Volume 9 Number 3 1981 Nucleic Acids Research

Evidence for a unique human fibroblast interferon (IFN-β_1) chromosomal gene, devoid of intervening sequences

Jan Tavernier, Rik Derynck and Walter Fiers

Laboratory of Molecular Biology, State University of Ghent, Ledeganckstraat 35, B-9000 Ghent, Belgium

Received 16 December 1980

ABSTRACT

Direct restriction analysis of the human genome, using the Southern transfer technique and hybridization with a human fibroblast interferon(IFN-β) complementary DNA insert probe, revealed the presence of a single gene; no additional closely related IFN-β genes could be detected. A λ-linked human gene library (Lawn et al., Cell, 15, 1157-1174 (1978)) was screened using the cDNA probe. Out of 600,000 recombinant phage examined, one single clone bearing interferon sequences was obtained. Restriction analysis of the relevant region revealed an identical restriction map as obtained for the IFN-β_1cDNA clones. No intervening sequences could be detected, either in the coding or the non-coding regions of the gene.

INTRODUCTION

Human interferons are divided into three distinct classes: α (leukocyte), β (fibroblast) and γ (immune) interferon. Differences in induction, cellular origin and specificity, as well as the physicochemical and serological properties of these interferons are the basis for this classification (see ref. 1 for a review). Both α and β interferon preparations are physically heterogeneous (e.g., see refs. 2-4). This could be due to a varying degree of glycosylation (4-6), to the presence of a series of different polypeptides, or to a combination of both phenomena.

Using recombinant DNA technology bacterial clones have been constructed which contain cDNA complementary to interferon α mRNA (7-9) or β mRNA (10-12). Sequence analysis of the cDNAs revealed 45% homology at the nucleotide level between the coding regions of these interferon types (13). Recently, in the case of IFN-α, at least eight different genes were demonstrated in a human gene bank and the existence of 10 or even more IFN-α-related genes was postulated (14). Analysis at the protein level provided evidence for at least five different but related primary structures for IFN-α (15). On the

© IRL Press Limited, 1 Falconberg Court, London W1V 5FG, U.K.

other hand, all nucleotide sequencing data so far obtained for cloned IFN-β cDNA reveal a nearly identical sequence (10-12), although there is some evidence to suggest the existence of a second, but not closely related fibroblast interferon mRNA species (16).

In the present paper we present evidence, based on hybridization data, for the occurrence of a single IFN-β_1 gene in the human genome. We have also isolated and characterized the IFN-β_1 gene from a λ-linked human gene library. All data obtained are in total agreement with the known nucleotide sequence of IFN-β_1 cDNA. As is the case for IFN-α_1 (14), no intervening sequences seem to be present in the IFN-β_1 chromosomal gene.

MATERIALS AND METHODS

Procedures for preparing DNA

High molecular weight DNA was prepared from VGS human diploid fibroblasts (17). The cells were grown to confluency in bulk vessels (Continental Pharma). They were washed three times with phosphate-buffered saline without Ca^{2+} or Mg^{2+}, scraped off and collected in ice-cold lysis buffer (0.01 M NaCl, 0.01 M Tris-HCl, 0.03 M $MgCl_2$, pH 8.5). NP-40 was added to a final concentration of 1% to lyse the cells (15 min on ice with occasional gentle shaking). Nuclei were collected by centrifugation (5 min at 3000 rpm, 4°C), resuspended in 10 volumes of 1 x SE buffer (0.15 M NaCl, 0.1 M EDTA, pH 8.0) and homogenized. Further treatment was essentially as described by Jeffreys and Flavell (18).

Charon 4A phages were grown and purified, and phage DNA was prepared essentially according to a procedure of Dr. F. Blattner (personal communication). Plasmid DNA was prepared following a standard procedure (19) which included CsCl equilibrium gradient centrifugation and, if necessary, further purification on a 5-20% sucrose gradient. Clone analysis was performed using an alkali lysis procedure described by Birnboim and Doly (20).

