The absence of introns within a human fibroblast interferon gene


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ABSTRACT

Experiments in which immobilised restriction fragments of genomic DNA were hybridised with a cloned human fibroblast interferon cDNA indicate that the homologous chromosomal genes exist in only one basic arrangement. This is in marked contrast to recent studies by Nagata et al. (1) showing that there are at least eight gene arrangements for human leukocyte interferon.

Having isolated a chromosomal human fibroblast interferon gene from a gene bank, we conclude from nucleotide sequencing studies that there is a complete absence of introns within the mRNA-coding region. In view of a similar observation recently made for a human leukocyte interferon gene (1), it would appear as if interferon genes in general are unlike the vast majority of eukaryote genes in this respect.

INTRODUCTION

The important problem concerning how many genes encode human interferon proteins has recently received much illuminating investigation. Firstly, by comparing the nucleotide structure of a human leukocyte (IFN-α) and fibroblast (IFN-β) cDNA clone, Taniguchi et al. (2) concluded that these interferons are the products of two separate, but structurally related genes.

That there may be more than one gene coding for human IFN-α proteins and the α-type (3) human lymphoblastoid interferons, has been one explanation for the widely observed heterogeneity exhibited by these molecules (4,5). Direct evidence for the existence of multiple IFN-α genes first became available from work in which two different leukocyte interferon cDNAs were cloned (6), both of which have the potential to code for proteins with a different primary structure to that determined for a human lymphoblastoid interferon (7).

More recently, Allen and Fantes (8) purified and sequenced
a human lymphoblastoid interferon preparation and concluded that there must be several distinct genes coding for these diverse molecules. Consistent with this hypothesis is the parallel study by Nagata et al. (1) who used a leukocyte cDNA clone to screen a human gene bank and hence isolate at least 8 distinct, homologous gene arrangements. Furthermore, by detailed investigation of one of these chromosomal genes, they concluded that there was no evidence for the existence of introns. As such, this gene represents a rare exception to the widely observed phenomenon of introns within eukaryote genes (9).

The situation with human IFN-β genes is less advanced. Generally speaking, preparations of this interferon exhibit less heterogeneity than those of IFN-α (10) and only one particular protein sequence has been elucidated so far, although this is only a partial, amino-terminal sequence (11,12). DNA complementary to the mature mRNA molecule that codes for this protein sequence has been isolated and sequenced by three independent laboratories. There is only one difference between these sequences, being a 'silent' nucleotide change in a tyrosine-coding triplet (13-16).

In order to extend our knowledge of IFN-β gene organisation, we report here the results of experiments in which an IFN-β cDNA clone was used to characterise and isolate homologous chromosomal genes. The structure of one gene was analysed in detail in order to establish the presence or absence of introns.

**INTERFERON NOMENCLATURE**

The nomenclature used here was according to a recent proposal (17).

**MATERIALS**

Restriction enzymes and T₄ DNA ligase were purchased from New England Biolabs. T₄ polynucleotide kinase came from P-L Biochemicals Inc., while bacterial alkaline phosphatase (BAPF) was from Worthington Enzymes. Radioactive nucleotide derivatives were obtained from The Radiochemical Centre (U.K.).

The strain 17/1 fibroblast cell-line was derived from human embryo lung tissue. The Hae III/Alu I human chromosomal gene library was kindly donated by Dr. T. Maniatis.
METHODS

Cloning human IFN-β cDNA: The isolation of these clones and the production of biologically active interferon from them will be described in detail elsewhere (30).

Analysis of restriction digests of genomic DNA: High molecular weight DNA was isolated from strain 17/1 human fibroblasts by our previous methods (18). Following restriction of 10-15 µg with an excess of enzyme, DNA was purified by phenol/chloroform (1:1) extraction and precipitated with ethanol. It was then dissolved and electrophoresed through native (19) 1% agarose gels (20 x 14 x 0.5 cms) at 150V for 3 hours. The fragments were then transferred to a nitrocellulose filter in 20 x SSC (1 x SSC is 0.15M NaCl, 0.015M Na citrate, pH 7.6) as described by Southern (20). The filters were pre-treated and then hybridised with 'nick-translated' (21) cloned IFN-β cDNA (0.03 µg/ml; 2 x 10^6 dpm/µg) as described by Flavell et al. (22). The IFN-β cDNA was obtained by restricting the recombinant plasmid and excising the 730 base-pair fragment containing the cDNA from a polyacrylamide gel as previously described (23).

