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Structure of two forms of the interferon-induced (2′-5′) oligo A synthetase of human cells based on cDNAs and gene sequences

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The (2′-5′) oligo A synthetase E, one of the translational inhibitory enzymes whose synthesis is strongly induced by all interferons (IFNs), is shown to be encoded in human cells by a 13.5-kb gene. By a cell-specific differential splicing, between the seventh and an additional eighth exon of this gene, two active E mRNAs of 1.6 and 1.8 kb are produced, along with several longer transcripts. cDNA clones for the two mRNAs were obtained and their sequences indicate that the human (2′-5′) oligo A synthetase gene codes for two forms of the enzyme of mol. wt. 41 000 and 46 000, which differ only by their C-terminal ends. The product of the 1.6-kb RNA (E16) has a very hydrophobic C terminus, which is replaced by a longer acidic C-terminal sequence in the 1.8-kb RNA product (E18). The transcriptional start site of the gene was identified and 200 bp of the 5′ flanking region were sequenced. A strong homology was found between this region of the IFN-activated (2′-5′) oligo A synthetase gene and the corresponding region of the human fibroblast IFN-β1 gene, whose transcription is also stimulated by IFN priming. The gene has two polyadenylation sites which share a common undecanucleotide, but are used in a cell-specific manner to give rise to the 1.6- and 1.8-kb mRNAs.

Key words: interferon-induced proteins/(2′−5′)A synthetase/human gene/cDNA sequence/differential splicing

Introduction

The (2′-5′) oligo A system in IFN action

Many of the biological effects of interferons appear to be mediated by the induction of new mRNAs and proteins in cells exposed to IFNs (for review: Revel, 1984; Lebleu and Content, 1982; Baglioni and Nilsen, 1983). Among these IFN-induced proteins two groups appear particularly important: (i) translation regulatory enzymes [ds RNA-dependent protein kinase and (2′-5′) oligo A synthetase, (2′-5′) oligo A-activated nuclease, 2′-phosphodiesterase] and (ii) cell-surface antigens (HLA-A,B,C, β2-microglobulin, HLA-DR). Other cellular and excreted proteins probably play important roles as well (Weil *et al.*, 1983; Chebath *et al.*, 1983; Wallach *et al.*, 1983). With the exception of the HLA genes (Malissen *et al.*, 1982; Schamboeck *et al.*, 1983), the structure and sometimes the function of the IFN-induced proteins is unknown and so is the mechanism by which IFNs specifically activate these genes. To address these questions, several cDNAs from IFN-induced genes have been cloned recently (Chebath *et al.*, 1983; Merlin *et al.*, 1983; Friedman *et al.*, 1984; Samanta *et al.*, 1984). Of particular interest is the gene coding for the (2′-5′) oligo A synthetase, a ds RNA-activated enzyme that converts ATP into ppp(A2′pA)n oligomers (Kerr and Brown, 1978), which in turn bind to and activate the latent RNase F (Schmidt *et al.*, 1978). The (2′-5′) oligo A synthetase is strongly induced in human cells by all three types of IFNs, and its increase is a good marker of IFN activity (Wallach *et al.*, 1982). The enzyme is induced during differentiation of hematopoietic cells, as a result of an autocrine secretion of IFN-β (Yarden *et al.*, 1984). The enzyme is similarly induced late in the S phase of synchronized embryo fibroblasts (Wells and Mallucci, 1985). The enzyme activity drops when cell growth starts (Etienne-Smekens *et al.*, 1983; Creasey *et al.*, 1983) and appears to be involved in the antigrowth effect of IFN (Kimchi *et al.*, 1981). A partial loss of the antiviral effects of IFN was seen in cells deficient in the (2′-5′) oligo A synthetase (Salzberg *et al.*, 1983), deficient in the (2′-5′) oligo A-activated RNase F (Epstein *et al.*, 1981) or when this RNase was inhibited by (2′-5′)A analogs (Watling *et al.*, 1985) although this is probably not the only mechanism by which IFN inhibits virus growth (Lebleu and Content, 1982). The (2′-5′) oligo A nucleotides have been detected in many eukaryotic cells and even in bacteria (Laurence *et al.*, 1984) and 2′-specific synthetase may be widespread. The mammalian IFN-induced enzyme has been purified from mouse (Dougherty *et al.*, 1980) and human cells (Yang *et al.*, 1981; Revel *et al.*, 1981); a large and a small form of the enzyme have been observed (Revel *et al.*, 1982; St. Laurent *et al.*, 1983) but their structures were not elucidated.

Cloning of human (2′-5′) oligo A synthetase mRNAs

A partial cDNA clone (E1) for the (2′-5′) oligo A synthetase mRNA from human SV80 cells, was first obtained through its ability to select by hybridization a mRNA producing (2′-5′) oligo A synthetase activity upon translation in *Xenopus laevis* oocytes (Merlin *et al.*, 1983). The E1 cDNA insert (675 bp) hybridized to three RNA species of 1.6, 1.8 and 3.6 kb which are coinduced by IFN in SV80 cells, accumulate for 12 h and are found in the cytoplasmic polysomal fraction (Benech *et al.*, 1985). Additional early transcripts of 2.7 and 4 kb were seen in smaller amounts. Analysis of various types of human cells has shown that these RNAs are differentially expressed in a cell-specific manner. In B lymphoblastoid cells (Namalva, Daudi) only the 1.8-kb RNA accumulates, while in amniotic WISH cells, in histiocytic lymphoma U937 cells and in HeLa cells, the 1.6-kb RNA is predominantly induced by IFN with some 3.6 kb RNA but little 1.8 kb RNA. In diploid fibroblasts FS11, in SV80 fibroblastoid cells and in the T-cell line CEMT, all three stable RNAs are expressed (Benech *et al.*, 1985; unpublished results). The type of (2′-5′) oligo A synthetase RNA expressed does not depend on the species of IFN used (α, β, γ) but rather seems to be developmentally regulated in the cell. The different (2′-5′) oligo A synthetase transcripts could be mapped to a single gene (Benech *et al.*, 1985). Restriction mapping showed (i) that the E1 cDNA corresponds to the 3′ end of the 1.6-kb RNA, (ii) that the 1.8-kb RNA has a different 3′ end than the 1.6-kb RNA and contains an additional downstream exon, (iii) that the 3.6-kb RNA has the same 3′ end as the 1.8-kb RNA but is incompletely spliced. Hybridization-translation experiments using specific genomic DNA fragments also demonstrated that both the 1.8- and 1.6-kb

RNAs actively code for (2'-5') oligo A synthetase (Benech *et al.*, 1985).

