and IFN-α4 were detected; other IFN-α specific transcripts were not observed. Thus the number of IFN-β

TABLE 2

Source of Interferon	Inducer (H) After Induction	Antiviral Activity (IU/ml)	IFN mRNAs (copies/cells)* α 1	α 2	α 4	α 5	α 6	α 7	α 8	α 14	β	Total
Expt.1 & 2												
Leukocytes	Sendai Virus (2h)	3.5×10^3	1200	1100	800	100	-	200	100	ND	600	4100
Leukocytes	Sendai Virus (6h)	6×10^4	2500	1400	1600	300	-	150	300	100	1000	7350
Leukocytes	Sendai Virus (21h)	6×10^4	100	50	50	-	-	-	-	ND	100	300
Expt.3												
Namalwa	Sendai Virus (4h)	3×10^3	150	500	100	50	-	80	60	ND	600	1500
Namalwa	Sendai Virus (10h)	1×10^4	100	350	200	40	-	90	70	ND	500	1350
Namalwa	Sendai Virus (22h)	1×10^4	-	-	-	-	-	-	-	ND	200	200
Expt.4												
Namalwa	Sendai Virus (8h)	5×10^3	100	250	100	15	-	60	50	-	700	1300
Namalwa	NDV (8h)	3×10^3	50	100	75	+	-	+	+	ND	400	625
Namalwa	VSV (8h)	3×10^2	5	10	+	-	-	-	-	ND	20	35
Namalwa	Mengo Virus (8h)	1×10^2	5	10	+	-	-	-	-	ND	10	25
Expt.5												
Namalwa	High Density Incubation (24h)	-	-	-	-	ND	ND	ND	ND	ND	100	100
Expt.6												
GM2504	NDV (8h)	3×10^3	+	-	+	-	-	-	-	ND	1500	1500
GM2504	poly I:C (3h)	3×10^3	-	-	-	-	-	-	-	ND	1200	1200
Expt.7												
Hela S3	NDV (8h)	1×10^2	-	-	-	ND	ND	ND	ND	ND	500	500
Expt.8												
Namalwa	Sendai Virus (8h)	1×10^3	50	60	40	10	-	20	10	ND	300	490
Namalwa	BudR (25ug/ml) Sendai Virus	3×10^3	150	150	50	20	-	20	20	ND	150	560
Namalwa	Dexamethasone (10uM) Sendai Virus	3×10^3	150	100	50	20	-	30	30	ND	200	580
Namalwa	N-Butyrate (1mM) Sendai Virus	5×10^3	250	140	150	30	-	50	30	ND	300	960
Expt.9												
Mononuclear Cells(C.W.)	In Vivo	20	10	+	-	-	-	-	-	ND	-	10-15

Summary: Average values for expts. 1-4 (all times)

Source of Interferon	Inducer (H) After Induction	IFN mRNAs (values relative to IFN-α1)* α 1	α 2	α 4	α 5	α 6	α 7	α 8	α 14	β
Leukocytes	Sendai Virus	(100)	65±15	59±12	11±2	-	10±5	5±4	2	43±29
Namalwa	Sendai Virus	(100)	280±70	118±52	23±13	-	62±16	45±13	-	523±86

transcripts is greater by a factor of 100 than the IFN-α RNA in virus-induced fibroblasts.

