MULTIPLE HUMAN β INTERFERON GENES*

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The human α and β interferon (IFN) gene family is even more complex than has been generally appreciated. Human interferons are classified as α (leukocyte), β (fibroblast), and γ (immune) mainly on serologic grounds (1). Several investigators (2–7) have cloned and characterized at least 8 distinct cross-hybridizing species of IFN-α cDNA and a set of up to 16 cross-hybridizing IFN-α genes and pseudogenes. These genes do not have introns (3, 8), are localized to human chromosome 9 (9), and appear to correspond to mature polyadenylated mRNA of length ~0.8–1.4 kilobases (kb). These are collectively referred to by us as IFN-α_S mRNA (10, 11). We recently described a second heterodisperse set of translationally active IFN-α mRNA of length 1.6–3.5 kb (peak activity at 1.8 kb), designated IFN-α_L, which code for interferons that are serologically of the α type, but that do not appear to cross-hybridize with α_S-specific DNA probes (10, 11). Several investigators have also described the molecular cloning and characterization of a single species of human IFN-β cDNA ("β_1") (12) derived from poly(I)·poly(C)-induced diploid human fibroblasts (13–19). Characterization of the human chromosomal DNA indicates that there exists a single gene corresponding to IFN-β_1 that does not have introns (20–24) and that can also be localized to human chromosome 9 (9). Nevertheless, earlier results of genetic experiments with somatic cell hybrids had indicated that human IFN-β genes were located on human chromosomes 2, 5, and 9 and that the presence of any of these human chromosomes alone was sufficient for the expression of human IFN-β in somatic cell hybrids (25–30). However, the results of these somatic cell hybrid experiments have been controversial. On the one hand, Slate and Ruddle (27, 28) obtained evidence for the involvement of chromosomes 2, 5, and 9 in IFN-β production, while, on the other hand, Meager and his colleagues (29, 30) concluded that chromosome 9 alone was involved in IFN-β production. The latter investigators were unable to find evidence to implicate chromosomes 2 or 5, although low levels of human IFN production were observed in somatic cell hybrids lacking chromosome 9. Data presented in the present report indicate that the human IFN-β system is quite complex and that the chromosomal localization controversy can be resolved.

Materials and Methods

We prepared polyadenylated IFN mRNA by inducing confluent cultures of FS-4 cells and of several human-mouse somatic cell hybrids with poly(I)·poly(C) (P.-L. Biochemicals, Inc., Milwaukee, WI) and cycloheximide (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) for 4 h, extracting either the total cellular or the cytoplasmic RNA, followed by poly(U)-Sepharose (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) chromatography (12, 34, 35). A small aliquot (usually, 2 µl out of 25 µl) of each preparation has been first assayed for IFN mRNA activity using the *Xenopus* oocyte assay (12, 34, 35). Suitable mRNA samples (10–75 µg) are mixed with marker ^{32}P-labeled HeLa cytoplasmic RNA, denatured in 12.5 mM CH_3HgOH (in 45-µl volume), and analyzed by electrophoresis through a 2% agarose-10 mM CH_3OH tube gel (11, 12, 36, 37). Thus, RNA electrophoresis is carried out under stringent denaturing conditions. Because marker RNA species are located by Cerenkov counting of the same set of gel slices from which IFN mRNA is subsequently eluted (recovery, 50–80%), there is little or no ambiguity in relating the observed IFN mRNA to the marker RNA species used. The RNA length estimates in nucleotides for each observed species of IFN-β mRNA are indicated in each experiment. The IFN products synthesized by *Xenopus* oocytes microinjected with ~40% (2 µl out of 5 µl) of each gel-selected mRNA fraction have been assayed using the conventional semi-micro cytopathic effect inhibition assay with human trisomy 21 cells of the GM2504 or GM2767 strain (obtained from the Human Genetic Mutant Cell Repository, Camden, NJ) as cell substrate and with vesicular stomatitis virus as the challenge virus. Ordinarily, we can clearly detect IFN at a concentration of 2 reference U/ml in the oocyte incubation medium. Routinely, ~60% of each RNA fraction is available for additional studies. All of the oocyte IFN products tested and described in this article were completely neutralized by an anti-IFN-β₁ antiserum (raised by Dr. Y. H. Tan, University of Calgary, Canada, against homogeneous human IFN-β₁ protein) and none of those tested by an anti-IFN-α antiserum (obtained from Dr. E. A. Havell, Trudean Institute, Saranac Lake, NY).