In the present work, we report the sequences of cDNA clones for the 1.6- and 1.8-kb RNAs, which allow us to predict the complete amino-acid sequences of two forms of the IFN-induced (2'-5') oligo A synthetase in human cells. The two proteins differ in their C termini, which is hydrophobic in the 1.6-kb RNA product (E16) and acidic in the 1.8-kb RNA product (E18). A full-length (2'-5') oligo A synthetase gene was cloned from the human genome. Transcript mapping on this gene shows that the 1.6-kb RNA is coded by seven exons and the 1.8-kb RNA by eight exons. The sequence of the presumed transcription-initiation site and promoter region of the IFN-activated human (2'-5') oligo A synthetase gene was identified and shows a striking homology to the promoter region of the human IFN-β1 gene.

Results

Sequence of cDNA for the 1.6-kb (2'-5') oligo A synthetase mRNA

The partial E1 cDNA clone (Merlin *et al.*, 1983), shown to be the 3' end of the 1.6-kb (2'-5') oligo A synthetase mRNA induced by IFN in human cells (Benech *et al.*, 1985) was used to screen a lambda gt10 cDNA library from SV80 cell RNA (Wolf and Rotter, 1985). By restriction mapping, clone λ gt10 9-2 was found to contain the E1 cDNA at the 3' end of a 1.32-kb *Eco*RI insert (Figure 1A) which was subcloned in pBR (9-21 cDNA). Sequencing was carried out as outlined in Figure 1A and confirmed that the 9-21 cDNA contains the C terminus and 3' untranslated sequence previously reported for the E1 cDNA (Merlin *et al.*, 1983). The 9-21 cDNA sequence (Figure 2) predicts an open reading frame of 364 amino acids starting at an ATGATG sequence. A computer program based on the three-base periodicity of protein-coding sequences (Trifonov, 1984) indicated that the only compatible reading frame is the one starting from this ATGATG (not shown). It is possible that the translation initiates at the second ATG in this site, since it is the only one preceded by an A at −3 and having homology with the consensus translation initiation sequence (Kozak, 1984).

The enzyme thus coded by the 1.6-kb (2'-5') oligo A synthetase RNA would have a mol. wt. of 41 700, in good agreement with the apparent mol. wt. 38 000 protein seen when the *in vitro* translation product of RNA hybridized to E1 cDNA was analyzed by SDS-polyacrylamide gel electrophoresis (Merlin *et al.*, 1983). A synthetic peptide (amino acids 348 − 364) was prepared and used to immunize rabbits. The resulting antiserum specifically immunoprecipitated an IFN-induced mol. wt. 40 000 protein from extracts of ^{35}S-methionine labeled SV80 cells, as well as part of the (2'-5') oligo A synthetase activity; furthermore, the E16 9-21 cDNA produced ds RNA-binding (2'-5') oligo A synthetase activity in *E. coli* (Chebath *et al.*, 1985). The E16 cDNA contains, therefore, the information for an active form of the enzyme.

Sequence of cDNA for the 1.8-kb mRNA

A genomic DNA fragment corresponding to the additional exon of the 1.8-kb RNA (Benech *et al.*, 1985; see Figure 4) was used

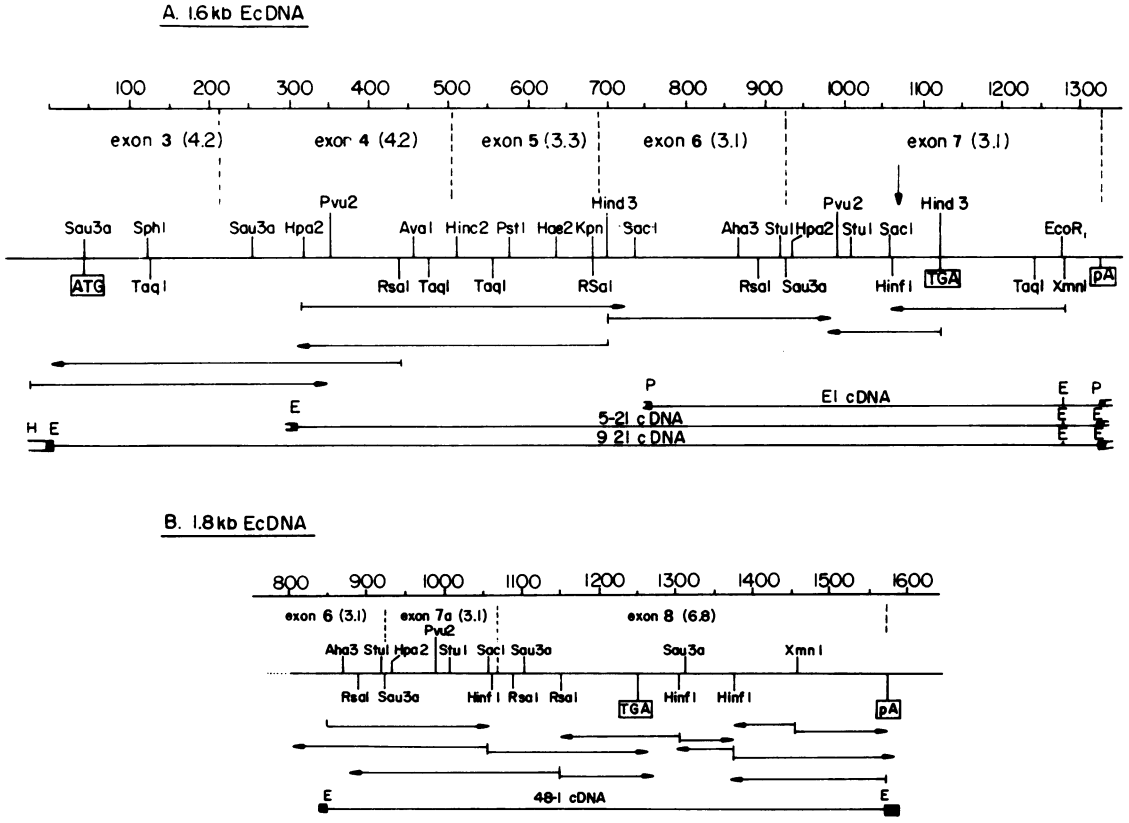

Fig. 1. Restriction map of cDNAs for the 1.6- and 1.8-kb (2'-5') oligo A synthetase mRNAs. **(A)** Map of the 1.6 kb cDNA. The position of the E1 cDNA (Merlin *et al.*, 1983) and of the λgt10 cDNAs is shown. pA is the polyadenylation site. The exon limits are shown by vertical dotted lines. The size of the genomic DNA fragments carrying each exon is given in parentheses. The vertical arrow shows the position of the additional splice site in the 1.8-kb RNA. The strategy for sequencing the 9-21 and 5-21 cDNAs is indicated. The sequence from the 3' *Eco*RI site (E) to the *Pst*I site (P) was determined in the E1 cDNA (Merlin *et al.*, 1983). **(B)** Map of the 1.8 kb cDNA. The λgt10 clone 48-1 was isolated using the *Pst*I-*Pst*I genomic fragment containing exon 8 of the 1.8-kb RNA (Figure 4). Exons are numbered as for the 1.6-kb E cDNA. The truncated exon 7 is designated 7a.