Following incubation at 65°C for 2 days in hybridisation buffer (3 x SSC, 0.2% (w/v) BSA, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) Ficoll 400, 0.1% (w/v) SDS, 10 µg/ml poly A, 50 µg/ml sheared, single-strand salmon sperm DNA, 0.03 µg/ml IFN-β cDNA), the filters were washed several times in hybridisation buffer at 65°C and then given 2 final washes in either 1 x SSC or 0.3 x SSC containing 0.1% (w/v) SDS at 65°C for 30 mins. each. Following blotting dry, filters were autoradiographed using Kodak XH-1 film in a Kodak regular intensifier cassette. Exposure was at -70°C for around 7 days.

Isolation and characterisation of the IFN-β chromosomal gene

The human gene library was propagated both on plates and in liquid culture in the host LE 392, obtained from Dr. P. Leder. The library was initially amplified by the PDS method (24) starting from a stock of 3 x 10^6 phage. First-round screening was carried out on 23 cm. square plates containing about 50,000 plaque-forming units/plate. Subsequent screening was on 9 cm. plates containing less than 200 pfu. Transfer of DNA to nitrocellulose for plaque hybridisation was as described (25).
DNA was transferred for 2-3 minutes and the filters then denatured in 0.5M NaOH, 1.5M NaCl for 2 minutes, neutralised for 4 minutes in 0.5M Tris-HCl pH 7.5, 1.5M NaCl and baked at 80°C for 2 hours. Filters were pre-treated and hybridised with "nick-translated" cloned IFN-β cDNA essentially as described above. Restriction digests of phage DNA were electrophoresed through agarose gels, transferred to nitrocellulose and 'probed' with the cloned IFN-β cDNA also essentially as described above.

Sub-cloning the 1550 base-pair EcoRI fragment of λ-H.IFN-β/Pad 3

The EcoRI fragment was eluted from a 5% native polyacrylamide gel as described (23). 0.01 μg was then ligated with 0.08 μg of pAT153 plasmid (49) that had been restricted with EcoRI and then treated with bacterial alkaline phosphatase in order to eliminate subsequent transformation with non-recombinant plasmid. These methods and the subsequent transformation into E. coli K12 HB101 are essentially as described elsewhere (26). Recombinant plasmid DNA was purified from cleared lysates by centrifugation through caesium gradients containing ethidium bromide (27).

Nucleotide sequencing of the pAT153/chromosomal IFN-β sub-clone.

Chemical cleavages were carried out according to Maxam and Gilbert (28) and the reaction products were analysed as described by Porter et al. (29). 10 μg of pure plasmid DNA were restricted with either EcoRI, Bgl II, BstE II, or Hga I and then extracted with phenol/chloroform and precipitated with ethanol. Following treatment with alkaline phosphatase and further extraction (see below), the DNA was incubated at 37°C for 30 minutes in 25 μl containing 50 mM Tris/HCl, pH 7.6, 10 mM MgCl₂, 5 mM DTT, 0.1 mM spermidine, 0.1 mM EDTA, 40 pmoles of [γ-32P] ATP (> 5000 Ci/mmol), and 10 units of T4 polynucleotide kinase. The mix was then brought to 2M NH₄Ac and precipitated with 3 volumes of absolute ethanol. After centrifugation (10,000 x g; 10 mins.), the DNA was either re-restricted with Pvu II (for the EcoRI label) or EcoRI (for the other 3 end-labels) and appropriate radioactive fragments (Fig. 6) were extracted from a native 5% polyacrylamide gel by excising the required gel slice and electrophoresing the DNA on to a small pad of DE52. The DNA was eluted by high salt, precipitated with ethanol and sequenced.