Results

We recently detected two distinct species of translationally active (in *Xenopus* oocytes) IFN-β mRNA in polyadenylated RNA preparations derived from poly(I)· poly(C)-induced human diploid fibroblasts (FS-4 strain) (12). The mRNA species were resolved by electrophoresis of RNA under stringent denaturing conditions in agarose-CH_3HgOH tube gels. IFN-β₁ (which corresponds to the Knight-Taniguchi protein and cDNA sequence) (14, 31) is of length ~0.9 kb and IFN-β₂ of length ~1.3 kb. Although IFN-β₁ and IFN-β₂ mRNA code for IFN proteins that are neutralized by anti-IFN-β₁ antisera, their nucleic acids do not cross-hybridize even under relaxed hybridization conditions (12). Similar observations have also been described by Weissenbach and his colleagues (32).

Noncoordinate Expression of IFN-β mRNA. After the discovery of human IFN-β₁ and β₂ mRNA in poly(I)·poly(C)-induced FS-4 cells, we proceeded to investigate various experimental conditions that might influence the expression of the two mRNA species. These studies led to the observation that different derivatives of the FS-4 cell strain can consistently express grossly different relative amounts of IFN-β₁ and β₂ mRNA after induction with poly(I)·poly(C) (Fig. 1). Most of our previous experiments dealing with the induction of IFN-β by poly(I)·poly(C) in cultures of diploid human fibroblasts (34–38) were carried out using a batch of FS-4 cells obtained from Dr. Jan Vilček (New York University School of Medicine) in 1973. We have already shown (12) that several different IFN mRNA preparations derived from these cells consist mainly of IFN-β₂. Fig. 1A is another example of the IFN-β mRNA profile in these cells after induction ("β₂ phenotype") (range of RNA length estimates 1,241–1,443 nucleotides; mean, 1.3 kb). A second batch of FS-4 cells obtained from Dr. Vilček in
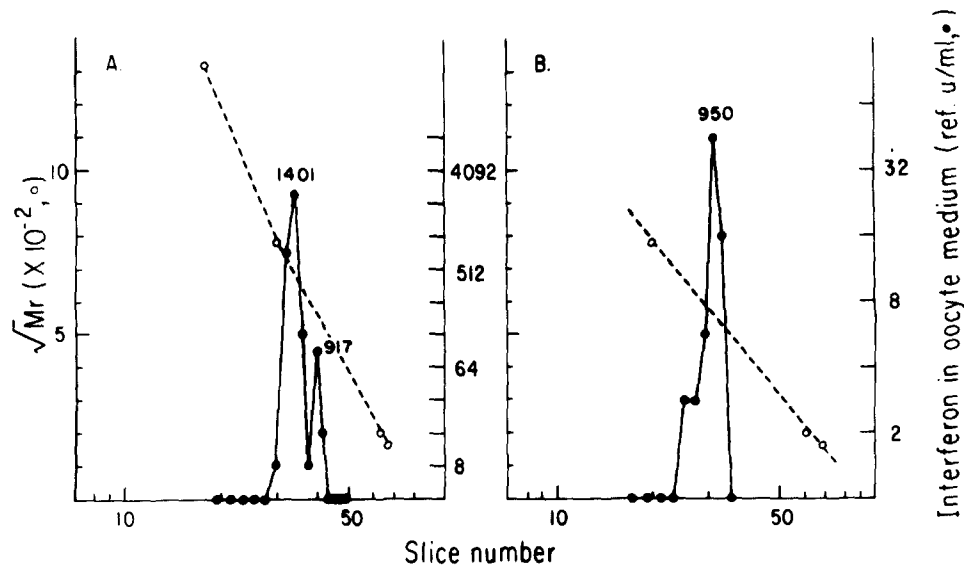
FIG. 1. Noncoordinate expression of IFN-β mRNA in poly(I)·poly(C)-induced FS-4 cells. Confluent cultures of FS-4 cells grown in Eagle's MEM supplemented with 10% bovine serum in 150-mm Falcon plastic petri dishes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) were induced with poly(I)·poly(C) (30 μg/ml) and cycloheximide (50 μg/ml) in 10 ml Eagle's MEM for 4 h, the cells harvested by trypsinization, polyadenylated RNA prepared by poly(U)-Sepharose chromatography of the extracted cellular RNA, appropriate amounts of the poly(U)-selected RNA (10–50 μg) mixed with ^{32}P-labeled HeLa cytoplasmic RNA, denatured in 12.5 mM CH_3HgOH for 5–10 min at room temperature in a 45-μl volume, and then electrophoresed through a 2% agarose-10-mM CH_3HgOH tube gel, using procedures that have been described earlier (11, 12, 36, 37). IFN mRNA eluted from pools of adjacent gel slices (two 1-mm slices/pool) was detected by microinjection of 2 μl of each RNA sample (of 5 μl) into *Xenopus* oocytes (10–15 oocytes in 0.2 ml Barth's medium incubated for 24–48 h), followed by determination of the IFN titer in the incubation medium (11, 12, 37, 42, 43), using GM2504 or GM2767 cells and vesicular stomatitis virus as the challenge virus. IFN titers (●) are expressed in terms of the 69/19 reference standard. The lengths in nucleotides of the observed IFN mRNA species are derived from the migration of marker 28 S, 18 S, 5 S, and 4 S RNA (○) in the same tube gel (12) and are indicated next to each peak. The IFN mRNA profile in RNA from induced cultures derived from the 1973 batch of FS-4 cells and that in RNA from the 1975 batch are indicated in panels A (45 μg RNA) and B (10 μg RNA), respectively.