Restriction endonuclease digestion, agarose gel electrophoresis and blotting experiments

Restriction enzymes were obtained from Boehringer Mannheim (EcoRI) and Biolabs (all others) and used as directed by the supplier. For direct genomic analysis, DNA samples (20 μg each) were digested in a final volume of 500 μl, phenolized, extracted twice with ether and precipitated with ethanol. DNA was redissolved in 10 mM Tris-HCl, pH 8.0 and loaded into 10 x 1 x 5 mm deep slots

in 20 x 20 x 0.6 cm horizontal agarose slab gels. The gels were run for 15 hr at 25 mA in 50 mM Tris-HAc, 20 mM NaAc, 2 mM EDTA, pH 7.8. The DNA was denatured and transferred to nitrocellulose filter paper essentially as described by Southern (21). Prior to hybridization, filters were baked for 2 hr at 80°C in vacuo.

Screening of a human gene library

A human gene library, described by Lawn et al. (22) and kindly provided by Dr. T. Maniatis, was screened using in situ plaque hybridization (23,24). A density of 25,000 plaques on a 14 cm petri dish was chosen. At this density plaques are in a subconfluent state after 16 hr incubation at 37°C. Approximately 600,000 recombinant phages were screened. Adsorption of phages and DNA was by sequentially placing two filters on each plate for 2 min and 5 min, respectively. Further treatment of the filter was as described by Benton and Davis (23). Prior to hybridization, filters were baked for 2 hr at 80°C in vacuo. A positive signal on the autoradiogram was correlated with a region of a plate extending over about 100 plaques. The whole region was picked and resuspended in λ buffer (0.01 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.2) containing 0.02% gelatin. After titration of the phage suspension a plate with approximately 500 plaques was rescreened. This plaque purification process was repeated twice.

Colony filter hybridization

We used the procedure of Grünstein and Hogness (25) as modified by Hanahan and Meselson (26). Clones were grown on nitrocellulose filters, lysed with 0.5 M NaOH, neutralized, dried on a filtration device and washed with ethanol. Filters were heated at 80°C for 2 hr in vacuo before hybridization.

Preparation of ^{32}P-labeled cDNA probes

The TaqI-BglII restriction fragment containing the nearly total 5'-untranslated region and the total coding region of the interferon cDNA clone pHFIF-21 was purified twice on low-melting-point agarose (Bethesda Research Laboratories) following the manufacturer's instructions. Nick translation of this DNA fragment was based on the procedure described by Rigby et al. (27). Twenty pmol of α-^{32}P-dATP (2000 to 3000 Ci/mmol) and 20 pmol of α-^{32}P-dGTP (2000 to 3000 Ci/mmol)(both obtained from The Radio-

chemical Centre, Amersham) were vacuum dried, and redissolved in 30 μl of 0.05 M Tris-HCl, 0.005 M $MgCl_2$, 0.01 M β-mercaptoethanol, pH 7.4, containing 30 μM TTP, 30 μM dCTP and 10 to 100 ng DNA. Two units of Escherichia coli DNA polymerase I (Worthington Co.) were added and the mix was incubated for 60 min at 15°C. EDTA was added to a final concentration of 0.08 M and the mixture was chromatographed on a Sephadex G-50 column. The excluded fractions were pooled and DNA was denatured at 95°C for 5 min before use. Approximately 2×10^8 cpm/μg was incorporated in the DNA.

Filter hybridization experiments

Nitrocellulose filters used were obtained from Schleicher and Schüll Co. (BA85 401180) or Millipore Co. (HATF 14250, HATF 09025). Conditions for plaque and colony hybridizations were as follows. Nitrocellulose filters were pretreated for 2 hr at 68°C in 5 x Denhardt's solution (0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% ficoll)(28) plus 1 x SET buffer (0.03 M Tris-HCl, 0.15 M NaCl, 0.001 M EDTA, pH 8.0). After rinsing the filters with hybridization solution (1 x Denhardt's, 5 x SET, 0.5% SDS) they were incubated for 16 hr at 68°C in the hybridization solution containing the ^{32}P-labeled probe DNA, and, for genomic DNA analysis, 50 μg/ml denatured herring sperm DNA (Koch-Light Laboratories Ltd.). Filters were washed for 4 hr at 68°C in 2 x SET, 0.5% SDS with one renewal of the solution.