For end-labelling the Pvu II cleavage site in the IFN-β
gene, 10 μg of plasmid were restricted with this enzyme and extracted as above. Following treatment with bacterial alkaline phosphatase by incubating at 65°C for 30 min. in 25 μl containing 20 mM Tris/HCl, pH 7.5, 0.1% (w/v) SDS, 0.7 mg/ml alkaline phosphatase, the DNA was extracted 3 times with phenol/chloroform, twice with ether and precipitated with ethanol. Efficient end-labelling was achieved by first dissolving the DNA in 22.5 μl containing 16 mM Tris/HCl, pH 9.5, 0.8 mM spermidine, 0.08 mM EDTA and denaturing at 90°C for 2 mins. It was then incubated immediately at 37°C for 30 min. in 25 μl containing 60 mM Tris/HCl, pH 9.5, 0.7 mM spermidine, 0.07 mM EDTA, 10 mM MgCl₂, 5 mM DTT, 5% (v/v) glycerol, 40 pmoles [γ-³²P] ATP (> 5000 Ci/mmole) and 10 units of T₄ polynucleotide kinase. Following ethanol precipitation, the DNA was dissolved in 50 μl containing 300 mM Tris/HCl, pH 7.5, 150 mM NaCl, 15 mM MgCl₂ and re-annealed at 67°C for 15 minutes. It was then re-restricted with EcoRI and the appropriate radioactive fragments (Fig. 6) were extracted from a native 5% polyacrylamide gel and sequenced as described above.

RESULTS

Our investigation into the organisation of IFN-β genes was initiated by digesting high molecular weight, total fibroblast DNA with various restriction enzymes and separating the products on agarose gels. Following transfer of the fragments to nitrocellulose filters, the latter were hybridised with the excised, cloned IFN-β cDNA that had been radioactively labelled to high specific activity using the process of nick-translation.

In order to prove that we obtained complete digestion with each restriction enzyme, we routinely used two incubation conditions. Although both incubations theoretically contained an excess of restriction enzyme, one was incubated for twice as long and with twice as much enzyme. Hence, the resulting similarity in restriction patterns indicates that completion of digestion was achieved. Double digests were carried out under the x2 conditions for each enzyme.

Fig. 1 shows the resulting autoradiograph obtained after digesting genomic DNA with either Hpa II, EcoRI or Hind III.
Figure 1: Hybridisation of 'nick-translated' IFN-β cDNA probe to various restriction digests of genomic DNA. Genomic DNA was restricted and the resulting fragments were electrophoresed and transferred to a nitrocellulose filter for hybridisation. The filter was given a final wash in 0.3 x SSC, 0.1% (w/v) SDS at 65°C before autoradiography. 'End-labelled' restriction fragments of phage PM2 DNA restricted with Hind III were used as size markers (14). The sizes in base-pairs of hybridising fragments were estimated as: Hpa II, 3800; EcoRI, 1900; Hind III, >5400. (A double digest of EcoRI and Hpa II is also shown next to the molecular weight markers.)

From the mature mRNA sequence determined in our and other laboratories (13-16), it can be determined that none of these enzymes should cleave the corresponding DNA sequence. The result shows that only one radioactive band was seen in all cases from which two conclusions can be drawn. Firstly, it is clear that there are no cross-hybridising IFN-β genes that contain a different restriction map, for the above enzymes, in their gene-flanking regions. One possible interpretation of this result is that there is actually only one type of IFN-β gene in the genome that
is capable of hybridising with our probe. Secondly, it could be noted that this result furnished no evidence for the presence of introns within the homologous chromosomal gene.

