THE IDENTITY OF INTERFERON-β_1 mRNA TRANSCRIPTS IN HUMAN FIBROBLASTS AND NAMALVA CELLS

Gerhard GROSS, Wolfgang BRUNS, Ulrich MAYR and John COLLINS
Gesellschaft für Biotechnologische Forschung mbH, Abt. Genetik, Mascheroder Weg, 3300 Braunschweig, FRG

Received 3 December 1981; revision received 1 February 1982

1. Introduction

Depending on the mode of induction and the cell-type, human cells are able to produce interferons, which are categorized in 3 different groups: interferon (IFN)-α, -β and -γ [1]. Studies at the DNA and protein level provide evidence that the α-group is not a homogenous population of molecules, but consists of several species, differing in gene sequence and in the physicochemical properties of the protein [2–4].

For IFN-α there exist $\geqslant$12 different genes, and 8 different gene-products have been identified [4]. In the case of IFN-β only 1 gene-product has been characterized [5–7], but under some conditions human fibroblasts can synthesize >1 species of IFN-β mRNA [8,9]. A Namalva (lymphoblastoid) cell line produces, upon induction with Sendai virus, both α- and β-types of interferon. Namalva IFN-β mRNA consists of 2 size-classes; 1.9 kilobase (kb) and 1.1 kb, both absent from fibroblasts [10]. This raises the question as to whether or not other IFN-β genes are turned on in Namalva cells, or if transcription is initiated and/or terminated at novel sites. We have used a cloned genomic DNA fragment containing the human IFN-β_1 gene, as a probe for S1-mapping of the IFN-β mRNA species produced in Sendai-induced Namalva cells. We find that this Namalva IFN-β mRNA is indistinguishable from the main species found in poly [r(I):r(C)]-induced fibroblasts, with respect to molecular size, and the starting and termination points of transcription.

2. Materials and methods

Interferon mRNA synthesis in human fibroblasts (FS4 cells) was induced in the presence of poly [r(I):r(C)] (50 μg/ml) and cycloheximide (50 μg/ml) for 4 h [11]. The induced cells were supplied by Rentschler Inc. (Laupheim). Lymphoblastoid Namalva cells (5×10^6 cells/ml) were induced by 40 HA-units/ml of Sendai virus for 5 h. Cells were washed and harvested.

The genomic clone pCosIFN-β_1 has been described [7]. DNA fragment *Eco*RI-F containing the whole IFN-β_1 gene has been subcloned in pBR325. This subclone has been used for S1-mapping. 3′-End labelling was done with 'Klenow' polymerase by 'filling in' [12]. 5′-End labelling was done with T4 DNA kinase [14].

Hybridisation and S1-mapping were performed as in [13].

DNA-sequencing was performed according to [14].

3. Results

Based on size determinations in a denaturing 2% agarose gel the size of IFN-β_1 mRNA seemed identical (± 50 nucleotides) for poly [r(I):r(C)]-induced fibroblasts and Sendai virus-induced Namalva cells (fig.1). To substantiate these findings we carried out S1-mapping experiments.

As a hybridisation probe we used the 1.9 kb *Eco*RI-F fragment of pCosIFN-β_1, described in [7]. This DNA fragment contains the entire IFN-β_1 gene as well as the flanking sequences at the 3′- and the 5′-end. The strategy used to determine the size of IFN-β_1 mRNA in poly [r(I):r(C)]-induced human fibroblasts (FS4) and Sendai-induced lymphoblastoid Namalva cells is shown in fig.2.

The size of the protected fragments is identical in poly [r(I):r(C)]-induced FS4 and in Sendai-induced Namalva cells (fig.3). The size of IFN-β_1 mRNA without the poly(A)-tail is 840 nucleotides. The 5′-end