the fall of 1975 was found to express mainly IFN-$β_1$ (Fig. 1 B, "$β_1$ phenotype") (range of RNA length estimates 861–1,019 nucleotides; mean, 0.9 kb). These two batches of cells were found to express the appropriate $β_2$ or $β_1$ phenotype in experiments over a period of several months (data not shown). These data suggest that the two IFN-β mRNA species can be expressed in a noncoordinate manner.

Further Heterogeneity of IFN-β mRNA Species. Figs. 2 and 3 reveal further heterogeneity of IFN-β mRNA species derived from some batches of FS-4 cells. Fig. 2 illustrates three different preparations from induced FS-4 cells that display IFN-β mRNA activity in RNA of length ~1.8 kb (close to the 18 S marker). The data in Fig. 2 B clearly demonstrate that the long IFN-β mRNA described in Fig. 2 A, which runs close to the 18 S marker, which in turn is included in the same agarose-CH_3HgOH tube gel, is not an aggregation artifact. For convenience, we refer to the species of IFN-β mRNA of length of ~1.8 kb (range, 1,628–1,939 nucleotides; mean, 1.8 kb) as IFN-$β_3$ mRNA (37). Fig. 3 illustrates three different preparations of IFN mRNA obtained from poly(I)·poly(C)-induced FS-4 cells that exhibit IFN mRNA of length
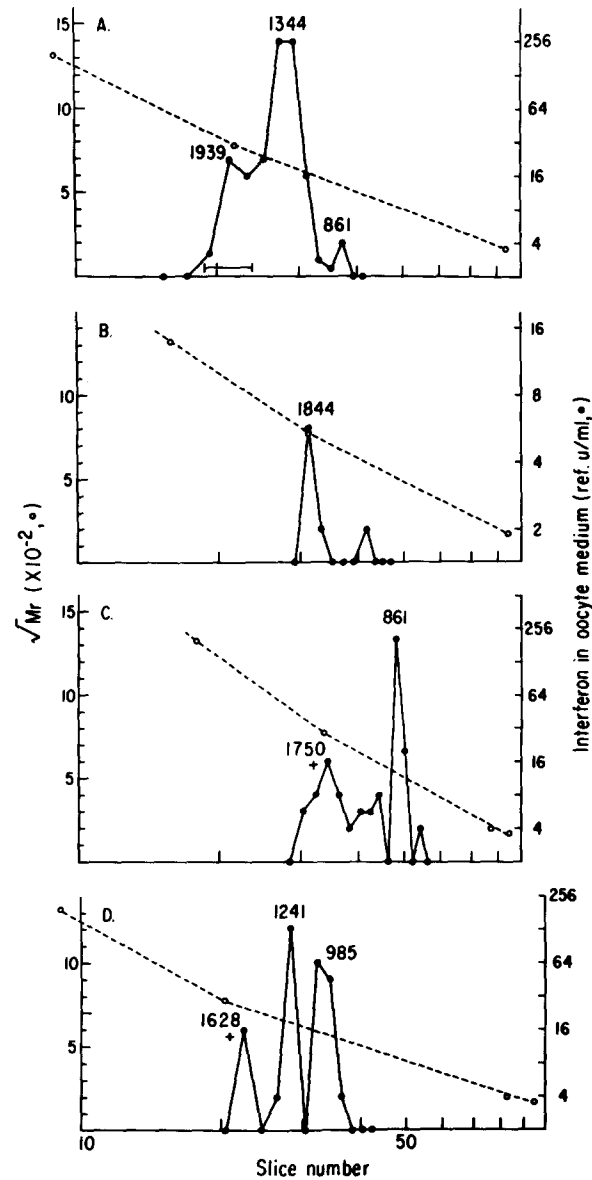
Fig. 2. Human IFN-β_3 mRNA. IFN mRNA (●) profile in polyadenylated RNA (30–50 μg) derived from FS-4 cells induced with poly(I)·poly(C) in the presence of cycloheximide and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) for 4 h (panels A, B, C) or with poly(I)·poly(C) alone (panel D). Panels A and D represent total cellular polyadenylated RNA preparations, whereas panel C is a cytoplasmic polyadenylated RNA preparation. 