In order to test these interpretations, we next repeated this type of experiment but this time using restriction enzymes (either in single or double digests) that are known to have cleavage sites in the mRNA-coding DNA sequence (13-16). For clarification, Fig. 2 shows the positions at which Mbo I, Pvu II and Tag I cleave the cDNA sequence and also the region which is homologous to our cDNA probe. For reasons of expression of the cDNA in bacteria (30), we specifically obtained a cDNA clone essentially corresponding to the mature protein-coding region and the 3' untranslated mRNA sequence. It therefore lacks most of the DNA coding for the signal peptide sequence and also all of the 5' untranslated mRNA sequence.

Mbo I and Pvu II enzymes each cleave the cDNA sequence in one position only and assuming that there is only one type of IFN-β gene present in the genome and that it has no introns containing cleavage sites for these enzymes, one would expect to see only two radioactive bands resulting from the type of experiment described above, when total DNA is digested with either enzyme. Fig. 3 shows that this was in fact the observed result.

Tag I enzyme also cleaves the cDNA sequence in one position only but since this site is only about 18 base-pairs from the terminus corresponding to the 5' terminus of the mature mRNA (Fig. 2), our cDNA probe will not be able to detect the upstream restriction fragment. The result in Fig. 3 shows that only one

Figure 2: Restriction map of IFN-β cDNA sequence. Sizes of fragments are given in base-pairs. The region which is homologous to our cloned IFN-β cDNA probe is also indicated.
Figure 3: Hybridisation of 'nick-translated' IFN-β cDNA probe to various restriction digests of genomic DNA. Genomic DNA was restricted with either one or two enzymes. The fragments were processed as in Fig. 1 except that the filter was given a final wash in 1 x SSC, 0.1% (w/v) SDS at 65°C. The sizes in base-pairs of hybridising fragments were estimated as: Mbo I/Pvu II, 1320, 1130 and 380; Pvu II, >5400 and 1850; Mbo I, 1500 and 1280; Mbo I/Taq I, 650 and 240; Taq I, 870.

major radioactive band was observed corresponding to a fragment of about 870 base-pairs. Since the cDNA sequence itself contains only 836 base-pairs, this indicates the presence of a Taq I cleavage site about 50 base-pairs downstream from the DNA.
coding for the 3' mRNA terminus, assuming the absence of introns within the chromosomal gene.

It is perhaps significant that some very faint radioactive bands were also apparent in this autoradiograph (Fig. 3). This filter was given a less-stringent, post-hybridisation wash than the filter autoradiographed in Fig. 1 and hence it is possible that under these conditions, the cDNA probe is allowed to mis-match with a different but related gene. There is significant homology between IFN-α and IFN-β genes (2) and it is possible that under these conditions, a low level of cross-hybridisation occurs. Alternatively, this effect may be due to the presence of other related IFN gene types.

Nonetheless, even in view of this uncertainty, it can be concluded from the collective data shown in Figs. 1 and 3 that there is only one chromosomal IFN-β gene-type that exhibits extensive homology with the cDNA probe.

A striking feature of this latter gene-type is that it would appear to contain either small introns or none at all. This is apparent not only from the size of the hybridising Taq I restriction fragment (Fig. 3) which is only about 50 base-pairs larger than the contribution from the mRNA-coding DNA, but also from the results obtained with double digestions with restriction enzymes. Fig. 3 shows that in the case of double digestions with Mbo I/Taq I and Mbo I/Pvu II, a particular hybridising restriction fragment of approximately 650 and 380 base-pairs was obtained, respectively. Assuming the absence of introns within this gene, the expected sizes of these fragments would be about 621 and 366 base-pairs, respectively (See Fig. 2).

Since the vast majority of eukaryote genes studied so far contain introns (9,31) and various important functions have been postulated for them (32), it was important to firmly establish the presence or absence of such sequences in this IFN-β gene. In order to do this, we isolated the chromosomal gene from a human DNA/λ phage-hybrid library and studied it's structure in detail.

The human library was constructed by Lawn et al. (33) by partially digesting foetal human chromosomal DNA with Alu I and Hae III and then isolating fragments of about 17,000 base-pairs.
Following an incubation with EcoRI methylase, synthetic EcoRI linkers were ligated to the ends of the human DNA which was then cloned into the λ Charon 4A phage vector.