SEQUENCE OF (2'-5') OLIGO A SYNTHETASE cDNA FOR 1.6 Kb RNA (E16,clone 9-21)

```
    1                                   30      (1)    Sau3a                 60                                  90
E16..GAG GCA GTT CTG TTG CCA CTC TCT CTC CTG TCA ATG ATG GAT CTC AGA AAT ACC CCA GCC AAA TCT CTG GAC AAG TTC ATT GAA GAC TAT
                                            MET MET ASP LEU ARG ASN THR PRO ALA LYS SER LEU ASP LYS PHE ILE GLU ASP TYR
   91                           Sph1   120     (31)                  150                                 180
E16  CTC TTG CCA GAC ACG TGT TTC CGC ATG CAA ATC GAC CAT GCC ATT GAC ATC ATC TGT GGG TTC CTG AAG GAA AGG TGC TTC CGA GGT AGC
     LEU LEU PRO ASP THR CYS PHE ARG MET GLN ILE ASP HIS ALA ILE ASP ILE ILE CYS GLY PHE LEU LYS GLU ARG CYS PHE ARG GLY SER
   181                  exon 3         210    ¦(61)       exon 4              240              Sau3a              270
E16  TCC TAC CCT GTG TGT GTG TCC AAG GTG GTA AAG¦GGT GGC TCC TCA GGC AAG GGC ACC ACC CTC AGA GGC CGA TCT GAC GCT GAC CTG GTT
     SER TYR PRO VAL CYS VAL SER LYS VAL VAL LYS GLY GLY SER SER GLY LYS GLY THR THR LEU ARG GLY ARG SER ASP ALA ASP LEU VAL
   271                                  300     (91)                  330                  Pvu2           360
E16  GTC TTC CTC AGT CCT CTC ACC ACT TTT CAG GAT CAG TTA AAT CGC CGG GGA GAG TTC ATC CAG GAA ATT AGG AGA CAG CTG GAA GCC TGT
     VAL PHE LEU SER PRO LEU THR THR PHE GLN ASP GLN LEU ASN ARG ARG GLY GLU PHE ILE GLN GLU ILE ARG ARG GLN LEU GLU ALA CYS
   361                                  390    (121)                  420                                 450
E16  CAA AGA GAG AGA GCA CTT TCC GTG AAG TTT GAG GTC CAG GCT CCA CGC TGG GGC AAC CCC CGT GCG CTC AGC TTC GTA CTG AGT TCG CTC
     GLN ARG GLU ARG ALA LEU SER VAL LYS PHE GLU VAL GLN ALA PRO ARG TRP GLY ASN PRO ARG ALA LEU SER PHE VAL LEU SER SER LEU
   451                                  480    (151)       exon 4      ¦exon 5 510                             540
E16  CAG CTC GGG GAG GGG GTG GAG TTC GAT GTG CTG CCT GCC TTT GAT GCC CTG GGT CAG TTG ACT GGC AGC TAT AAA CCT AAC CCC CAA ATC
     GLN LEU GLY GLU GLY VAL GLU PHE ASP VAL LEU PRO ALA PHE ASP ALA LEU GLY GLN LEU THR GLY SER TYR LYS PRO ASN PRO GLN ILE
   541                                  570 Pst1(181)                 600                                 630
E16  TAT GTC AAG CTC ATC GAG GAG TGC ACC GAC CTG CAG AAA GAG GGC GAG TTC TCC ACC TGC TTC ACA GAA CTA CAG AGA GAC TTC CTG AAG
     TYR VAL LYS LEU ILE GLU GLU CYS THR ASP LEU GLN LYS GLU GLY GLU PHE SER THR CYS PHE THR GLU LEU GLN ARG ASP PHE LEU LYS
   631                                  660    (211)          Kpn1   exon 5 ¦690  exon 6 Hind3                  720
E16  CAG CGC CCC ACC AAG CTC AAG AGC CTC ATC CGC CTA GTC AAG CAC TGG TAC CAA AAT¦TGT AAG AAG AAG CTT GGG AAG CTG CCA CCT CAG
     GLN ARG PRO THR LYS LEU LYS SER LEU ILE ARG LEU VAL LYS HIS TRP TYR GLN ASN CYS LYS LYS LYS LEU GLY LYS LEU PRO PRO GLN
   721          Sac1                    750    (241)                  780                                 810
E16  TAT GCC CTG GAG CTC CTG ACG GTC TAT GCT TGG GAG CGA GGG AGC ATG AAA ACA CAT TTC AAC ACA GCC CAA GGA TTT CGG ACG GTC TTG
     TYR ALA LEU GLU LEU LEU THR VAL TYR ALA TRP GLU ARG GLY SER MET LYS THR HIS PHE ASN THR ALA GLN GLY PHE ARG THR VAL LEU
   811                                  840    (271)                Aha3 870                                 900
E16  GAA TTA GTC ATA AAC TAC CAG CAA CTC TGC ATC TAC TGG ACA AAG TAT TAT GAC TTT AAA AAC CCC ATT ATT GAA AAG TAC CTG AGA AGG
     GLU LEU VAL ILE ASN TYR GLN GLN LEU CYS ILE TYR TRP THR LYS TYR TYR ASP PHE LYS ASN PRO ILE ILE GLU LYS TYR LEU ARG ARG
   901            exon 6 ¦exon 7  Sau3a 930    (301)                  960                              Pvu2 990
E16  CAG CTC ACG AAA CCC AGG CCT GTG ATC CTG GAC CCG GCG GAC CCT ACA GGA AAC TTG GGT GGT GGA GAC CCA AAG GGT TGG AGG CAG CTG
     GLN LEU THR LYS PRO ARG PRO VAL ILE LEU ASP PRO ALA ASP PRO THR GLY ASN LEU GLY GLY GLY ASP PRO LYS GLY TRP ARG GLN LEU
   991                                 1020    (331)                 1050   Sac1                              1080
E16  GCA CAA GAG GCT GAG GCC TGG CTG AAT TAC CCA TGC TTT AAG AAT TGG GAT GGG TCC CCA GTG AGC TCC TGG ATT CTG CTG GTG AGA CCT
     ALA GLN GLU ALA GLU ALA TRP LEU ASN TYR PRO CYS PHE LYS ASN TRP ASP GLY SER PRO VAL SER SER TRP ILE LEU LEU VAL ARG PRO
  1081                                 1110    (361)    Hind3        1140                                1170
E16  CCT GCT TCC TCC CTG CCA TTC ATC CCT GCC CCT CTC CAT GAA GCT TGA GAC ATA TAG CTG GAG ACC ATT CTT TCC AAA GAA CTT ACC TCT
     PRO ALA SER SER LEU PRO PHE ILE PRO ALA PRO LEU HIS GLU ALA END
  1171                                 1200                  1230                                1260
E16  TGC CAA AGG CCA TTT ATA TTC ATA TAG TGA CAG GCT GTG CTC CAT ATT TTA CAG TCA TTT TGG TCA CAA TCG AGG GTT TCT GGA ATT TTC
  1261              EcoR1-Xmn1        1290                  1320                                1350
E16  ACA TCC CTT GTC CAG AAT TCA TTC CCC TAA GAG TAA TAA TAA ATA ATC TCT AAC ACCAAAAA...
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SEQUENCE OF cDNA FOR E 1.8 Kb RNA (E18, clone 48-1)