Direct analysis of the human genome was according to procedures described by Wahl et al. (29). Nitrocellulose filters were finally air dried and autoradiographed by exposure to an Rx medical Fuji X-ray film. When necessary a Kyokko X-ray intensifying LH II screen was used with exposure at -80°C.

RESULTS

Detection of restriction fragments of human DNA containing the IFN-β_1 gene

Detection of picogram amounts of filter-bound DNA makes it possible to study specific eukaryotic genes directly at the genomic DNA level (18). DNA extracted from diploid VGS cells was digested to completion with a number of restriction endonucleases. The DNA fragments were transferred onto a nitrocellulose filter using the method described by Southern (21). Filter-bound DNA was then hybridized with denatured, radioactively labeled IFN-β_1 complementary DNA (cDNA). The cDNA probe used was a TaqI-BglII restriction fragment isolated from the recombinant plasmid pHFIF-21, which contains the nearly total

5'-untranslated region and the total coding region of the IFN-β_1 gene. Fig. 1 shows the cellular DNA fragments hybridizing to the cDNA probe. One single band was detected in each of the following digests of cellular DNA: BamHI (16.0 kilobase fragment), KpnI (20.0 kb), EcoRI (1.9 kb), HindIII (9.4 kb) and SacI (2.6 kb). Since these enzymes do not cut within the cloned IFN-β_1 gene, the results are consistent with the presence of a single chromosomal IFN-β_1 gene devoid of intervening sequences. Two bands are seen after digestion of cellular DNA with PstI (1.7 kb and 2.5 kb) or PvuII (1.8 kb and 9.6 kb). This result is not unexpected, as both enzymes have one recognition site within the IFN-β_1 gene. Cellular DNA cleaved with BglII also yields one band (12.6 kb) although there is a BglII recognition site within the IFN-β_1 gene. However, since the cDNA probe was prepared by BglII cleavage, it should

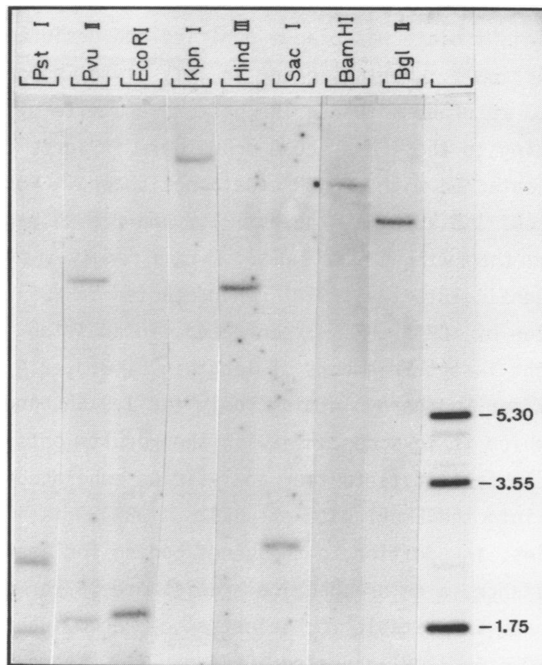

Fig. 1. Detection of human genomic DNA fragments containing an IFN-β_1 gene. Twenty-μg samples of human DNA were digested to completion with the indicated restriction endonucleases and, after electrophoresis in a horizontal 1.0% agarose slab gel, transferred onto a nitrocellulose filter. Hybridization conditions are as described in Materials and Methods. A nick-translated TaqI-BglII fragment from the cDNA clone pHFIF-21 was used as probe (see text). At the right of the autoradiogram are indicated the sizes in kb (kilo base pairs) of the IFN-β_1 cDNA clone pHFIF-21 digested with EcoRI or PstI-BamHI and included in the gel as size markers.

overlap with only one of the BglII fragments generated.