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

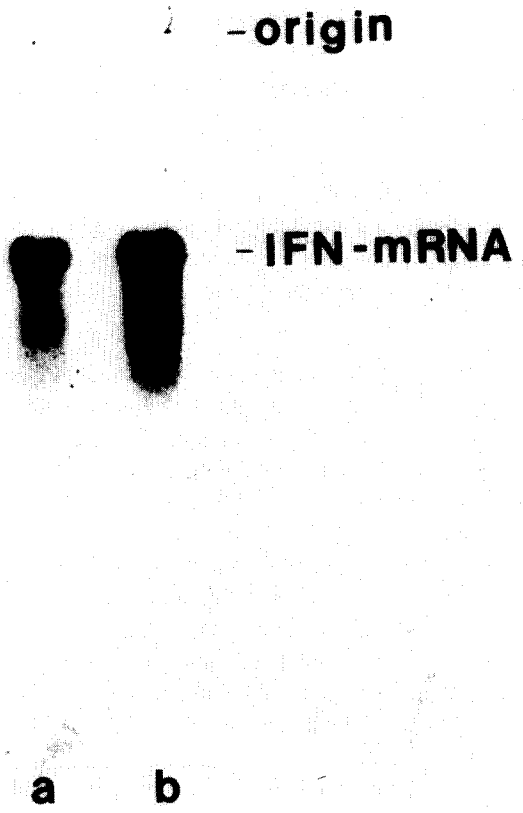
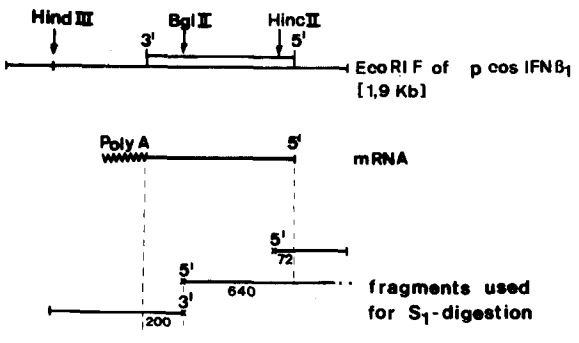


Fig.1. Size comparison of human IFN-β_1 mRNA from poly [r(I):r(C)]-induced fibroblasts and Sendai-induced Namalva cells under denaturing conditions. Poly(A) RNA (1 μg) was separated electrophoretically according to size in 2% agarose (2 M formaldehyde, 40 mM Na-acetate (pH 4.5), 2 mM EDTA and blotted to a nitrocellulose membrane [15]. IFN-β_1 mRNA was visualized by hybridisation of ^{32}P-labelled nick-translated *Eco*RI-F fragment of pCosIFN-β_1, inserted in pBR325 to the blotted RNA. (a) Poly(A) RNA from Sendai-induced Namalva cells; (b) poly(A) RNA from poly [r(I):r(C)]-induced FS4 cells. Poly(A) RNA from uninduced FS4 cells does not show any hybridisation to *Eco*RI-F fragment under the conditions used (not shown).



labelled *Bgl*II fragment gives rise to a 640 nucleotide fragment and the 3'-end-labelled *Bgl*II fragment gives rise to a 200 bp fragment protected against S1-digestion. Also, the initiation point of IFN-β_1 mRNA is identical in both induction systems. It is located at the sequence:

$$\overset{-79}{\downarrow}\quad\quad\overset{-75}{\downarrow}$$
$$5'\ \mathrm{A\ A\ G\ G\ A\ C\ A}\ldots$$

based on the sequence in [7]. The highest rate of transcription seems to be initiated at map position −75 (fig.4).

4. Discussion

The size of human IFN-β_1 mRNA has been reported to vary according to the cell type induced [10]. However, we show here that IFN-β_1 mRNA in poly [r(I):r(C)]-induced FS4 cells is identical to that found in Sendai-induced Namalva cells. The larger IFN mRNA species in [10] might be an experimental artefact, represent one of the other fibroblast IFN species postulated in [9], or could be based on enormous variations in the length of the poly(A)-tail, which we did not find.

Fig.2. Strategy of S1-mapping. DNA fragment *Eco*RI-F from pCosIFN-β_1 subcloned in pBR325 has been described in [7]. This plasmid was cut with *Bgl*II and 3'-end-labelled by 'filling in' with 'Klenow' polymerase [12] and redigested with *Hin*dIII. The resulting 800 bp *Bgl*II–*Hin*dIII fragment containing the 3'-end sequences of the IFN-β_1 gene was used to map the 3'-end of the IFN-β_1 mRNA. After hybridisation to IFN-β_1 mRNA a 200 bp fragment should be protected against S1-digestion. To map the 5'-end of the IFN-β_1 mRNA the *Bgl*II-digested plasmid was 5'-end-labelled with T4 polynucleotide kinase [14] and redigested with *Hin*dIII. The 5800 bp fragment containing the 5'-end flanking sequences of the IFN-β_1 gene as well as some pBR325 vector sequences was used for S1-mapping. After hybridisation to IFN-β_1 mRNA a 640 bp fragment should be protected against S1-digestion. To determine the exact initiation site of IFN-β_1 mRNA the plasmid was cut with *Hinc*II and 5'-end-labelled with T4 polynucleotide kinase. After redigestion with *Eco*RI the 350 bp fragment containing the 5'-end sequence of the IFN-β_1 gene was isolated and hybridised to IFN-β_1 mRNA. S1-digestion gives rise to a protected DNA fragment ~62 bases long.