Before screening this gene bank for IFN-β genes, the phage were grown by infection of host bacteria in liquid culture. 300,000 recombinants were then screened in duplicate, by plaque hybridisation (25) using the 'nick-translated' cloned cDNA as probe.

7 plaques strongly and reproducibly hybridised with the probe and DNA was subsequently prepared from 6 of these. Surprisingly, EcoRI restriction resulted in the production of identical fragment sizes in all 6 cases indicating that these recombinants were identical. In view of the methods employed in the construction of the human library, this result is unexpected and presumably means that during the initial amplification of the stock phage, only a small sub-population efficiently multiplied in the host bacteria. A similar phenomenon has been observed previously during isolation of Xenopus Laevis globin genes (34).

One of these clones was selected and used for mapping and sequencing studies and was designated λ-IFN-β/Pad 3 (H denotes the human origin of the gene and 'Pad 3' refers to the given number of the clone isolated at St. Mary's Hospital, Paddington).

Fig. 4 shows the autoradiograph obtained by restricting λ-IFN-β/Pad 3 with various enzymes, electrophoresing the fragments through an agarose gel and then transferring them to a nitrocellulose filter before hybridising with the 'nick-translated', cloned cDNA probe. The sizes of the radioactive fragments are given in the legend to Fig. 4.

It can be seen that in the case of the Bgl II/Pst I double digest, one of the radioactive fragments (380 base-pairs) is very similar to the actual distance separating these two unique cleavage sites (360 base-pairs) in the cDNA sequence (13-16). This result is consistent with our earlier results and again suggests the presence of only very small introns, if any, within the gene.

Based on the relative position of the Bgl II and Pst I cleavage sites within the gene, the data shown in Fig. 4 allow
Figure 4: Hybridisation of 'nick-translated' IFN-β cDNA probe to various restriction digests of λ-H IFN-β/Pad 3 DNA. Restriction fragments were processed as in Fig. 1. The sizes in base-pairs of the hybridising fragments were: a) EcoRI, 1550, b) EcoRI + Hsu I, 1250, c) EcoRI + Bgl II, 900 + 650, d) Bgl II, 2600 + 2250, e) Hsu I + Bgl II, 2200 + 660, f) Hsu I, 6800, g) Pst I, 7800 + 2350, h) Hsu I + Pst I, 5600 + 980, i) EcoRI + Pst I, 1250 + 340, j) Bgl II + Pst I, 1900 (doublet) + 380.

This allows us to determine the positions of the nearest EcoRI, Hsu I, Bgl II and Pst I cleavage sites within the flanking regions of the chromosomal gene.
This restriction map is shown in Fig. 5 along with an equivalent restriction map for the right arm of the λ Charon 4A vector DNA. It can be seen that the relative positions of the EcoRI, Bgl II and Hsu I sites in the latter are the same as in the upstream flanking region of the cloned chromosomal gene. There is also an Sst I cleavage site in the latter region corresponding to the one indicated in Fig. 5 in the vector DNA (I. Jackson - data not shown). It would seem therefore, that the EcoRI cleavage site which maps very close to that terminus of the cloned chromosomal gene corresponding to the 5' terminus of the mature mRNA, is in fact at the human DNA/vector DNA boundary and hence should be an artificial EcoRI site inserted as a result of using EcoRI linkers in the original cloning methods.