```
  901            exon 6 ¦exon 7a Sau3a 930    (301)                  960                              Pvu2 990
E18..CAG CTC ACG AAA CCC AGG CCT GTG ATC CTG GAC CCG GCG GAC CCT ACA GGA AAC TTG GGT GGT GGA GAC CCA AAG GGT TGG AGG CAG CTG
  ..GLN LEU THR LYS PRO ARG PRO VAL ILE LEU ASP PRO ALA ASP PRO THR GLY ASN LEU GLY GLY GLY ASP PRO LYS GLY TRP ARG GLN LEU
  ------------------------------------------------------------------------------------------------------------------------
  991                                 1020    (331)                 1050   Sac1          exon 7a ¦exon 8 1080
E18  GCA CAA GAG GCT GAG GCC TGG CTG AAT TAC CCA TGC TTT AAG AAT TGG GAT GGG TCC CCA GTG AGC TCC TGG ATT CTG CTG¦GCT GAA AGC
     ALA GLN GLU ALA GLU ALA TRP LEU ASN TYR PRO CYS PHE LYS ASN TRP ASP GLY SER PRO VAL SER SER TRP ILE LEU LEU ALA GLU SER
  ------------------------------------------------------------------------------------------------------------------------
 10@1 {glycos.}               Sau3a 1110    (361)                 1140                                1170
E18  AAC AGT ACA GAC GAT GAG ACC GAC GAT CCC AGG ACG TAT CAG AAA TAT GGT TAC ATT GGA ACA CAT GAG TAC CCT CAT TTC TCT CAT AGA
     ASN SER THR ASP ASP GLU THR ASP ASP PRO ARG THR TYR GLN LYS TYR GLY TYR ILE GLY THR HIS GLU TYR PRO HIS PHE SER HIS ARG
  1171                                 1200    (391)                 1230                                1260
E18  CCC AGC ACG CTC CAG GCA GCA TCC ACC CCA CAG GCA GAA GAG GAC TGG ACC TGC ACC ATC CTC TGA ATG CCA GTG CAT CTT GGG GGA AAG
     PRO SER THR LEU GLN ALA ALA SER THR PRO GLN ALA GLU GLU ASP TRP THR CYS THR ILE LEU END
  1261                                 1290                 Sau3a  1320                                1350
E18  GGC TCC AGT GTT ATC TGG ACC AGT TCC TTC ATT TTC AGG TGG GAC TCT TGA TCC AGA GAA GAC AAA GCT CCT CAG TGA GCT GGT GTA TAA
  1351                                 1380                  1410                                1440
E18  TCC AAG ACA GAA CCC AAG TCT CCT GAC TCC TGG CCT TCT ATG CCC TCT ATC CTA TCA TAG ATA ACA TTC TCC ACA GCC TCA CTT CAT TCC
  1441           Xmn1                  1470                  1500                                1530
E18  ACC TAT TCT CTG AAA ATA TTC CCT GAG AGA GAA CAG AGA GAT TTA GAT AAG AGA ATG AAA TTC CAG CCT TGA CTT TCT TCT GTG CAC CTG
  1531                                 1560                  1590                                1620
E18  ATG GGA GGG TAA TGT CTA ATG TAT TAT CAA TAA CAA TAA AAA TAA AGC AAA TAC CAAAAA...
```

Fig. 2. Nucleotide sequences of the two (2′-5′) oligo A synthetase cDNAs. The nucleotides of the 1.8-kb cDNA clone 48-1 are numbered as for the 1.6-kb cDNA clone 9-21. Amino acid numbering is given in parenthesis. Translation could start at the first or second codon of the ATGATG sequence. Limits between exons are shown by vertical bars. {Glycos.} indicates a possible glycosylation site in E18. Single base variations, possibly allelic differences, were detected between clones or genomic DNA in the 1.6-kb sequence at 376 (T for C), 525 (G for A), 807 (G for C), 811 (A for G); in the 1.8-kb sequence at 1087 (G for A), 1115 (G for C).

as probe to isolate an E18 cDNA clone, 48-1, from the same λgt10 cDNA library of SV80 RNA. The restriction map of the E18 cDNA clone (Figure 1B) confirmed that its 5′ end is part of the E16 cDNA but that its 3′ end differs. Sequencing (Figure 2) revealed that the junction is at nucleotide 1071 of the E16 9-21 cDNA clone, the last 247 nucleotides of E16 being replaced by a 515 nucleotide-long sequence terminated by a different polyadenylation site. This difference accounts for the 0.2-kb difference in size between the two mRNAs seen on Northern blots. The 5′ portion of the E18 cDNA shows no base change from the sequence of the E16 cDNA, but is incomplete. The gene mapping described below, indicates that both 1.6- and 1.8-kb mRNAs have the same 5′ end.

The sequence of the 1.8-kb RNA predicts a protein which differs from the product of the 1.6-kb RNA. The 3′ region of the E18 cDNA diverges from the E16 C terminus in its last 18

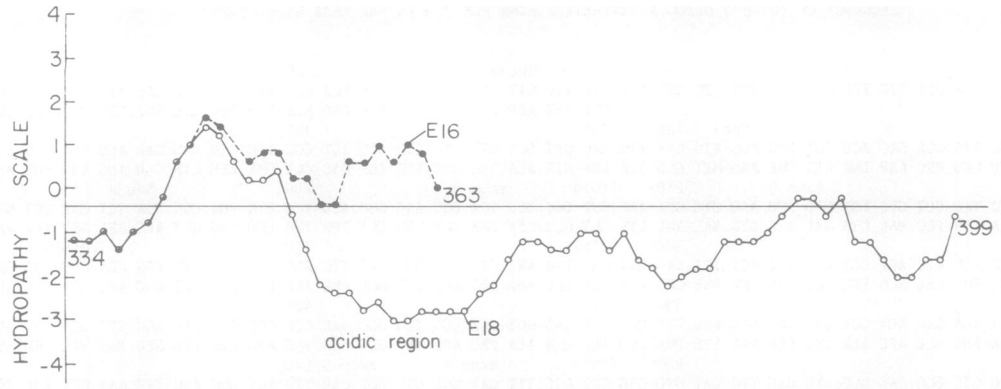

Fig. 3. Hydropathy plot of the C termini of the E16 and E18 (2′-5′) oligo A synthetases. The computer program of Kyte and Doolittle (1982) was used. Hydrophobic regions are over the midline. The acidic region in E18 corresponds to amino acids 353–358 in Figure 2.

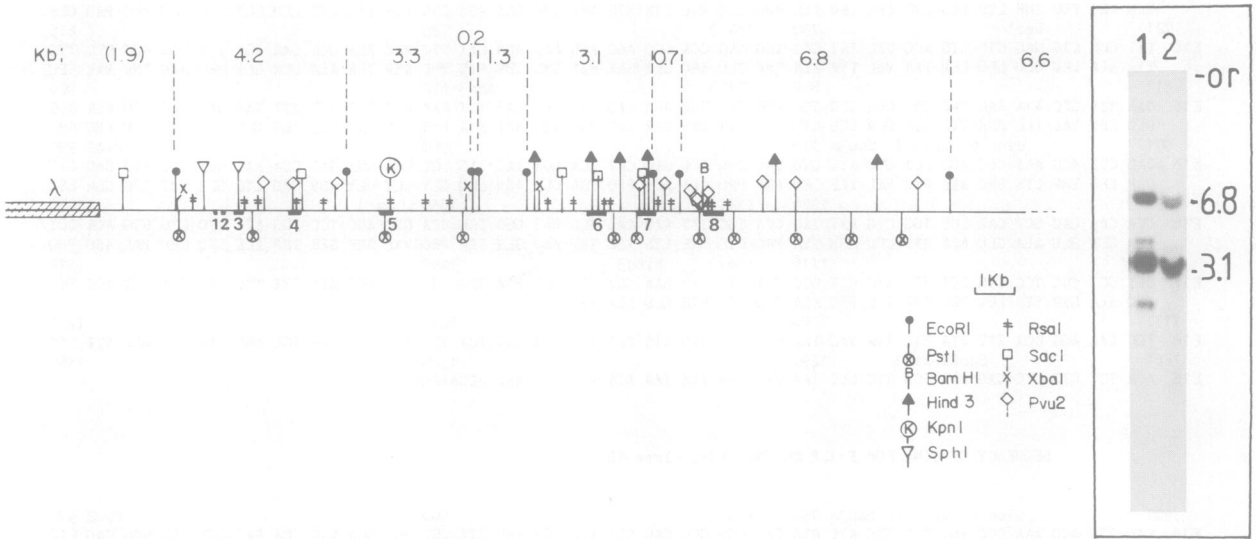

Fig. 4. Restriction map of the human (2′-5′) oligo A synthetase gene. A map constructed from three overlapping genomic clones is shown with the position of the 7 exons of the 1.6-kb RNA and the additional 8th exon of the 1.8-kb RNA (black bars). The insert shows a Southern blot of genomic DNA with the 48-1 cDNA as probe. **Slot 1,** Daudi DNA; **slot 2,** diploid fibroblast FS11 DNA.

codons, and contains an open reading frame ending after 54 codons. This reading frame, which leaves a 350 nucleotides-long untranslated region, was confirmed by the computer program based on the three base periodicity of protein-coding sequences (Trifonov, 1984). An alternative longer open reading frame would not be in the same computed phase as the 5′ portion common with the E16 cDNA. A hydropathy plot (Kyte and Doolittle, 1982) on the predicted C termini of the 1.6- and 1.8-kb mRNA protein products, indicates a striking difference between the two forms of the (2′-5′) oligo A synthetase (Figure 3). The C terminus of the E16 protein is very hydrophobic, while that of the E18 protein is hydrophilic and contains two acidic regions (Asp-Asp-Glu-Thr-Asp-Asp and Glu-Glu-Asp) (Figure 2). Furthermore, a possible glycosylation site is present in the C terminus of the E18 product (Figure 2).

Organisation of the human (2′-5′) oligo A synthetase gene

Three overlapping genomic clones were isolated using the E1 cDNA as probe (Benech *et al.*, 1985), one from the library of partial *Eco*RI digest of human blood-cell DNA (Mory *et al.*, 1981) and two from a library of partial *Alu*I and *Hae*III digests of embryonic human DNA (Maniatis *et al.*, 1978). The genomic clones represent about 29 kb of human DNA and no evidence for more than one E gene was found while screening the libraries. Southern blots of genomic DNA are consistent with the existence of a single gene (Figure 4). By Northern-blot analysis using genomic DNA fragments as probes, by S1 nuclease mapping and by sequencing, the E16 cDNA 9-21 was shown to correspond to five exons on the gene (Figure 4). The ATGATG sequence is found in exon 3, while the termination codon and 3′ untranslated region with the polyadenylation site of the 1.6-kb RNA are found in exon 7. The structure of the more 5′ exons 1 and 2 is described below. The sequences of the intron-exon boundaries were determined (Table I) and follow the CAG and GT rule for the splice acceptor and donor sites (Breathnach and Chambon, 1981). A sequence CTGAC/T is commonly found not far from the splice acceptor, as reviewed recently by Keller (1984). It is notable that the CTGAC/T region shows base complementarity to the sequence of the intron/exon 3′ boundary (acceptor site: Table I), in addition to the complementarity of the intron donor site with the CTGAC sequence pointed out by Keller (1984) as playing a role in the lariat model.

The sequences of the five exons containing the coding region of the (2′-5′) oligo A synthetase produced by the 1.6-kb mRNA,

would indicate that the enzyme is composed of domains with differing amino-acid compositions (Figure 2). The first exonic domain (60 amino acids) is rich in aspartic acid (10%), in the second (amino acids 61 – 156) arginine is predominant (10.4%), the two next exons (amino acids 157 – 218 and 219 – 295) are lysine rich (11.5%) and the C terminus of the E16 product (296 – 364) is very rich in proline (16%) and alanine (10.1%).

Table I. Exon-intron boundaries in the human (2'-5') oligo A synthetase gene