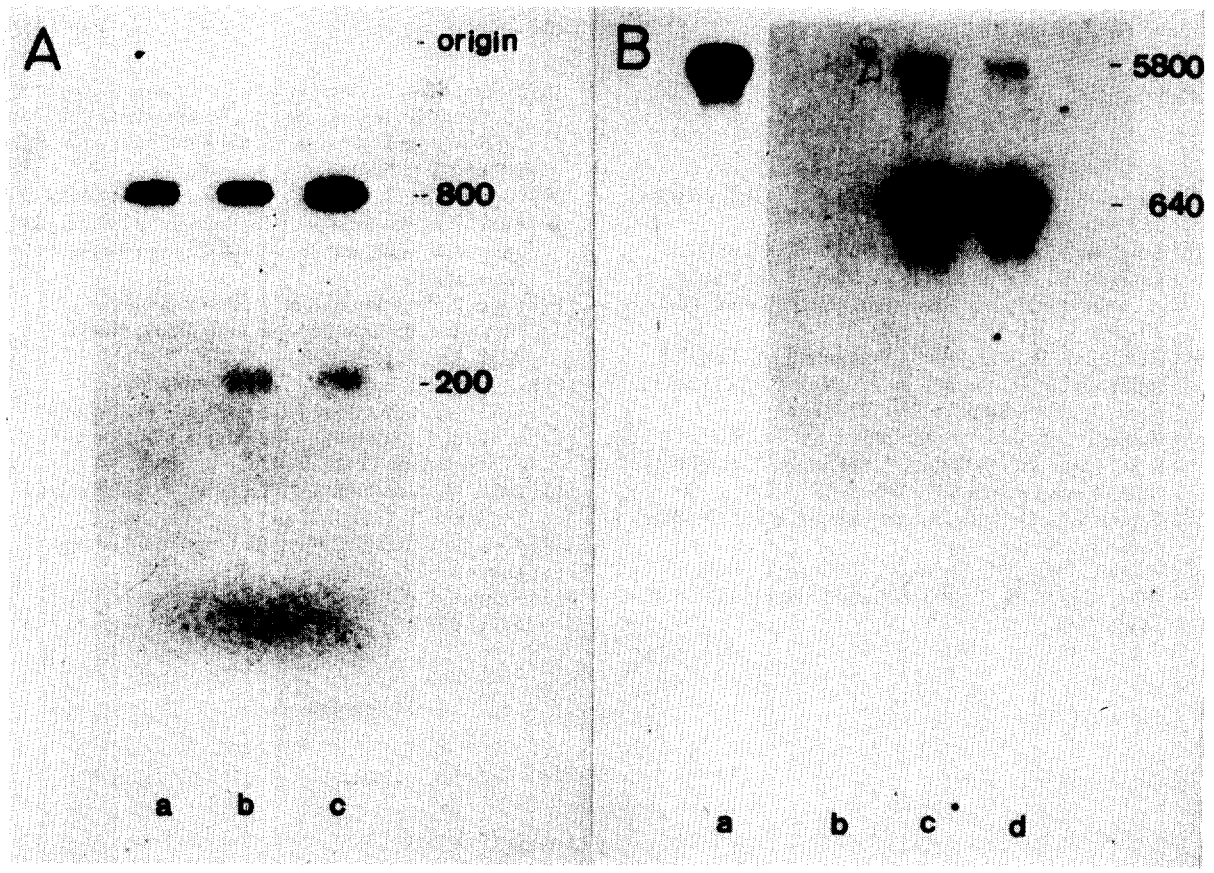
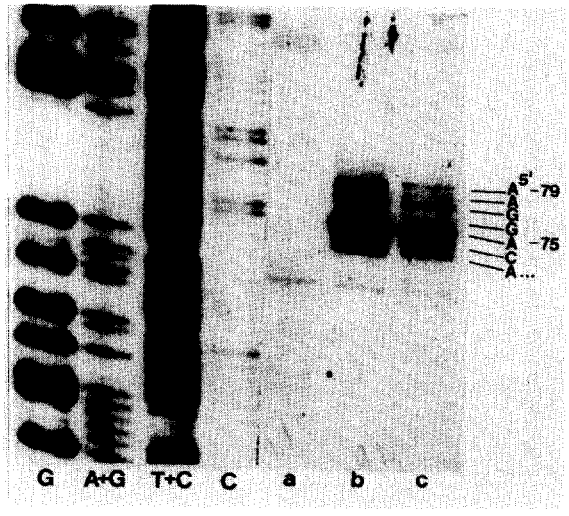


