MOLECULAR CLONING OF TWO GENES INDUCED BY α-INTERFERON IN HUMAN CELLS.

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INTRODUCTION

IFN-induced proteins as well as IFN-induced enzymes have been considered for several years as possible mediators of the various interferon actions (reviewed in Sen, 1984 ; Galabru et Hovanessian, 1984; De Clercq et al., 1984 ; Johnston and Torrence, 1984). However, despite numerous attempts to compare cellular variants that display an altered response to interferon, no clearcut functional characterization of the various biochemical pathways described so far has emerged (Lebleu and Content, 1982). Interferon-induced proteins of unknown function have been described in murine and human cells (Gupta et al., 1979). Interestingly they are the subject of a temporal regulation (Gupta et al., 1981) and their pattern of expression depends on the cells or tissue used and also on the kind of IFN used (Weil et al., 1983 ; Epstein et al., 1983). It became thus interesting to attempt to isolate cDNA clones corresponding to some of these interferon-induced genes. This has been realized by several groups : Chebath et al. (1983) have cloned a cDNA corresponding to a 56,000 dalton protein induced by IFN in human cells, and later on, a cDNA clone corresponding to a 38,000 dalton/human 2-5A synthetase (Merlin et al., 1983). A similar 56 K cDNA clone has been isolated by Larner et al. (1984) from human diploid cells. A murine, perhaps homologous, 56 Kdalton cDNA clone has also been described by Samanta et al. (1984). Recently, Friedman et al. (1984) have isolated cDNA clones corresponding to seven different α-interferon induced sequences from T98G human neuroblastoma cells. With the exception for the 600 bp metallothionein-II cDNA (Friedman et al., 1984), most of the

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clones described so far were incomplete, representing usually less than 50% of the size anticipated for full size cDNA clones.
Studies on the regulation, gene organisation, transcriptional processing, as well as functional characterization through expression would be greatly facilitated if full-length cDNAs corresponding to some of these IFN-induced genes became available. For this reason we have first attempted to obtain such large cDNA clones corresponding to the previously described 56-kDa protein and 38-kDa 2-5A synthetase which have been cloned from human cells mRNA by Chebath et al. (1983) and Merlin et al. (1983). In this paper we describe two presumably full size cDNA clones that have been selected out a human cDNA library of α-IFN induced genes.

PREPARATION OF A cDNA LIBRARY FROM α-IFN TREATED CELLS

Total cellular poly A+ RNA was prepared by extraction in 8M guanidine-hydrochloride (Pavlovec et al., 1978) from human amniotic U cells (UAC) that had been grown in roller bottles and treated for 6 hrs at 37°C with 200 U/ml partially purified α-IFN. After oligo(dT) cellulose chromatography, this RNA was fractionated on a 5-20% sucrose 50% formamide gradient as described and the 16-25S region of the gradient was used for cDNA synthesis.
We used the method described by Gubler and Hoffman (1983) with the following modifications : 1) In the first strand cDNA synthesis, Na.pyrophosphate was omitted ; 2) for the second strand synthesis, T4DNA ligase (100 U/ml) was used instead of E.coli DNA ligase and the incubation was carried out overnight at 14°C; 3) cDNA was tailed with dCTP and annealed to G-tailed Pst I-cut-pBR322 (purchased from BRL). Transformation was done on $CaCl_2$ treated MC1061 E.coli and the cells were plated and handled at high density exactly as described by Hanahan and Meselson (1983). About 18,000 clones were replicated from a single 82 mm nitrocellulose filter. Replicas were screened by hybridization with two oligonucleotides that were synthesized following the procedure of Sproat and Bannwarth (1983), following the sequence published for the human 56K cDNA (a) and human 38K 2-5A cDNA (b) (Chebath et al. 1983 ; Merlin et al., 1983). Their sequence is :
a) 5'CTAAGGACCTTGTCTCACAGAAGTTC3'
b) 5'TCAAGCTTCATGGAGAGGGGCAGGG3'
These oligonucleotides were labeled by kination with γ-ATP-p^{32} to 2-5 x 10^8 cpm/μg (Maniatis et al., 1982). Hybridization was in the conditions described by Hanahan and Meselson (1983) at 45°C for (a) and 52°C for (b) except that the medium contained

heparine (50 U/ml) and 6xSSC instead of 6xSET. After hybridization, the filters were washed four times in 6xSSC, 0.5% SDS at 20°C, and for 5 min at 45°C (a) or 52°C (b). From 18,000 clones two were selected, one hybridizing with each oligonucleotide. After two rounds of purification and hybridization at intermediate and low density, the two clones were amplified and the plasmid DNA's were analysed by restriction analysis.