This contention is supported by the estimated positions of the nearest EcoRI cleavage sites to the strong cross-reacting chromosomal genes observed in Figs. 1 and 3. Although the position of the EcoRI sites could not be determined from these particular experiments, we were later able to locate these sites (by carrying out double digests with EcoRI and either Pvu II, Taq I or Mbo I) at approximately 300 base-pairs upstream and 740 base-pairs downstream from the mature mRNA-coding DNA (M. Houghton - unpublished data). This indicates that the

![Diagram of restriction map](image)

**Figure 5: Nearest restriction site map of λ-H.IFN-6/Pad 3 DNA**

This map was constructed from data shown in Fig. 4. A restriction map of the right arm of λ Charon 4A DNA is shown underneath. The code is as follows: B, Bgl II; E, EcoRI; H, Hsu I; P, Pst I; S, Sst I. The black rectangle indicates the assumed location of the mature 6 mRNA coding sequence.
upstream EcoRI site in λ-H. IFN-β/Pad 3 is indeed due to the presence of a synthetic EcoRI linker molecule. It should also be noted that the estimated position of the nearest downstream EcoRI site coincides with the position of this cleavage site mapped in the chromosomal clone (Fig. 5).

In order to facilitate sequencing of the nucleotide structure of the 1550 base-pair EcoRI fragment of λ-H. IFN-β/Pad 3, we sub-cloned this particular fragment into the plasmid vector pAT153 and then isolated selected 'end-labelled' restriction fragments for sequencing according to the method of Maxam and Gilbert (28). A schematic representation of the sequencing strategy is shown in Fig. 6 and this allowed the elucidation of the complete sequence from the EcoRI site (in the synthetic linker molecule) down to a point that was past the site of transcription termination. The individual sequences were overlapped by at least 25 nucleotides and in the case of the Bgl II and Pvu II 'end-labelled' fragments, we also checked the sequence at and around these cleavage sites by sequencing from neighbouring BstE II and Hga I sites, respectively (Fig. 6). The seq-

![Figure 6: Scheme of the nucleotide sequencing strategy employed for the pAT153/H. IFN-β sub-clone. The restriction enzyme map of the 1550 base-pair EcoRI fragment cloned into the plasmid vector pAT153 (3650 base-pairs) is shown. Distances between cleavage sites are given in base-pairs. The 'end-labelled' restriction sites used for sequencing are indicated underneath by vertical lines. The direction of sequencing and the approximate region covered are also indicated.](image)
quence could be clearly deduced from each autoradiograph (e.g. Fig. 7) and in this way we were able to establish the presence of an uninterrupted mRNA-coding DNA sequence identical to the one established collectively by this laboratory (14,15), and by the work of Derynck et al. (16). We could confidently rule out the presence of any size of intron within this sequence.

Fig. 8 shows a comparison of the upstream flanking region of the IFN-β gene with the corresponding region recently determined for an IFN-α gene (1). The sequences can be aligned to show considerable homology (~60% total).

The pre-determined 5' termini of the mature mRNAs (1,14) may not be complete if reverse transcriptase did not copy right to the extreme ends. Furthermore, it is conceivable that the 5' terminus of the primary transcript is separated from the rest of the mRNA molecule by an excisable intervening sequence. While possible, this would seem unlikely in view of the relative location of the apparent 'Hogness box' in the α gene to the tentative 5' terminus of the mature mRNA (Fig. 8). The distance separating these two features (27 nucleotides) is very similar to that which separates the 'Hogness box' from the 'cap' site in many genes (35,36,41) and there is evidence that the 'cap' site is actually the 5' terminus of the primary transcript RNA (37,50,51). Therefore, the likelihood of an intervening sequence in the 5' terminal region of the IFN-α mRNA is very small and because of the obvious homology between the α and β genes (Fig. 8), the same contention can be applied to the latter.

Fig. 8 also compares the downstream flanking regions of the α and β genes and shows that unlike the upstream flanking regions, the level of homology does not appear to be very significant. Both downstream regions are very A-T rich and as such are similar to the 3' untranslated mRNA sequences (6, 13, 16).