Fig.3. S1-mapping of IFN-β_1 mRNA. *Eco*RI-F inserted in pBR325 was cut with *Bgl*II/*Hin*dIII. The resulting 800 bp fragment was used for S1-mapping of the 3'-end of IFN-β_1 mRNA (A). The 5800 bp fragment was used for S1-mapping of the 5'-end (B). Poly(A) RNA (1 μg) was dried down with the respective labelled DNA fragments (see fig.2). The pellet was dissolved in 10 μl hybridisation mixture (80% formamide, 0.04 M Pipes (pH 6.4), 0.4 M NaCl, 2 mM EDTA) and sealed in capillaries. After boiling for 5 min the capillaries were transferred to 53°C for 10 h. S1-digestion was performed in 250 μl (0.3 M NaCl, 0.05 M Na-acetate (pH 4.5), 0.003 M $ZnSO_4$, 2.5 μg/ml herring sperm DNA) with 75 U S1-nuclease (Sigma) at room temperature for 3 h. (A) Comparison of the 3'-ends of IFN-β_1 mRNA by S1-mapping of DNA hybridised to: (a) poly(A) RNA from uninduced FS4 cells; (b) poly(A) RNA from poly [r(I):r(C)]-induced FS4 cells; (c) poly(A) RNA from Sendai-induced Namalva cells. (B) Comparison of the 5'-end of IFN-β_1 mRNA by S1-mapping of DNA hybridised to (b) poly(A) RNA from uninduced FS4 cells; (c) poly(A) RNA from poly [r(I):r(C)]-induced FS4 cells; (d) poly(A) RNA from Sendai-induced Namalva cells. The DNA fragment used for S1-mapping was run in (a). Separation of protected fragments was carried out in a 2% agarose gel (30 mM NaOH, 2 mM EDTA). Size marker was pBR322 digested with *Hin*fI or *Rsa*I.



Acknowledgements

We express our gratitude to H. Mohr for supplying us with Sendai virus and to Rentschler AG for the induced FS4 cells. We appreciate the expert technical assistance of W. Westphal.

Fig.4. Determination of the start point of IFN-β_1 mRNA. *Hin*cII–*Eco*RI (350 bp) labelled fragment (see fig.2) covering the 5'-end of IFN-β_1 mRNA was either subjected to Maxam-Gilbert DNA sequencing reactions or used for S1-mapping (a–c). S1-mapping of the DNA fragment hybridised to: (a) poly(A) RNA from uninduced RNA; (b) poly(A) RNA from poly [r(I):r(C)]-induced FS4 cells; (c) poly(A) RNA from Sendai-induced Namalva cells. DNA fragments were separated according to length in a 10% acrylamide gel (7 M urea, 0.1 M Tris–borate (pH 8.3)).

References

[1] Editorial (1980) J. Interferon Res. 1, vi–vii.

[2] Nagata, S., Mantei, N. and Weissmann, C. (1980) Nature 287, 401–408.

[3] Goeddel, D. V., Leung, D. W., Dull, T. J., Gross, M., Lawn, R. M., McCandliss, R., Seeburg, P. H., Ullrich, A., Yelverton, E. and Gray, P. W. (1981) Nature 290, 20–26.

[4] Rubinstein, M., Levy, W. P., Moschera, J. A., Lai, C. Y., Hershberg, R. D., Bartlett, R. T. and Pestka, S. (1981) Arch. Biochem. Biophys. 210, 307–318.

[5] Houghton, M., Jackson, I. J., Porter, A. G., Doel, S. M., Catlin, G. H., Barber, C. and Carey, N. H. (1981) Nucleic Acids Res. 9, 247–265.

[6] Tavernier, J., Derynck, R. and Fiers, W. (1981) Nucleic Acids Res. 9, 461–471.

[7] Gross, G., Mayr, U., Bruns, W., Grosveld, F., Dahl, H. M. and Collins, J. (1981) Nucleic Acids Res. 9, 2495–2507.

[8] Weissenbach, J., Chernajowsky, Y., Zeevi, M., Shulman, L., Soreq, H., Nir, U., Wallach, D., Perricaudet, M., Tiollais, P. and Revel, M. (1980) Proc. Natl. Acad. Sci. USA 77, 7152–7156.

[9] Seghal, P. B., Sagar, A. D., Braude, I. A. and Smith, D. (1981) in: The Biology of The Interferon System (De Maeyer, E. et al. eds) pp. 43–46, Elsevier Biomedical, Amsterdam.

[10] Sagar, A. D., Pickering, L. A., Sussmann-Berger, P., Stewart II, W. E. and Seghal, P. B. (1981) Nucleic Acids Res. 9, 149–160.

[11] Cavalieri, R., Havell, E., Vilcek, J. and Pestka, S. (1977) Proc. Natl. Acad. Sci. USA 74, 4415–4419.

[12] Schwarz, E., Scherer, G., Hobom, G. and Kössel, H. (1978) Nature 272, 410–414.

[13] Berck, A. J. and Sharp, P. A. (1978) Proc. Natl. Acad. Sci. USA 75, 1274–1278.

[14] Maxam, A. M. and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.

[15] Thomas, P. S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201–5205.