60% of the RNA eluted from gel slices indicated in panel A by the horizontal bar were pooled, denatured a second time in CH₃HgOH, and reelectrophoresed through a second agarose-CH₃HgOH tube gel (panel B). +, indicates that oocyte IFN products derived from the 1.7–1.8 kb RNA, shown in panels C and D, were neutralized by an anti-IFN-β_1 antiserum. The IFN titers in the absence of antiserum were 8 and 16 reference U/ml, whereas the residual titers after mixing with anti-IFN-β_1 antiserum were <2 and <2 reference U/ml for the products of the long mRNA in panels C and D, respectively. Antiserum controls are illustrated in Table I. Marker RNA (○).
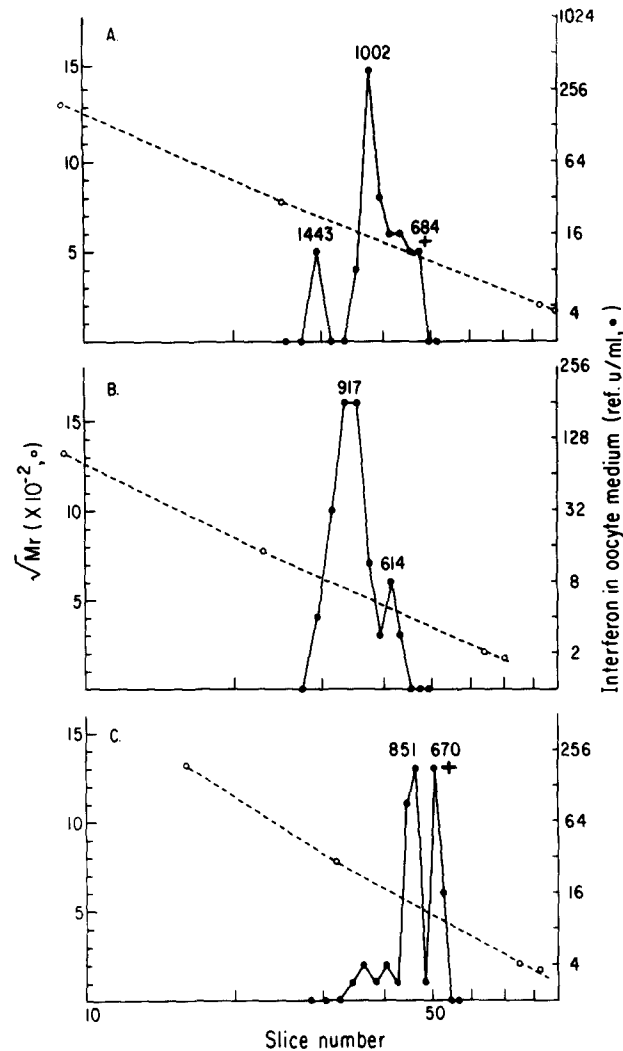
FIG. 3. Human IFN-β₄ mRNA. IFN mRNA (●) profile in cellular (panels A and B) and cytoplasmic (panel C) polyadenylated RNA (30–60 μg) derived from FS-4 cells induced with poly(I) · poly(C) in the presence of cycloheximide (panel A includes DRB). +, indicates that the oocyte IFN products derived from the 0.6–0.7 kb mRNA shown in panels A and C were neutralized by an anti-IFN-β₁ antiserum. The IFN titers in the absence of antiserum were 32 and 32 reference U/ml, whereas the residual titers after mixing with anti-IFN-β₁ antiserum were <2 and <2 reference U/ml for the products of the shortest IFN mRNAs shown in panels A and C, respectively. Antiserum controls are illustrated in Table I. Marker RNA (○).