It should be noted that there is some uncertainty attached to the three G residues closest to the end of the downstream β sequence, owing to the fact that this region is well over 200 nucleotides from the 'end-labelled' Bgl II site and is therefore approaching the normal limit of this sequencing method. However, it can be seen that one of these tentative G residues contributes
Figure 7: Autoradiograph of a nucleotide sequencing gel: The result obtained by sequencing the 300 base-pair (approximately) EcoRI-Pvu II restriction fragment shown in Fig. 6. The EcoRI site in the upstream region of the cloned β gene is 'end-labelled'. The cleavage specificity of each slot in the gel is as before (14). Only one loading of the gel is shown being that from which the upstream sequence of the β gene could be deduced.
Figure 8: A comparison of the upstream and downstream regions of the H. IFN-c and B chromosomal genes. The sequence of the chromosomal gene was determined by Nagata et al. (1) and compared with the B gene sequence elucidated here. Boxes indicate homologies. The rest of the mRNA-coding sequence for the B gene is as previously published (14-16). The (G) in the upstream sequence can be deduced from the fact that it forms part of the Hae III site (GGCC) that was presumably cleaved during the cloning of H. IFN-c/Pad 3 (33).
to a Taq I cleavage site, the location of which is close to that expected from our previous mapping data (Fig. 3).

DISCUSSION

Using a single IFN-α cDNA to probe a human gene library, Nagata et al. (1) readily observed at least eight different classes of chromosomal genes, as judged by restriction site distribution. Using our IFN-β cDNA as a probe for total genomic DNA restricted with a variety of different restriction enzymes, we observed only one major type of β chromosomal gene organisation. Combined with previous studies demonstrating heterogeneity in leukocyte IFN cDNA (6) and in lymphoblastoid interferon structures (8), our data clearly suggests a less diverse arrangement of IFN-β genes and corresponding protein products.

However, recent data by Sehgal and Sagar (38) show that in induced human fibroblasts, there are two different mRNA molecules that code for proteins with interferon activity. One of these mRNAs seems to be complementary to the cDNA probe used in our study while the other one shows little cross-hybridisation (if any) with this cDNA. This would explain why the corresponding gene sequence was not obvious in our experiments, although it should be noted that the very faint radioactive bands observed in Fig. 3 (where the filter was given a low stringency wash) may reflect the presence of partially homologous interferon genes. In view of the work by Sehgal and Sagar, perhaps it is unwise to be dogmatic about the diversity of human fibroblast interferon genes at this stage.

It is also relevant here to discuss the 'silent' single nucleotide change observed between the IFN-β sequence of Taniguchi et al. (13) and that of ourselves (15) and Derynck et al. (16). We have since discovered that both sequences are present in our cDNA preparations (30) thus confirming the result of Taniguchi et al. A simple explanation could be that this reflects polymorphism at one locus although it could be interpreted in terms of two separate IFN-β genes.

A conclusive result from the work presented here is that the chromosomal IFN-β gene isolated, does not contain introns in the DNA sequence coding for the primary structure of the inter-
feron protein, the 3' untranslated region of the mRNA and most (if not all) of the 5' untranslated mRNA region. For reasons mentioned earlier, it is very unlikely that there is an intron within the 5'-terminal region of the mRNA-coding sequence.

When considered in the light of an identical conclusion recently made for an IFN-α gene (1), the data suggests that interferon genes in general, may lack introns. Apart from histone genes in a sea urchin (39) and a particular mitochondrial (40) and adenovirus gene (41), virtually all other eukaryote genes studied to date appear to contain introns (9,31) although their function is very much open to debate (31,32).

Studies with SV40 hybrids have inferred that introns are required in this system for the ultimate production of stable, translatable mRNA (42,43). Clearly, this is not the case for the production of interferon mRNA in human fibroblasts.

Another attractive theory put forward for the function of introns is that they provide an efficient means of evolving new or modified protein functions by stimulating exon reassortment between different genes (32). Evidence has been presented in the cases of immunoglobulin (44), globin (45) and lysozyme (46) that introns may separate exons coding for particular protein domains with distinct functions in the final polypeptide molecule. Given the varied function of interferon molecules (47) and the fact that it has been postulated that the areas of homology between IFN-α and β proteins may correspond to particular functions displayed by the whole polypeptide (2,48), it is especially surprising to find that these genes do not contain introns.

Whether the interferon genes are special cases or whether the above theories of intron function are not tenable, remains to be seen.

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