Isolation of the IFN-β_1 gene from a human library

The human gene library constructed by Lawn et al. (22) was used in these experiments. The library was derived from human fetal liver DNA partially cleaved with the restriction enzymes HaeIII and AluI. Fragments of 15-20 kb were isolated, methylated with EcoRI methylase, and with the aid of EcoRI oligonucleotide linkers inserted into the bacteriophage λ replacement vector Charon 4A (30).

We screened the library by the in situ plaque hybridization procedure (23,24) using the TaqI-BglII cDNA restriction fragment described above which was ^{32}P-labeled by nick translation. Six hundred thousand recombinant phages were screened and only one single plaque bearing IFN-β_1-specific information was identified. This isolate was plaque purified and designated λCH4A-gHFIF-1. The size of restriction fragments present in this clone indicated that it contains about 16.3 kb of human DNA. The pattern and size of restriction fragments hybridizing to the IFN-β_1 cDNA probe were in agreement with the results in Fig. 1 obtained with VGS DNA (data not shown). For example, we found again the EcoRI 1.9 kb band, the PstI 1.7 and 2.5 kb bands, the BglII 12.6 kb band and the PvuII 1.8 kb band. This strongly indicated that the gene we isolated was identical to the IFN-β_1 gene detected in VGS chromosomal DNA.

EcoRI digestion of λCH4A-gHFIF-1 generated, in addition to the two Charon 4A phage arms, eight insert fragments of length 4.6, 3.5, 2.4, 1.9, 1.3, 1.2, 0.8 and 0.6 kb. After Southern blotting, only the 1.9 kb band hybridized with the IFN-β_1 cDNA, which is in accordance with the results obtained with VGS chromosomal DNA. To facilitate further analysis we subcloned this 1.9 kb fragment directly into the EcoRI site of pBR325. pBR325 is a derivative of pBR322 which carries, in addition to the genes coding for ampicillin and tetracycline resistance, a chloramphenicol resistance marker containing a single EcoRI site and is suitable for selection of recombinant plasmids (31). After ligation of 0.6 μg EcoRI-digested λCH4A-gHFIF-1 DNA to 100 ng of pBR325 and transformation of E. coli HB101, several bacterial clones were selected. Sixteen colonies were examined further; all genomic EcoRI fragments present in λCH4A-gHFIF-1 were found in different pBR325 subclones (Fig. 2). As expected, only the subclones containing the 1.9 kb band hybridized to the IFN-β_1 cDNA probe. We designated a representative subclone p(325)-gHFIF-4.