between 0.6 and 0.7 kb. For convenience, we refer to this IFN-β mRNA (range, 614–684 nucleotides; mean, 0.65 kb) as IFN-β₄ mRNA.

The range of RNA length estimates for the individual IFN-β mRNA observed in numerous independent experiments over a period of 2 yr is nonoverlapping and in each instance corresponds to one fraction or a 2-mm distance out of 80–90 mm in a gel (distance from top of gel to 4 S marker RNA). Although our overall RNA recovery is in the range of 50–80% from one gel to the next, the extent of RNA recovery from

gel slices derived from the same gel is rather uniform. Because we mix in ^{32}P-labeled HeLa cytoplasmic RNA with IFN mRNA preparations before the gel run, we can directly monitor RNA recoveries across each gel. We are thus able to exclude the possibility that the data in Figs. 1 A, 2 D, 3 B, 3 C, and 4 C result from differences in RNA recovery.

With the discovery that translationally active human IFN-β mRNA are heterogeneous in RNA length and the realization that the expression of these mRNA can be consistently noncoordinate, it was apparent that we had the theoretical elements for the resolution of the chromosomal localization controversy in hand (25–30). The somatic cell hybrids analyzed by Meager et al. (29, 30) might have consistently expressed mainly IFN-β_1 derived from chromosome 9, while those analyzed by Slate and Ruddle (27, 28) might have expressed additional IFN-β mRNA derived from other chromosomes. We therefore proceeded to carry out gel analyses of IFN mRNA preparations derived from poly(I)·poly(C)-induced human-mouse somatic cell hybrids.

Human Interferon mRNA in Induced Human-Mouse Somatic Cell Hybrids. A series of human-mouse somatic cell hybrids that had been extensively investigated in the past for human interferon production and whose karyotype had been characterized were selected for study (Table I) (27, 28). These hybrids contained different combinations of human chromosomes 2, 5, and 9 and were known to be inducible by poly(I)·poly(C) for human IFN-β production in cell culture (27, 28). Appropriate cultures were induced with poly(I)·poly(C) in the presence of cycloheximide and cellular polyadenylated RNA prepared by poly(U)-Sepharose chromatography. An appropriate aliquot (2 μl out of 25 μl) was assayed in *Xenopus* oocytes for IFN mRNA activity. Poly(I)·poly(C) induction of the parental murine cells fails to induce any IFN or mRNA coding for IFN active on human cells (27, 28, 40). Table I shows that not only did many of the hybrids express human IFN mRNA activity, but also that all of the detectable IFN activity was of the β type. Thus, all of the hybrids listed in Table I express human IFN-β mRNA, even though most of them lack human chromosome 9. Similar results have also been obtained by Pitha et al. (40). It was rather surprising to find that BDA 17b17/DpT-2, a hybrid that appears to lack human chromosomes 2, 5, and 9, expressed IFN-β mRNA activity. Many of the IFN-β mRNA preparations listed in Table I were then subjected to analysis by agarose-CH$_3$HgOH gel electrophoresis. Fig. 4 summarizes some of the results obtained.

Gel Electrophoresis of IFN mRNA from Somatic Cell Hybrids. Fig. 4 A describes the IFN-β mRNA species observed in a hybrid that, against a murine background, contains a complex human chromosome involving translocated pieces of human chromosomes 9, 11, and X (28). This hybrid expresses an IFN-β mRNA of length ~0.9 kb. This result is consistent with the assignment of IFN-β_1 to human chromosome 9 (9).