```
                    (4.2)|-50
..CCCTTCTGAGGAAACGAAACCAACAG|CAGTCCAAG....
                         exon 3          (4.2)
                        ..AAG.GTG.GTA.AAG|GTGAGCGG.... 1.3 kb
                                    213|
      (4.2)..GGTTTGCCTTACTAAG|214
CATCAATTATTATTTTTGTTCTTTTTTCAG|GGT.GGC.TCC.TCA..
                         exon 4          (4.2)
                          ..GAT.GCC.CTG.G|GTGAGAGCTC... 2.3 kb
                                    502|
          (3.3)..GAAGAGCTGAC|503
 ACCTAAGTTGTAAGTTTTACCCAGACAG|GT.CAG.TTG.ACT..
                         exon 5          (3.3)
                        ..TGG.TAC.CAA.AAT|GTATGGTTT.... 5.3 kb
                                    687|
         (3.1)..TGAGCAAACCAA|688
TTTTTTTCTGATTGTTTTTCCTCTTCTCAG|TGT.AAG.AAG.AAG..
                         exon 6          (3.1)
                          ..ACG.AAA.CCC.AG|GTATGCTATCCCACATGGCTTG.. 0.9kb
                                    916|
         (3.1)       -Pst1-|917
TACCTGTCCTCTCTAAATGCTGCTCTGCAG|G.CCT.GTG.ATC..
                         exon 7a         (3.1)       EcoR1- (0.7)
                        ..TGG.ATT.CTG.CTG|GTGAGACCT....GAATTCATTCCCCTAAG
                                    1071|AGTAATAATAAATAATCTCTAACACCATTT
                                        |ATTGACTGTCTGCTTCGGGCTC.. 1.4kb
             (6.8)   BamH1-             |     (whole intron = 1.6 kb)
                  ....GGATCCAG|
ATGGCATGTCACAGTATACTAAATGCTCAC|
TGAATCCAGCTGCAATGCAGGAAGACTCCC|1072    exon 8 (1.8 KB RNA)    1585
CTGATGTGATCATGTGTCTCACCCTTTCAG|GCT.GAA.AGC...AATAAAAATAAAGCAAATACCATTTATTGGGTG..
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For exon numbering see Figures 2 and 4. The self-complementary regions between the CTGAT/C, or CTTAC, CTGTC (Keller, 1984) and splice acceptor CAG are underlined. The polyadenylation sites with a conserved undecanucleotide of the 1.6- and 1.8-kb RNAs (see Figure 2) are underscored by dots. The numbers in parentheses are the size of the *Eco*RI genomic fragments carrying the introns or exons (see Figure 4). The start and end of each exon is numbered as in the 9-21 E cDNA of Figure 2.