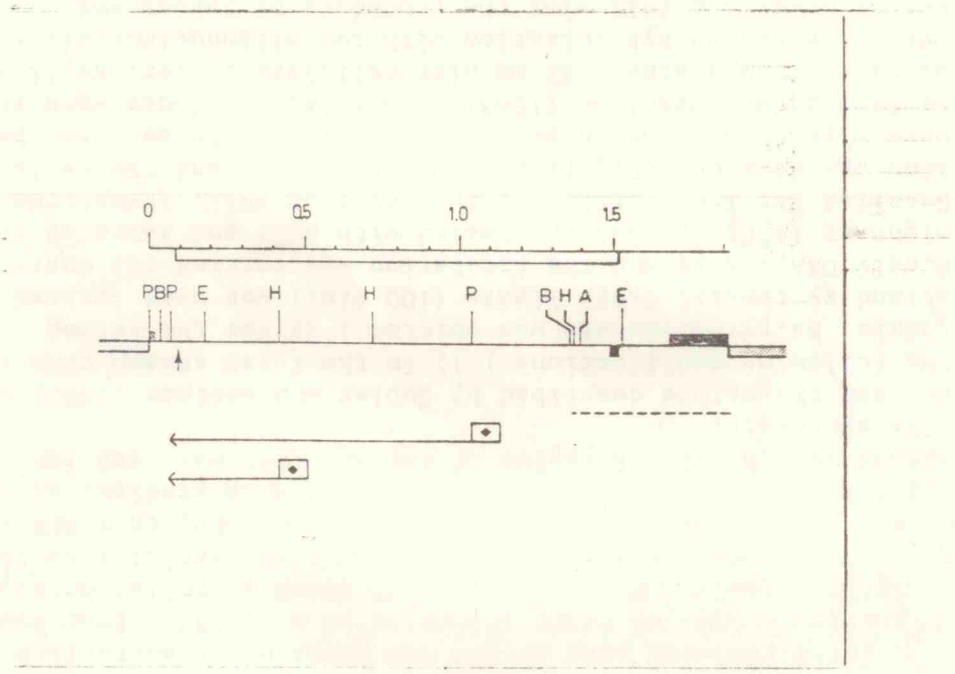

FIGURE 1. Restriction map of the 56 K cDNA clone (p56-1). Only the sites which have been determined are indicated (those derived from the sequence are not represented). The dashed line indicates the size of the clone sequenced by Chebath et al. (1983). The black square corresponds to the location of the synthetic (25 mer) oligonucleotide (a) used for the screening of our cDNA library. The arrow indicates the location of the initiator AUG. The SP64 constructions used for the preparation of P^{32} RNA probe (top) or antisense (bottom) are also represented. ◆ = SP6 promotor. The upper scale is in kilodaltons. Abbreviations are as follows : P = Pst I ; B = Bgl II ; E = EcoR I ; H = Hind III ; A = Acc I ; EV : EcoR V. The coding region is indicated by a white box ; GC and AT tails hatched box and the part of pBR 322 vector by dots.

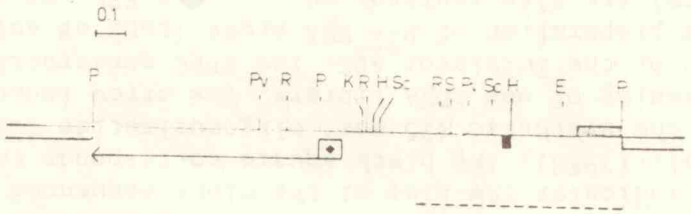

FIGURE 2. Restriction map of the 2-5A (38K) cDNA clone (p2-5A-1).
The dashed line shows the size of the clone described and sequenced by Merlin et al. (1983). The black square indicates the location of the synthetic (25 mer) oligonucleotide (b) used for the screening of our cDNA library. The SP64 construction used for preparing a 5'P^{32} RNA probe is represented.
Pv = Pvu II ; R = RSa I ; K = Kpn I, Sc = Sac I ; S = Sau3AI; other symbols and abbreviations are as in figure 1.

In both cases, we have isolated a cDNA corresponding to a large insert of 1.85 kb for the 56 K cDNA and 1.7 kb for the 2-5A cDNA (including the homopolymeric tails). Detailed analysis of the restriction maps from plasmid DNA digestion and hybridization on Southern blots with oligonucleotides (a) and (b) confirmed entirely the maps described previously (Chebath et al., 1983 ; Merlin et al., 1983), indicating that we had indeed obtained very large extensions of the previously described human 56K and 38K-2-5A cDNA's (see fig. 1 and 2).