Fig. 4 B and C describe two independently derived hybrids that have been extensively studied by isozyme patterns and karyotype analyses and are known to lack human chromosome 9. Nevertheless, both these hybrids express an IFN-β mRNA of length 0.9 kb. A derivative of AIM15aB1 that has also been extensively verified to lack human chromosome 9 (Fig. 4 D) also expresses an IFN-β mRNA of length 0.9 kb. Furthermore, a sister clone of AIM15aB1 (AIM15aA3) and a second batch of BDA10a3 cells have been found to be devoid of IFN-β_1-specific DNA, and transla-

TABLE I

Human IFN mRNA in poly (I)·poly (C)-induced Human-Mouse Somatic Cell Hybrids

Human-mouse hybrid	Human chromosome composition	Residual interferon titer in oocyte medium		
		No anti-serum	Anti-α	Anti-β₁
		reference U/ml		
29/21-30b SR	X, 9, 11	48, 32	ND*	<4
AIM 15aB1	2⁺5⁺9⁻	48, 32	ND	<4
		48, 16	32	<4
AIM 15aB1/DpT	2⁺5⁻9⁻	128, 64	ND	<4
BDA 10a3	2⁺5⁻9⁻ (10% 5⁺)	32, 32	ND	<4
		16, 32	64	<4
BDA 10a3/DpT	2⁺5⁻9⁻	12, 32	32	<4
BDA 17b17	2⁺5⁺9⁺	64, 128	ND	<4
		16, 32	32	<4
		8, 12	12	≤3
BDA 17b17/DpT-2	2⁻5⁻9⁻	64	64	<4
		16, 12	24	≤3
Antiserum controls				
IFN-α (leukocyte)		>256	4	>256
IFN β (fibroblast)		128	128	<2

Batches of appropriate human-mouse somatic cell hybrids were grown to confluence either in 850-cm² Corning plastic roller bottles (8–10 per batch) or in 150-mm Falcon plastic petri dishes (60–100 per batch) in Eagle's MEM supplemented with 5–10% fetal bovine serum and then induced with poly(I)·poly(C) (100 µg/ml) and cycloheximide (50 µg/ml) for 4 h. The cells were harvested by trypsinization, and cellular polyadenylated RNA was prepared (34, 35). Each mRNA sample (35–50 µg RNA) was dissolved in 25 µl sterile distilled water, and a 2-µl aliquot was assayed for IFN mRNA activity using the *Xenopus* oocyte assay (12). The serology of IFN in oocyte incubation medium was verified by the addition of 10 µl of anti-IFN-α antiserum (obtained from Dr. E. A. Havell, Trudean Institute, Saranac Lake, NY) or 2 µl of an anti-IFN-β₁ antiserum (obtained from Dr. Y. H. Tan, University of Calgary, Canada), as described earlier (12, 34, 37). Human leukocyte IFN (IFN-α) and human fibroblast IFN (IFN-β) samples were obtained from Dr. W. E. Stewart II and Dr. W. A. Carter, respectively. Each IFN titer represents a separate assay (42, 43), and each data line represents a different RNA preparation. The human chromosome composition of each hybrid was determined to be as follows: 29/21-30bSR: "X, 9, 11 translocated chromosome;" AIM15aB1: 1,2,5,7,11,12,13,14,15,17,18,20,21,X; AIM15aB1/ DpT: 1,2,7,11,12,13,14,15,17,18,20,21,X; BDA 10a3: 2,6,10,12,13,16,18,20,X,5 at 10% and a low frequency (<20%) of 3,4,8,11,17; BDA 10a3/DpT: 2,6,8,10,11,12,13,16,17,20,X and a low frequency (<20%) of 3,11; BDA 17b17: 1,2,4,5,6,9,12,13,14,16,18,20,21,X and trace of 3; BDA 17b17/DpT-2: 4,13,21.
* Not done.

tionally active IFN-β mRNA preparations derived from these particular hybrids failed to hybridize with IFN-β₁ cDNA probes (40). Similarly, a second sister clone of AIM15aB1 (AIM15aA1) and another batch of BDA10a3 cells were found to be devoid of IFN-α₁-specific DNA sequences that are also known to map to chromosome 9 (9, 44). This excludes the possibility that aberrant fragments of chromosome 9 containing the IFN-β₁ gene are retained in the AIM15a and the BDA10a3 series of human-mouse hybrids.