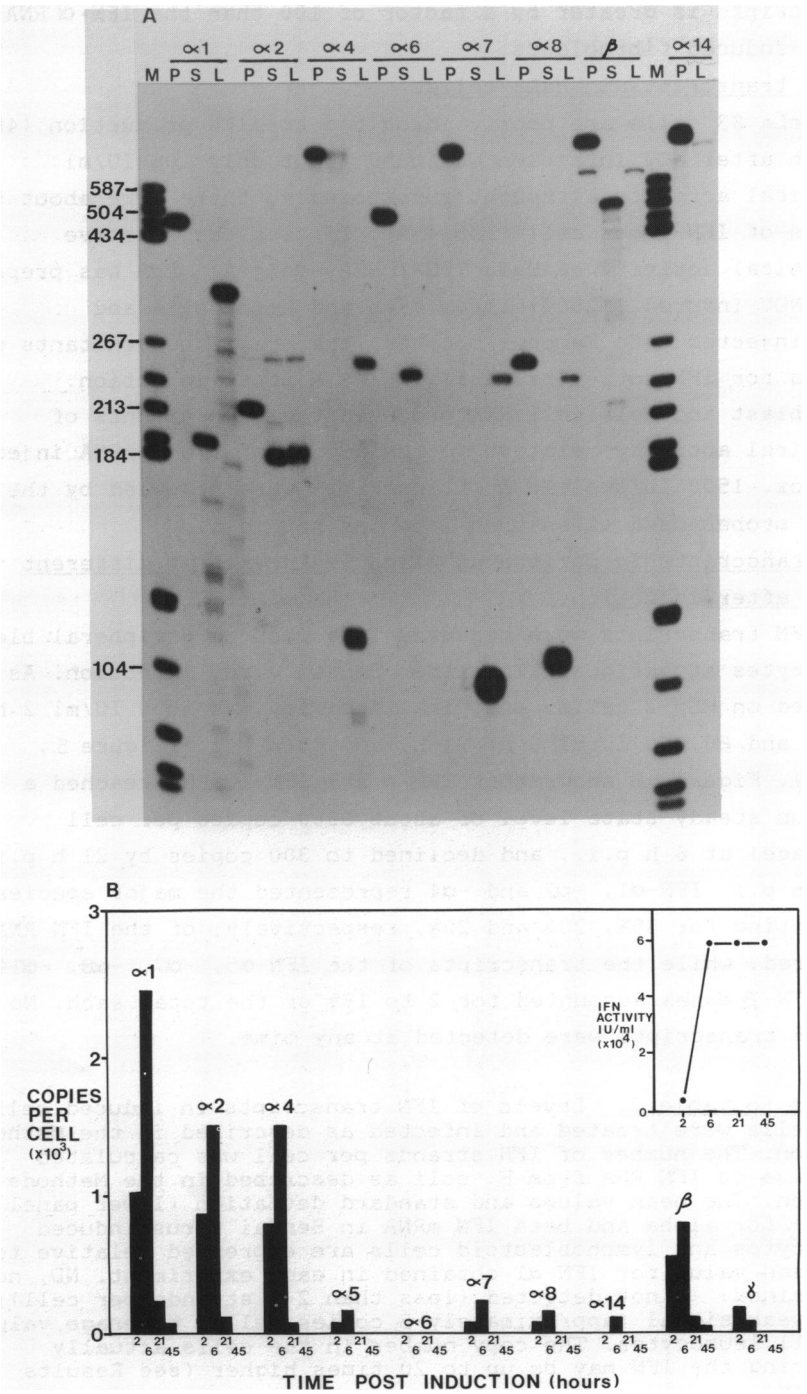
IFN-. transcripts in HeLa cells.

HeLa S3 cells are poorly inducible for IFN production (45). At 8 h after NDV infection we found about only 100 IU/ml antiviral activity although, surprisingly, there were about 500 copies of IFN-β per cell (Table 2). To test the relative biological activity of Hela IFN-β RNA, poly(A)$^+$ RNA was prepared from NDV-induced GM2504 fibroblasts and Hela cells and microinjected into Xenopus oocytes. The oocyte supernatants were tested for IFN anti-viral activity 48 h after injection. Fibroblast and Hela cell RNA produced the same amounts of antiviral activity relative to the amount of IFN-β mRNA injected (approx. 1500 IU/pmole). No transcripts were revealed by the IFN-α probes used (less than 2 copies per cell).

IFN transcripts in peripheral blood leukocytes at different times after induction.

IFN transcripts were measured in a pool of peripheral blood leukocytes at various times after Sendai virus infection. As assayed on HEp-2 cells, antiviral activity was 3500 IU/ml 2 h p.i., and 60,000 IU/ml 6 h, 21 h, and 45 h p.i. (Figure 5, inset). Figure 5B shows that IFN-α and IFN-β mRNA reached a maximum steady state level of about 6000 copies per cell (average) at 6 h p.i., and declined to 300 copies by 21 h p.i. At 6 h p.i. IFN-α1, -α2 and -α4 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 to 15% of the total each. No IFN-α6 transcripts were detected at any time.

Legend to Table 2. Levels of IFN transcripts in induced cells.
Cells were treated and infected as described in the Methods section. The number of IFN strands per cell was calculated relative to IFN RNA from E. coli as described in the Methods section. The mean values and standard deviation (lower panel of Table) for alpha and beta IFN mRNA in Sendai virus induced leukocytes and lymphoblastoid cells are expressed relative to the mean value for IFN-α1 obtained in each experiment. ND, not determined; -, not detected (less than 2-5 strands per cell); +, very weak signal (approximately 5 copies/cell); *average value for all leukocytes. The copy number in the cells actually producing the IFN may be up to 20 times higher (see Results section).