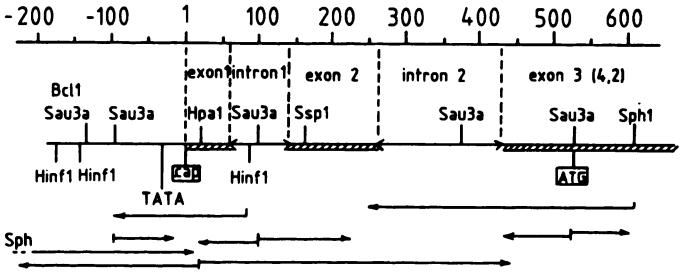

Fig. 5. Promoter region of the human (2'-5') oligo A synthetase gene. A restriction map of the *Sph*I-*Sph*I 0.85-kb fragment from the 4.2-kb *Eco*RI genomic DNA segment in Figure 4 is shown. The 5' end of the mRNAs are marked as cap.

Although the E18 cDNA 48-1 is incomplete, we found that exons 1 – 6 (Figure 4) hybridize to the 1.8-kb mRNA as well as to the 1.6-kb mRNA on Northern blots (not shown). The structure of the two RNAs is most likely identical up to exon 7. The additional splicing from the middle of exon 7 to exon 8 characterizing the E18 cDNA, was confirmed by sequencing these intron-exon boundaries in the genomic DNA clone (Table I). The truncated exon 7a present in the E18 cDNA is followed by a 1.6-kb intron containing the polyadenylation site of the 1.6-kb RNA. Exon 8 begins 98 bp downstream from the unique *Bam*HI site of the gene (Table I, Figure 4). The genomic exon 8 ends by the polyadenylation site of the 1.8-kb RNA, characterized by a tandem repeat of the AATAAA signal. Although exon 7 and 8 have no homology, a conserved undecanucleotide ACCATTTATTG, in which the third cytidine is polyadenylated, is present at the end of both exons (Table I). As pointed out previously (Benech *et al.*, 1985), a hairpin-loop structure can be formed in both cases between this conserved undecanucleotide and the AATAAA region; such structures may participate in the cell-specific mechanism which determines whether cleavage and polyadenylation of the transcripts occur at the end of exon 7 or at the end of exon 8.

Based on the above gene mapping, the enzyme coded for by the 1.8-kb mRNA should be identical to the E16 product in the first 346 amino acids, which are followed by a specific 54 amino-acid region, rich in aspartic acid (9.3%), glutamic acid (9.3%) and threonine (14.8%). The 400 amino-acid E18 enzyme would have a mol. wt. of 46 000.