Because from Table I, we know that all of the IFN mRNA species described in Fig. 4B, C, and D are of the β type, for convenience, we refer to the 0.9 kb mRNA that we are unable to assign to chromosome 9 as IFN-β₅ mRNA. Thus, in a formal sense,
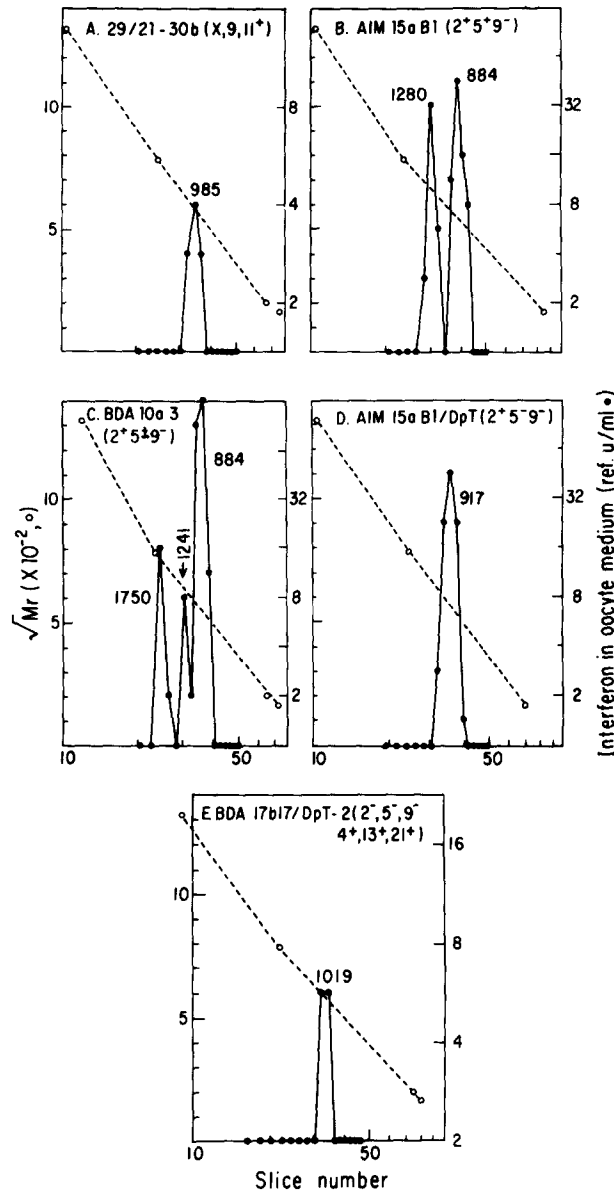
Fig. 4. IFN-β mRNA (●) species in a selection of human-mouse somatic cell hybrids induced with poly(I)·poly(C) in the presence of cycloheximide. Appropriate preparations (35–50 μg) of translationally active IFN-β mRNA illustrated in Table I were analyzed by agarose-CH$_3$HgOH gel electrophoresis. mRNA eluted from gel slice pools was dissolved in 2 μl water, and the entire sample was assayed by microinjection into *Xenopus* oocytes. Panel A, 29/21-30bSR cells containing the X, 9, 11 translocated chromosome; panel B, AIM 15a B1:$2^+5^+9^-$; panel D, its diptheria toxin-treated derivative (AIM 15aB1/DpT), which is therefore $2^+5^-9^-$; and panel C, BDA 10a3, designated $2^+5^-9^-$ by isozyme pattern. 10% of the cells in BDA 10a3 contain chromosome 5, as assayed by diptheria toxin-mediated cell killing and chromosome analyses; panel E, the diptheria-toxin derivative, BDA 17b17/DpT-2, which is $2^-5^-9^-$. Marker RNA (○).

the 0.9 kb IFN-β mRNA peak observed in preparations from induced human diploid fibroblasts (12) could represent IFN-β_1 or β_5 or both.