A
∝1 ∝2 ∝4 ∝6 ∝7 ∝8 β ∝14
M P S L P S L P S L P S L P S L P S L P S L M P L
587
504
434
267
213
184
104
B
COPIES PER CELL (x10³)
IFN ACTIVITY IU/ml (x10⁴)
∝1
∝2
∝4
∝5
∝6
∝7
∝8
∝14
β
δ
2 6 21 45
TIME POST INDUCTION (hours)

IFN-γ mRNA was present at 30 and 15 copies per cell (average) at 2 h and 6 h p.i., respectively (Figures 5b), and is likely the consequence of a mixed leukocyte reaction (46). As most if not all of the IFN-α and IFN-β is produced by monocytes (J.H. and V. Hofmann, unpublished results), which constitute about 5% of the leukocyte preparation, the number of cognate mRNAs per producing cell may be as much as twenty times that indicated as average value. For analogous reasons, the copy number of IFN-γ mRNA in the mononuclear cells actually producing IFN-γ may also be higher.

IFN transcripts in human peripheral mononuclear cells producing interferon in vivo.

Lymphocytes and monocytes from 30ml of heparinized blood from a patient (C.W.) in the febrile stage of an influenza-like infection were isolated by Ficoll gradient purification (47); total RNA was prepared from about 4 X 10^7 mononuclear cells and analyzed by S_1 mapping. Acid stable IFN activity was detectable in the serum at 10-20 IU/ml. About 10 copies of IFN-α1 and 2-5 copies of IFN-α2 per cell were estimated to be present (Table 2). Other individual IFN mRNAs were undetectable, i.e. below the level of about 5 copies per cell.

Figure 5. IFN transcripts in Sendai virus-induced leukocytes
Leukocytes were infected with Sendai virus (19), samples were taken at 2, 6, 21 and 45 h, the antiviral activity of the supernatants was determined and poly(A^+) RNA was prepared (32) and subjected to quantitative S_1 analysis, using the probes described in Fig. 1. A. Autoradiogram of an S_1 analysis. P, probe specific for the IFN species indicated (specific radioactivity, 3-10x10^6 cpm/μg; S, reference IFN RNA from E.coli (0.05-2 μg); L, poly(A^+) RNA (100 ng) isolated 6 h after infection. In the experiment shown in this figure the lane with the IFN-β probe (not digested with S_1) shows a band due to renatured DNA which has a mobility similar to that of the protected probe in lane β-L. The signal in this lane is not due to renatured probe because S_1 digestion efficiently removes the 5'-^{32}P-label from renatured probe; moreover, in several repeat experiments no renatured probe was found, but a signal for mRNA of similar intensity as shown in this figure was noted. B. The transcript level of each type of IFN at different times was calculated as described in the Methods section and was plotted as a bar graph. Inset: antiviral activity as a function of time.

DISCUSSION

We have studied the transcription of beta, gamma and several alpha IFN genes in different cells under a variety of induction conditions. We found that alpha IFN genes as a group on the one hand and the IFN-β gene on the other are expressed in a cell specific manner; the predominant species in induced fibroblasts and HeLa cells was IFN-β mRNA, while induced leukocytes and lymphoblastoid cells contained alpha as well as beta IFN transcripts. The presence of IFN-β in lymphoblastoid cell IFN has been documented (40). As only a low level of IFN-β has been detected in buffy coat IFN (51), it is possible that the IFN-β mRNA in leukocytes is not expressed efficiently, as in the case of Hela cells (see below). When expressed, the individual alpha IFN mRNA species were present at widely different levels: in the cells described in this paper (leukocytes from pooled human blood, 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 5-20 fold lower levels. We have recently found that in buffy coat leukocytes 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 (K.Todokoro, D.Kioussis and C.W., submitted for publication). Most likely, the two transcripts, which differ only in a few nucleotides in the 5' and 3' non-coding regions, cannot be distinguished by our S_1 mapping procedure, so that what we designate as IFN-α1 transcripts in this paper may well 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 (13). Although expression of the IFN-α6 coding sequence in E.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 leukocytes, 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 (Table 2). We have recently found that in leukocytes from at least some individual leukemic patients IFN-α1 is a minor component (J.H. and V.Hofmann, unpublished results). In human IFN-producing mononuclear cells from a febrile patient only IFN-α1 and IFN-α2 RNA was identified, and diploid fibroblasts (GM2504) after induction with NDV (but not after induction with poly(I)-poly(C)) contained IFN-α1 and IFN-α4, but no detectable IFN-α2 RNA. In the last two cases the levels were so low that failure to detect other alpha IFN RNAs may have been incidental to the borderline detectability. Goeddel et al. (3) screened a cDNA clone bank derived from the myeloblastoid cell line KG-1 (49) with a fragment of IFN-A cDNA and identified 33 related clones, of which 70% were IFN-A and IFN-D (which correspond to IFN-α2 and IFN-α1, respectively), while 1 or 2 each of the remainder were IFN-G, -B and -H (IFN-α5, -α8 and -α14, respectively) and IFN-E (a pseudogene), IFN-C and IFN-F which have no counterpart in our collection (4). This suggests that KG-1 cells contain mainly IFN-α1 and IFN-α2, but little or no IFN-α4 mRNA.