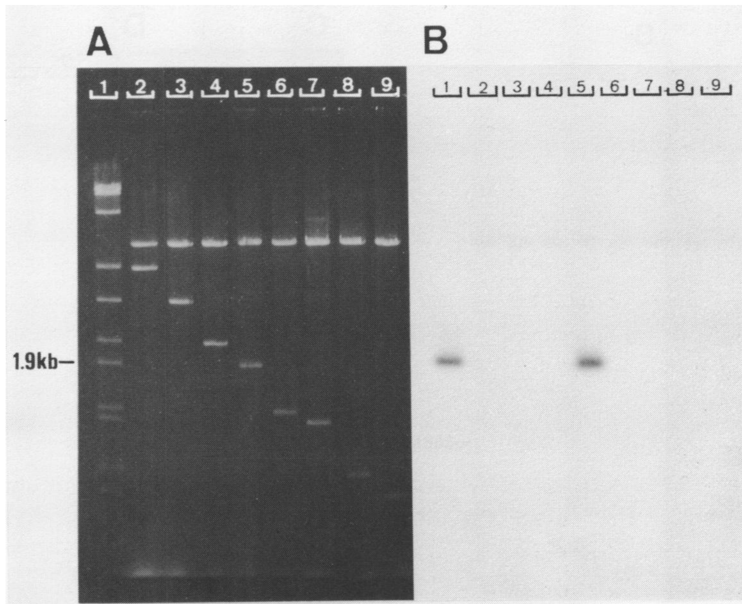

Fig. 2. Presence of an IFN-β_1 gene in λCH4A-gHFIF-1 and p(325)-gHFIF-4. The EcoRI restriction pattern of λCH4A-gHFIF-1 after electrophoresis in a 1% agarose slab gel is shown in panel A, lane 1. Lanes 2 to 9 represent the DNA patterns obtained after EcoRI cleavage of plasmid subclones p(325)-gHFIF-1 to 8. DNA was blotted onto a nitrocellulose filter and hybridized with the TaqI-BglII cDNA probe. Results are shown in panel B: only the 1.9 kb band contains the IFN-β_1 gene and is, after subcloning, present in p(325)-gHFIF-4.

Comparison of the cDNA clone pHFIF-21 with the chromosomal DNA clone p(325)-gHFIF-4

Comparison of restriction fragments derived from the IFN-β_1 cDNA clone and the chromosomal DNA clone provides a direct method to investigate the colinearity between gene and corresponding mRNA. If intervening sequences occur in a gene they will be detected by the enlargement of a particular restriction fragment or the fragment will be split into two or more fragments if recognition sites for the restriction enzyme used are present within an intervening sequence. We first compared the fragments resulting from double digestion with HindII and BglII (Fig. 3). Such cleavage of pHFIF-21 yields a fragment containing the entire coding region of IFN-β_1. As can be seen in panel A, a fragment of the same size (568 base pairs) is also generated by digestion of the genomic clone (indicated by an arrow). Southern blotting and hybridization with the TaqI-BglII probe demonstrates that this genomic