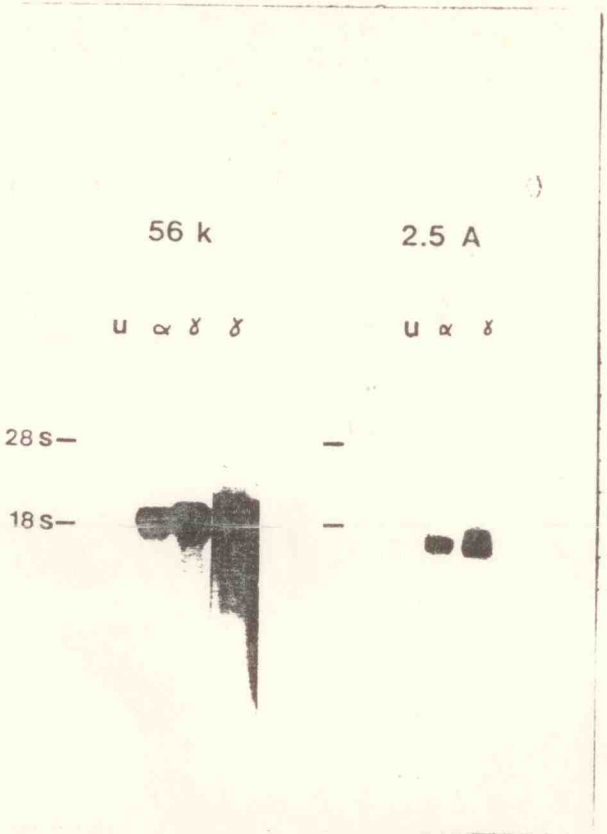

FIGURE 3. Northern blot analysis of RNA from IFN-treated or untreated human U amniotic cells.
The conditions for Northern blot analysis were as described (Poupart et al., 1984). The left panel corresponds to an hybridization with a 56K cDNA derived probe whereas the right panel corresponds to an hybridization with a 2-5A cDNA derived probe. Both P^{32}-RNA probes were transcribed from the SP64 constructions described in figures 1 and 2, using the conditions described by Melton et al. (1984) and used at a concentration of $5x10^6$ cpm/ml. Each lane corresponds to 1.5 µg of total cellular poly A+ RNA. Treatment was for 6 hrs with 200 U/µl of α-IFN (lane α) and for 12 hrs with 200 U/ml of HuIFN-γ (lane γ). U = untreated.
In the second lane (γ), in the left panel, the film has been overexposed to visualize the high molecular weight bands.

Northern blot analysis of control and α or γ-IFN treated UAC cells shows that

1) both kinds of interferons induce the accumulation of a major transcript of about 2kb which hybridizes with a 56K cDNA probe. This is compatible with the notion that the cDNA isolated could be a nearly full size transcript of the 56K protein mRNA. In addition, we observe two additional transcripts of 3-5 kb in this case which could correspond to partially spliced pre-mRNA or other intermediates ;

2) concerning the 2-5A cDNA probe, both kinds of interferon treatment induced also the accumulation of a major transcript of 1.6-1.7 kb together with minor amounts of large RNA's as described by Merlin et al. (1983) and Benech et al. (1985).

The size of the major transcripts, detected with the two kinds of probes corresponds thus quite well with that of the cDNA inserts, indicating that both might be nearly full-size cDNA copies. In the case of the 56K cDNA clone, this was confirmed by determining the full sequence of the cDNA insert (Whatelet et al., in preparation). We found an AUG close to the 5' Pst I site (Fig. 1), preceded by an untranslated region at least 60 nucleotides, and 4 out 5 of the consensus nucleotides preceding usually a functional initiator AUG (Kozak et al., 1984). This AUG is followed by an uninterrupted reading frame of 1437 nucleotides coding for 479 amino acids, corresponding to a calculated molecular weight of 55,386.

FUNCTIONAL STUDIES WITH ANTI-SENSE 56K RNA

Melton (1985) described recently the possibility to inhibit selectively the translation of globin mRNA into Xenopus oocytes by pre-injection of anti-sense RNA (prepared by SP6 polymerase transcription) provided the anti-sense RNA covers the 5' unstranslated region of the target mRNA. We prepared a construction corresponding to the 5' end of our 56 K cDNA clone by inserting the 5' Hind III-Pst I fragment in the SP64 vector. This construction should cover the initiator AUG and the 24 last nucleotides of the 5' untranslated region. RNA was transcribed with SP6 polymerase using the conditions of Melton et al. (1984) on the purified plasmid DNA. A unique RNA of about 400 nucleotides was obtained, purified and a 0.2 mg/ml solution was microinjected (Huez et al., 1983) into α-IFN (2,000 U/ml) treated Rsa cells (Vandenbussche et al, 1981). The amount of RNA injected corresponded to 10^5-5.10^5 molecules/cell. After seven hours treatment, the cells were infected with VSV at a multiplicity of infection of 10. Preliminary

results show a clear "deprotection" effect of this anti-sense 56K RNA. When the yield of VSV produced was compared in the different situations, we observed that interferon reduced the VSV yield at least 400,000 fold.In contrast, in cells micro-injected with anti-sense 56K DNA, the antiviral effect was considerably reduced (VSV yield was only reduced 100 fold). These experiments are curently controled with several RNAs originating from various independent constructions. If the results are confirmed, they may strongly indicate that translation of the 56K protein is required for expression of an antiviral state in this cell virus system. There is no other precedent of such a relationship, except for the well-characterized Mx protein/gene system which is required for expressing the antiviral effect of interferon specifically towards influenza virus (Horisberger and Hochkeppel, 1985 ; Staeheli et al., 1985). Furthermore, this approach could provide an interesting way to study the respective role of the various interferon-induced genes as soon as their 5' terminal end is available. Large size cDNA clones are also of major interest for studying the expression of the cloned cDNA in various host cell systems and for the study and isolation of genomic regulatory sequences. The method described here for the preparation of cDNA library of interferon-induced genes is a simple and valuable tool to achieve this goal.

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