Promoter region of the (2'-5') oligo A synthetase gene

The *Sph*I-*Sph*I fragment of 0.85 kb (Figure 5) from the genomic 4.2-kb *Eco*RI fragment (Figure 4) which contains part of exon 3 of the E16 cDNA 9-21 clone, hybridized in Northern blots with the 1.6-, 1.8-, 2.7- and 3.6-kb RNAs. However, upstream regions did not hybridize. Several experiments allowed localization of the RNA transcriptional start site in this fragment. S1-nuclease analysis first showed that exon 3 starts about 50 nucleotides upstream of the end of the 9-21 cDNA. A primer-extension experiment using an oligonucleotide from the end of the 9-21 cDNA, indicated that the 5' end of the mRNA is about 230 nucleotides from the 5' end of this cDNA. RNA hybridization with riboprobes produced in SP6 (Green *et al.*, 1983) and RNase digestion indicated two exons of 70 and 110 nucleotides preceding exon 3. By S1-nuclease analysis with a probe labeled at the unique *Hpa*I site (Figure 4), the 5' end of the mRNA was finally located 17 nucleotides upstream from the *Hpa*I site. The sequence of this region is shown in Figure 6. The location of the transcription initiation site 17 residues before the *Hpa*I site is supported by the presence of a TATAA box at position −30. A striking feature of the upstream sequences is the high purine content (69.6%), mostly adenine (58.9%). A run of a homology matrix with other known upstream promoter sequences reveal-

```
          Sau3a          -80        -70        -60        -50        -40
E     AAGATCCTGT CTCCAAAAAA TAATAAAATA AAATAAAAAT CTACTAATTG AAAGGGAAAA AAG------- ---------- ----------
          -130       |  | |||  || |||||   ||    | ||    -90|| || ||||||| ||   |          -60        -50
IFN-β AAATAAAGAG TTTTAGAAAC TACTAAAATG TAAATGACAT AGGAAAACTG AAAGGGAGAA GTGAAAGTGG GAAATTCCTC TGAATAGAGA

                    -30        -20        -10          1         10         Hpa1       30
E     ------CAT- -AGTATAATA CCATTCTTAA CAAAAAGAAA AGAGACCTGT GTTTGTGTGT GTGTTAACAT TTGAAAAAAA
          -40 |||      |||||      -20       ||   |||||  || |       10         20         30
IFN-β GAGGACCATC TCATATAAAT AGGCCATACC CATGGAGAAA GGACATTCTA ACTGCAACCT TTCGAAGCCT TTGCTCTGGC
                         =======
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Fig. 6. Sequence of the human (2'-5') oligo A synthetase promoter region. The sequence of the *Sau*3a-*Hpa*I segment of Figure 5 is shown, aligned for comparison with the promoter region of the human IFN-β1 gene (Degrave *et al.*, 1981). Numbering is from the presumed cap site. A purine-rich transcription-regulatory sequence around −75 in the IFN-β1 promoter (Zinn *et al.*, 1983), repeated at −10, is underlined. The TATA box is doubly underlined.

ed a surprising homology with the human IFN promoters, in particular with the sequence of the IFN-β gene promoter (Degrave *et al.*, 1981). The purine-rich region from −75 to −85 of the IFN-β1 promoter, which contains the essential transcription signal desribed by Zinn *et al.* (1983), shows 90% homology with the region of the presumed promoter of the (2′-5′) oligo A synthetase just upstream of the TATAA box (−40 to −50) (Figure 6). This purine-rich signal is repeated in the IFN-β1 promoter within the segment between the TATAA box and the cap site; in this region, which may also have regulatory functions (Nir *et al.*, 1984) the homology between the IFN-β1 gene and the (2′-5′) oligo A synthetase gene is high. In contrast, search for homology with promoters of other genes, such as HLA genes (Malissen *et al.*, 1982; Schamboeck *et al.*, 1983) and the metallothionein II gene (Karin and Richards, 1982) which are activated by IFNs (Fellous *et al.*, 1982; Rosa *et al.*, 1983b; Friedman *et al.*, 1984) showed no apparent sequence relationship in this region of the (2′-5′) oligo A synthetase gene promoter. Also, no significant homology was seen with the body of the IFN-β1 gene.

The 5′ untranslated leader of the (2′-5′) oligo A synthetase mRNA (exon 1, 2 and part of exon 3) is formed by splicing out two short introns whose position was tentatively determined by S1 analysis as shown in Figure 5. The entire human (2′-5′) oligo A synthetase gene is about 13.5-kb long (Figure 4) and the sum of the exons agrees with the observed sizes of the mRNAs.

Discussion

Primary structure and properties of (2′-5′) oligo A synthetases

The (2′-5′) oligo A synthetase, one of the translational inhibitors induced in cells exposed to IFNs (Hovanessian *et al.*, 1977; Zilberstein *et al.*, 1978) has a number of unusual properties. Its main activity is the synthesis from ATP of 5′ triphosphorylated short oligo-A chains (of up to 15As, consisting mainly of dimers to pentamers), but in contrast to other RNA polymerases, it adds adenylate or one other nucleotide specifically to the 2′OH of adenylate in oligo A (Kerr and Brown, 1978; Samanta *et al.*, 1980), or to other (oligo) nucleotides with a free 2′OH adenylate such as NAD (Ball, 1980) or even tRNA (Ferbus *et al.*, 1981). To be active, the enzyme has to bind to double-stranded RNA stretches of minimum 50 bp (Minks *et al.*, 1979), and must therefore possess several binding sites: for nucleoside triphosphates, for 2′OH adenosine polynucleotides and for double-stranded RNA. The enzyme binds to 2′,5′ ADP-Sepharose (Johnston *et al.*, 1980), to poly (rI)(rC)-agarose (Hovanessian *et al.*, 1977) and to Cibacron Blue-Sepharose (Revel *et al.*, 1981). In different cells, the (2′-5′) oligo A synthetase activity is in the cytosol (Revel *et al.*, 1981) or in ribosomal salt washes (Dougherty *et al.*, 1980), as well as in the nuclear sap (Nilsen *et al.*, 1982b) and even in large amounts in the nuclear matrix (A. Kimchi, personal communication). It is notable that cellular RNAs can replace poly (rI)(rC) for activation of the enzyme (Revel *et al.*, 1980) and the synthetase may even have a role in HnRNA processing (Nilsen *et al.*, 1982a). Some (2′-5′) oligo A synthetase is bound to plasma membranes and can be incorporated in budding virions (Wallach and Revel, 1980). These complex interactions may ensure a localized action of the (2′-5′) oligo A system (Baglioni and Nilsen, 1983) and explain its suggested multiple roles in normal and virus-infected cells. The synthetase amounts to less than 0.1% of the protein in IFN-treated cells, and its structure could not be determined directly.