The data summarized in Fig. 4 are consistent with the tentative assignments of four of the five observed IFN-β mRNA to human chromosomes 2, 5, and 9. Those in Fig. 4 A are consistent with the assignment of IFN-β_1 to chromosome 9. Fig. 4 B shows that a hybrid that is $2^+5^+9^-$ expresses IFN-β_2 and β_5, whereas its diptheria toxin-treated derivative that has lost chromosome 5 (Fig. 4 D) expresses only IFN-β_5. Thus, data in Fig. 4 B and D are consistent with the assignment of IFN-β_2 to chromosome 5. Fig. 4 C represents an independent confirmation of this assignment. Approximately 10% of BDA 10a3 cells, which as a population are $2^+5^-9^-$ (by isozymes), contain chromosome 5 (by diptheria toxin cell-killing and by chromosome analyses). These cells express a small amount of IFN-β_2 (Fig. 4 C). The data in Fig. 4 C and D are also consistent with the assignment of IFN-β_3 and β_5 to chromosome 2. In addition, the data in Fig. 4 E suggest that the human IFN-β system might be even more complex. A human-mouse hybrid (BDA 17b17/DpT-2), which appears to lack human chromosomes 2, 5, and 9, expresses IFN-β mRNA of length ~1 kb. The karyotype of this hybrid is presently under detailed investigation. Perhaps Fig. 4 E provides the first indication of a sixth species of human IFN-β mRNA that may derive from a chromosome other than 2, 5, or 9.

These data clearly show that IFN-β genes are located on at least three different human chromosomes. However the particular assignments are still tentative and will need to be confirmed by the molecular cloning of the individual IFN-β mRNA species and by the chromosomal mapping of these genes using recombinant DNA procedures. At the present time, we cannot exclude the possibility that IFN-β_3 and β_5 derive from the same gene, nor can we exclude the possibility that IFN-β_4 derives from one of the other IFN-β genes.

Discussion

Our studies have provided a basis for the resolution of the chromosomal localization problem that has existed in the IFN-β field for the last 7 yr. Briefly we consider that the observations reported by previous investigators (25–30) were essentially correct but that the earlier interpretations of the then available data did not fully recognize the complexities involved in the structure and expression of human IFN-β genes. The IFN-β gene family, of which only IFN-β_1 has been cloned and characterized convincingly (13–24), is dispersed in the human genome. We have shown earlier (12) that an IFN-β_1 cDNA probe does not cross-hybridize IFN-β_2 mRNA, even under rather relaxed hybridization conditions. The failure to pick up chromosomal DNA clones other than IFN-β_1 by screening human DNA gene banks with IFN-β_1 cDNA probes (20–24, 33) suggests that the other dispersed IFN-β genes do not cross-hybridize IFN-β_1 DNA, even though they code for proteins that are neutralized by an antiserum raised against homogeneous IFN-β_1 protein. Furthermore, it appears that these dispersed genes can be expressed independently of each other. Though the IFN-β_1 is a gene without introns (20–24, 33), there is suggestive evidence that IFN-β_2 might be a gene with introns (41, and Michel Revel, personal communication). It is clear that a great deal still remains to be learned about the structure and expression of human IFN-β genes.

Are IFN proteins corresponding to the IFN-β mRNA observed by us produced in

cell culture? The fact that attention was focused on somatic cell hybrids described in Table I, mainly because they were found to produce IFN-β in cell culture after induction with poly(I)·poly(C) (27, 28), suggests that the answer to this question is in the affirmative. Furthermore, in preliminary experiments carried out using FS-4 cells known to exhibit the β_2 phenotype, we (P. B. Sehgal, Y. H. Tan, and colleagues, unpublished data) have detected an IFN-β-specific polypeptide distinguishable by its mobility in a polyacrylamide gel from authentic IFN-β_1 in the culture medium of superinduced FS-4 cells using a polyacrylamide gel blotting procedure. It will be of interest to determine whether these different IFN are tailored to represent specific host-defense responses to combat specific situations.

Summary

Analysis of human β interferon (IFN) mRNA preparations obtained from poly(I)·poly(C)-induced human diploid fibroblasts (FS-4) and from several similarly induced human-mouse somatic cell hybrids by electrophoresis through agarose-CH$_3$HgOH tube gels led to the detection of at least five translationally active human IFN-β mRNA species. The results obtained are consistent with the existence of IFN-β genes on different human chromosomes. Marked cell-dependent variability in the expression of these IFN mRNA species was observed.

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