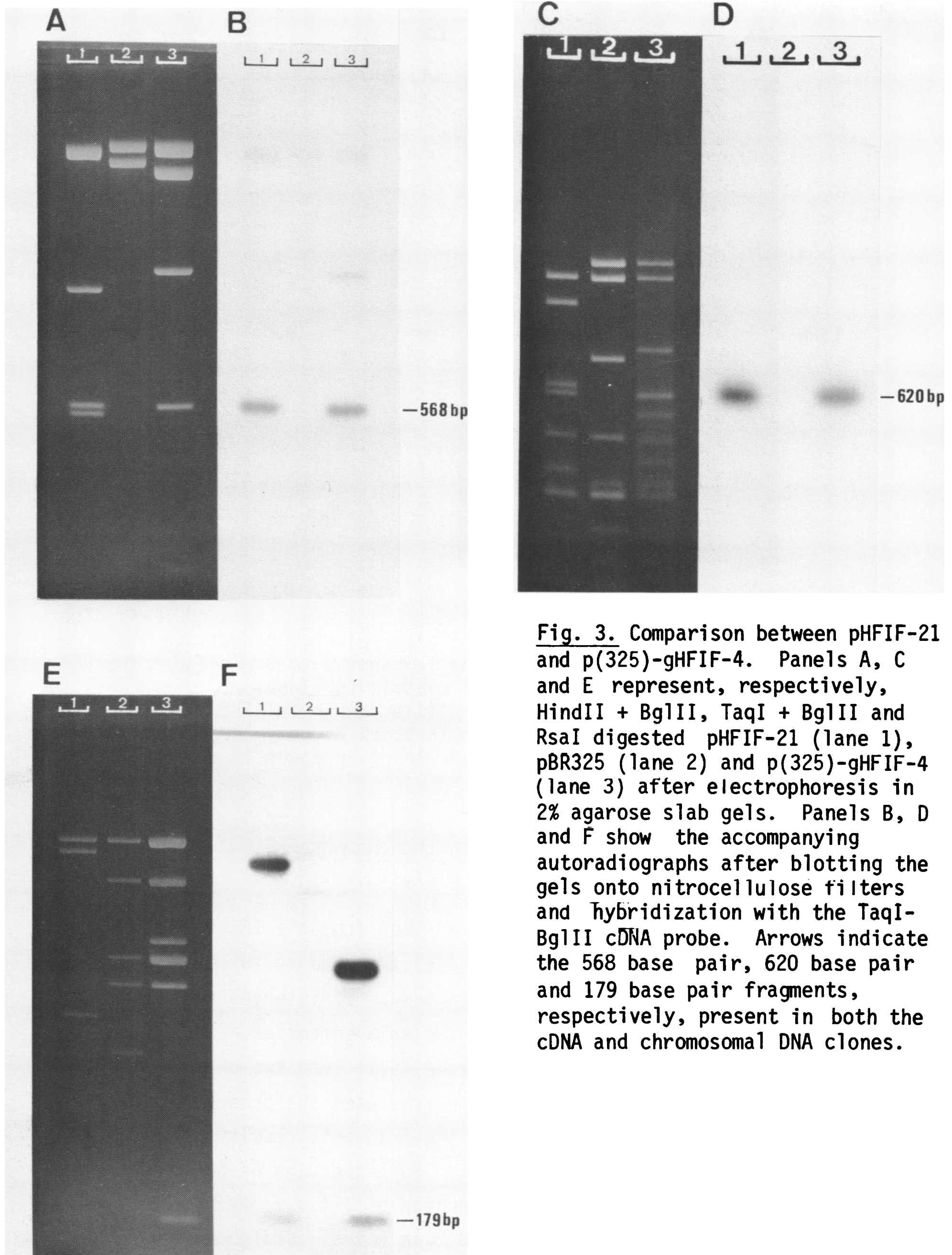

Fig. 3. Comparison between pHFIF-21 and p(325)-gHFIF-4. Panels A, C and E represent, respectively, HindII + BglII, TaqI + BglII and RsaI digested pHFIF-21 (lane 1), pBR325 (lane 2) and p(325)-gHFIF-4 (lane 3) after electrophoresis in 2% agarose slab gels. Panels B, D and F show the accompanying autoradiographs after blotting the gels onto nitrocellulose filters and hybridization with the TaqI-BglII cDNA probe. Arrows indicate the 568 base pair, 620 base pair and 179 base pair fragments, respectively, present in both the cDNA and chromosomal DNA clones.

fragment indeed contains the coding region (panel B). In the same way we showed complete homology between the cDNA clone and the genomic DNA clone for the 620 base pair TaqI-BglII fragment and for the 179 base pair RsaI fragment (Fig. 3, C to F). The TaqI-BglII fragment overlaps nearly the total 5'-untranslated region while the RsaI fragment extends 91 base pairs into the 3'-untranslated region. Thus there appear to be no intervening sequences between the TaqI site at position 9 of the cDNA insert and the RsaI site at position 717. All restriction data are summarized in Fig. 4.

DISCUSSION

Interpretation of the results obtained by direct hybridization analysis of the human genome using an IFN-β_1-specific cDNA probe suggests the existence of a single IFN-β_1 gene. The isolation from a human gene library of only one phage containing IFN-β information is consistent with this view. Indeed, this single recombinant phage was found to contain DNA information identical to the already isolated IFN-β_1 cDNA clones. The NH_2-terminal amino acid sequence of IFN-β_1 (32,33) and nucleotide sequences derived from three independently cloned IFN-β cDNAs (10-12) and from IFN-β mRNA (34) also are in agreement with the existence of a single gene for IFN-β. In contrast to the situation with