Cell specific differences in the levels of alpha and beta IFN mRNA could come about by different mechanisms: (1) the inducibility of the individual genes could, as a consequence of developmental control, be different in different cell types; (2) there could be a cell specific difference in the degradation rate of the different IFN mRNAs; or (3) there could be more than one pathway of induction, one of which would be prevalent in a certain cell type.

Induction of alpha and beta IFNs is believed to be brought about by double-stranded RNA, introduced either from without or generated intracellularly by invading viruses. We have however confirmed, at the transcriptional level, previous observations showing that acid stable IFN is produced by untreated Namalwa cells incubated at high density (28) and noted that only IFN-β mRNA is accumulated, whereas viral induction gives rise to both alpha and beta IFN mRNAs in these cells. If this effect is due to selective transcription of IFN-β mRNA and not to preferential degradation of IFN-α mRNAs this could mean that there are

independent pathways for the induction of IFN-α and IFN-β. No clear differences in the mRNA patterns were observed following induction with different viruses or using enhancers of induction.

What bearing do the transcript levels have on the level of the different IFNs produced by induced cells? One cannot, a priori, expect a strict correlation between mRNA level and extracellular IFN accumulation, as many factors, such as efficiency of translation, processing and secretion, as well as unequal protein stability may affect the final IFN levels. In fact, in the case of IFN-β the transcript level was not proportional to the amount of antiviral activity found in the supernatants: both fibroblasts and HeLa cells induced with NDV contained about 500-1000 IFN-β-specific RNA molecules per cell, however the antiviral potency of the fibroblast supernatants was 20-30 times greater than that of the HeLa cells. Poly(A)$^+$ RNA from Hela cells and fibroblasts had similar IFN-β mRNA activity, as shown by oocyte injection experiments; thus the failure of Hela cells to produce antiviral activity may be due to events at the translational or post-translational level. Similarly, density-induced lymphoblastoid cells had 100 IFN-β transcripts per cell, but no detectable level (less than 10 U/ml) of antiviral activity; the unexpectedly high level of IFN-β mRNA in virus-induced leukocytes has been mentioned above. There is, however, no indication of major discrepancies in the case of IFN-α. Allen and Fantes (23) purified IFN from Sendai virus-induced Namalwa cells and, by partial amino acid sequencing identified IFN-α2 (IFN-A) and -α1 (IFN-D), as well as peptides ascribed to three additional IFNs, C, F and G (48). Levy et al. (24) separated IFN from leukocytes of patients with chronic myelogenous leukemia into 10 fractions by HPLC; amino acid sequencing revealed that at least two of the distinct species had the same sequence, namely that of IFN-α2. Thus, analyses at both the transcript and the protein level indicate that IFN-α1 and IFN-α2 are the predominant IFN-α species in natural IFN preparations.

ACKNOWLEDGMENTS

This work was supported by the Kanton of Zürich and the Schweizerische Nationalfonds (No. 3.147.81). J.H. was a Fellow of the Leukemia Society of America. We thank the following for generous gifts: M. Aguet (NDV, VSV); W. Fiers (IFN-β plasmid); J. and A. Fujisawa (IFN-α5 plasmid, IFN-γ clones); K. Henco (IFN-α4 plasmid); H. Ragg (IFN-α1 plasmid); J. Schmid (IFN-α6 plasmid); and M. Streuli (IFN-α2 plasmid, IFN-γ clones); Biogen S.A., Geneva for IFN expression plasmids.

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