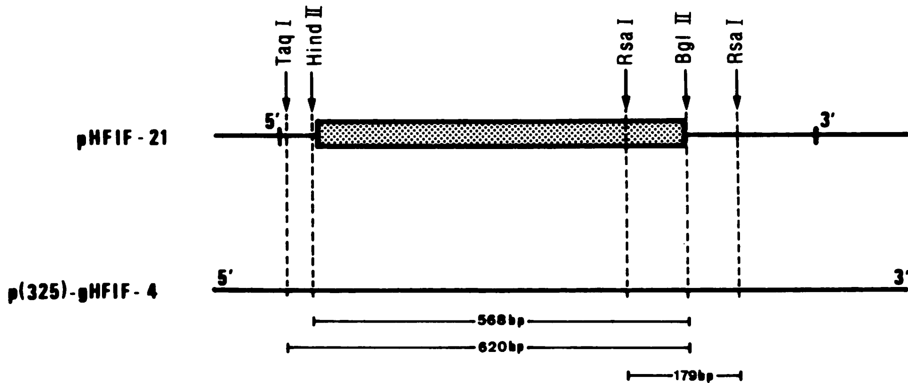

Fig. 4. Restriction map showing the colinearity between pHFIF-21 and p(325)-gHFIF-4. The upper part of the diagram shows the organization of the IFN-β_1 cDNA-containing hybrid plasmid pHFIF-21, including the 5'-untranslated region, the boxed coding region and the 3'-untranslated region of the IFN-β_1 gene. The lower line represents the genomic DNA clone p(325)-gHFIF-4, which contains the IFN-β_1 gene. Restriction sites present in both clones are indicated. The common restriction fragments as revealed by Southern blot analysis (Fig. 3) are shown below.

β interferons, α-type interferons are coded for by a family of multiple genes which are at least partially linked (14). Our results provide no evidence for a second β-type interferon, as recently suggested by Sehgal and Sagar (16). This can be explained by the inability to obtain cross hybridization between the putative two genes. Hybridization under less stringent conditions perhaps may help to answer this question. Heterogeneity of human fibroblast interferon has been reported (4), but this could be due to secondary modification(s).

Our results also indicate the absence of intervening sequences within the IFN-β_1 gene. Colinearity is found between IFN-β_1 chromosomal DNA and cloned cDNA from the TaqI site at nucleotide position 9 up to the RsaI site at position 717 (Fig. 4). The presence of introns in the remaining parts of the non-translated regions is rather unlikely but their absence can only be established by nucleotide sequence analysis. IFN-β_1 resembles the members of the IFN-α group and both types most probably are descendants of a common ancestral gene. The nucleotide sequence of the IFN-α_1 gene gives no evidence for an intervening sequence either in the coding region or in the 5'- and 3'-untranslated regions. Moreover, experiments with 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), a drug which blocks synthesis of most mRNAs probably by inhibiting an intron-related event, do not result in a decreased synthesis of IFN-α mRNA (P. Sehgal, personal communication) or adenovirus 2 polypeptide IX mRNA (35), both coded for by genes devoid of intervening sequences. Likewise the yield of IFN-β_1 mRNA is not lowered but rather is enhanced after treatment with DRB (36).

ACKNOWLEDGEMENTS

We are indebted to T. Maniatis for generously providing us with a human gene library, to M. Holsters and F. Van Vliet for advice, and W. Kuziel for help with the manuscript. J.T. holds a fellowship from the I.W.O.N.L. The research was supported by grants from the Gekoncerteerde Akties of the Belgian Ministry of Science and from Biogen N.V.

REFERENCES

1. Stewart, W. E. II (1979) The Interferon System (Springer, Berlin).
2. Berg, K. and Heron, I. (1980) Scand. J. Immunol. 11, 489-502.
3. Levy, W. P., Shively, J., Rubinstein, M., Del Valle, U. and Pestka, S. (1980). Proc. Nat. Acad. Sci. U.S.A. 77, 5102-5104.
4. Havell, E. A., Yamazaki, S. and Vilcek, J. (1977) J. Biol. Chem. 252, 4425-4427.

5. Jankowski, W. J., Davey, M. W., O'Malley, J. A., Sulkowski, E. and Carter, W. A. (1975) J. Virol. 16, 1124-1130.
6. Bose, S., Guarari-Rotman, D., Ruegg, U. T., Corley, L. and Anfinsen, C. B. (1976) J. Biol. Chem. 251, 1659-1662.
7. Nagata, S., Taira, H., Hall, A., Johnsrud, L., Streuli, M., Ecsödi, J., Boll, W., Cantell, K. and Weissmann, C. (1980) Nature, 284, 316-320.
8. Streuli, M., Nagata, S. and Weissmann, C. (1980) Science, 209, 1343-1347.
9. Goeddel, D. V., Yelverton, E., Ullrich, A., Heyneker, H. L., Miozarri, G., Holmes, W., Seeburg, P. H., Dull, T., May, L., Stebbing, N., Crea, R., Maeda, S., McCandless, R., Sloma, A., Tabor, J. M., Gross, M., Familletti, P. C. and Pestka, S. (1980) Nature, 287, 411-416.
10. Derynck, R., Content, J., De Clercq, E., Volckaert, G., Tavernier, J., Devos, R. and Fiers, W. (1980) Nature, 285, 542-547.
11. Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y. and Muramatsu, M. (1980) Gene, 10, 11-15.
12. Goeddel, D. V., Shepard, H. M., Yelverton, E., Leung, D., Crea, R., Sloma, A. and Pestka, S. (1980) Nucl. Acids Res. 8, 4057-4074.
13. Taniguchi, T., Mantei, N., Schwarzstein, M., Nagata, S., Muramatsu, M. and Weissmann, C. (1980) Nature, 285, 547-549.
14. Nagata, S., Mantei, N. and Weissmann, C. (1980) Nature, 287, 401-408.
15. Allen, G. and Fantes, K. H. (1980) Nature, 287, 408-410.
16. Sehgal, P. B. and Sagar, A. D. (1980) Nature, 288, 95-97.
17. Billiau, A., Joniau, M. and De Somer, P. (1973) J. Gen. Virol. 19, 1-8.
18. Jeffreys, A. J. and Flavell, R. A. (1977) Cell, 12, 429-439.
19. Kahn, M., Kolter, R., Thomas, C., Figurski, D., Meyer, R., Remaut, E. and Helinski, D. R. (1979) in Methods in Enzymology, vol. 68, pp. 268-280.
20. Birnboim, H. C. and Doly, J. (1979) Nucl. Acids Res. 7, 1513-1523.
21. Southern, E. M. (1975) J. Mol. Biol. 98, 503-517.
22. Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. and Maniatis, T. (1978) Cell, 15, 1157-1174.
23. Benton, W. D. and Davis, R. W. (1977) Science, 196, 180-182.
24. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. and Efstratiadis, A. (1978) Cell, 15, 687-701.
25. Grünstein, M. and Hogness, D. S. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 3961-3965.
26. Hanahan, D. and Meselson, M. (1980) Gene, 10, 63-67.
27. Rigby, P. W. J., Dieckmann, H., Rhodes, C. and Berg, P. (1977); J. Mol. Biol. 13, 237-251.
28. Denhardt, D. T. (1966) Biochem. Biophys. Res. Commun. 23, 461-467.
29. Wahl, G. M., Stern, M. and Stark, G. R. (1979) Proc. Nat. Acad. Sci. U.S.A. 76, 3683-3687.
30. Blattner, F. R., Williams, B. G., Blechl, A. E., Denniston-Thompson, K., Faber, H. E., Furlong, L-A., Grunwald, D. J., Kiefer, D. O., Moore, D. D., Schumm, J. W., Sheldon, E. L. and Smithies, O. (1977) Science, 196, 161-169.
31. Bolivar, F. (1978) Gene, 4, 121-136.
32. Knight, E., Jr., Hunkapillar, M. W., Korant, B. D., Hardy, R. W. F. and Hood, L. E. (1980) Science, 207, 525-526.
33. Stein, S., Kenny, C., Friesen, H-J., Shively, J., Del Valle, U. and Pestka, S. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5716-5719.
34. Houghton, M., Eaton, M. A. W., Stewart, A. G., Smith, J. C., Doel, S. M., Catlin, G. H., Lewis, H. M., Patel, T. P., Emtage, J. S., Carey, N. H. and Porter, A. G. (1980) Nucl. Acids Res. 8, 2885-2894.
35. Vennström, B., Persson, H., Pettersson, U. and Philipson, L. (1979) Nucl. Acids Res. 7, 1405-1418.
36. Sehgal, P., Lyles, D. and Tamm, I. (1978) Virology, 89, 186-198.