The present sequence analysis of complete cDNA and genomic DNA clones, allows us to predict the amino-acid sequences of two different forms of the enzyme, which are coded respectively by a 1.6- and 1.8-kb RNA. The 1.6-kb RNA codes for a protein (E16) of 363 or 364 amino-acids and mol. wt. 41 500. The 1.8-kb RNA, which is produced by a differential splicing process (Benech *et al.*, 1985) predicts a slightly larger protein (E18) of 399−400 residues and mol. wt. 46 000, identical to E16 in the first 346 residues but with a different C terminus. We have found no difference in the enzymatic activity of the E16 and E18 produced by translation of mRNAs selected by hybridization to specific DNA fragments: both are bound to poly (rI)(rC)-agarose and synthesize the same ppp(A2′pA)n oligonucleotides (Benech *et al.*, 1985). An interesting possibility is that the differing C termini of E16 and E18 determines their ability to dimerize, to interact with other proteins and with different cellular structures. For example, the hydrophobic end of E16 could bind to lipid membranes, while the acidic longer C terminus of E18 may allow interaction with ribosomes or basic proteins of the nuclear matrix. The common N-terminal portion would then contain the region involved in 2′, 5′ADP, NTP and dsRNA binding. This portion of the (2′-5′) oligo A synthetase seems to be composed of an N-terminal acidic domain followed by three basic regions, in particular between amino acids 199 and 225 where there is a Lys-Lys-Lys sequence, also found in viral RNA polymerases (Kitamura *et al.*, 1981). The last domain common to the E16 and E18 proteins is in contrast rich in glycine and proline.

Multiple forms of the enzyme

How do the E16 and E18 sequences relate to previous attempts to purify the human (2′-5′) oligo A synthetase? Gel filtration indicated that IFN-treated HeLa or SV80 cells contain two forms of the enzyme activity of mol. wts. 60−80 000 and 30−40 000 (Revel *et al.*, 1982). The smaller form corresponds in size to the proteins defined here by cDNA sequencing and to the *in vitro* translation product of E1 cDNA-selected mRNA which migrates as 38 000 dalton protein in SDS (Merlin *et al.*, 1983). Purified preparations of enzyme from Namalva and chronic myelogenous leukemia (CML) cells, where the small enzyme form predominates (Revel *et al.*, 1981,1982), also contain a mol. wt. 42 000 band in SDS (unpublished results). The identity of the larger of the two native enzyme forms remains an open question. A simple possibility is that the large enzyme is a dimer of one of the smaller proteins. However, purified preparations from HeLa cells were reported to contain a mol. wt. 100 000 protein in SDS gel electrophoresis (Yang *et al.*, 1981). The 3.6- or 2.7-kb RNA transcripts of the cloned E gene could code for such a protein. Their cDNAs remain to be sequenced to investigate their open reading frames, but gene mapping of the 3.6-kb RNA suggests that it is a partially spliced precursor of the 1.8-kb RNA which does not appear to be translated in *Xenopus* oocytes (Benech *et al.*, 1985). Furthermore, using the oocyte translation, we have not found significant (2′-5′) oligo A synthetase mRNA activity in human cell RNA fractions heavier than 1.8 kb in denaturating gel electrophoresis or sucrose gradients (Merlin *et al.*, 1983). This would also not favor the existence of another large mRNA unrelated to the E cDNAs. The situation may differ in mouse cells, where in denaturating gels a 3.8-kb RNA was found to be actively translated into an 80 000 dalton form of the enzyme upon injection to oocytes,while a 1.5-kb RNA produced a smaller 30 000 dalton form of the enzyme (St. Laurent *et al.*, 1983). The large enzyme appeared mainly to be cytoplasmic, while the small enzyme was found mainly in the nucleus of mouse cells. These two 3.8-kb and 1.5-kb mouse RNAs are indeed detected

by hybridization to the human E cDNA 9−21 (Mallucci *et al.*, 1985). Possibly, a large (2'-5') oligo A synthetase polypeptide is formed in mouse cells, which is replaced in human cells by the product of the further processed 1.8-kb RNA.

Differential splicing of the human (2'-5') oligo A synthetase gene

The single gene for the IFN-induced (2'-5') oligo A synthetase, described here, appears to be the origin of several RNA transcripts which are differentially expressed in various types of human cells. B lymphoblastoid cells, which form the 1.8-kb RNA, produce active cytoplasmic enzyme in response to IFN as do WISH or U937 histiocytic lymphoma cells which have mainly the 1.6- and 3.6-kb RNA species (Benech *et al.*, 1985). The cell-specific differential splicing process giving rise to the 1.6- and 1.8-kb RNAs is clearly demonstrated by the present sequence comparisons. Saunders and Williams (1984) also isolated from Daudi cell RNA, a cDNA clone showing the same splicing pattern as the 1.8-kb E RNA described here. From the sequence comparison of the E16 and E18 cDNAs, it appears that transcript cleavage and polyadenylation can occur either at the promoter-proximal site at the end of exon 7, giving rise to the 1.6-kb RNA, or at the end of exon 8 giving rise to the 1.8- and 3.6-kb RNAs. If the 3.6-kb RNA is a precursor of the 1.8-kb RNA (Benech *et al.*, 1985), absence of splicing in the middle of exon 7 would explain why some cells accumulate 1.6- and 3.6-kb RNAs. Cells producing mainly 1.8-kb RNA, would only polyadenylate at the end of exon 8 and rapidly process the 3.6-kb RNA into the 1.8-kb RNA. The hairpin-loop structures which can be formed around both polyadenylation sites could determine where processing will occur (Benech *et al.*, 1985). This model has to be verified in gene transfection experiments.

Gene promoter region

One of the major reasons for studying the human (2'-5') oligo A synthetase gene is to understand the mechanism by which its expression is induced by IFNs. By S1-nuclease analysis, we have mapped the putative transcription initiation site at a position compatible with the sizes of the transcripts. A TATA box is present 30 bp upstream from this RNA start site, and the sequence of about 200 upstream nucleotides was determined. This region, which could be a part of the promoter, is very rich in adenines, and has no obvious resemblance to corresponding regions of HLA genes (Strachan *et al.*, 1984) or the metallothionein II gene (Karin and Richards, 1982), which are stimulated by IFNs (Fellous *et al.*, 1982; Friedman *et al.*, 1984) and contain a consensus sequence (Friedman and Stark, 1985). In the (2'-5') oligo A synthetase gene, which is very strongly activated by IFNs, a homology matrix computer program revealed a striking degree of homology of nucleotides −37 to −85 with the −72 to −120 region of the human IFN-β1 gene (Degrave *et al.*, 1981). In particular, there is a marked homology with a transcription control sequence around −75 in the IFN-β1 gene (Zinn *et al.*, 1983). It is interesting to note that the human IFN-β1 gene is autoregulated by IFN, leading to the so-called priming effect (Raj and Pitha, 1981). By *in vitro* nuclear run-off transcription experiments, we have shown (Nir *et al.*, 1984) that the transcription of the IFN-β1 gene is strongly stimulated by priming. The significance of this sequence homology will have to be evaluated by functional studies. Induction of the synthetase has been reported in glucocorticoid-treated lymphocytes (Krishnan and Baglioni, 1980) and after hormone withdrawal in oviduct (Stark *et al.*, 1979), but the main control of the expression of the (2'-5') oligo A synthetase gene is without doubt by the various IFN species added either exogenously, or produced endogenously by the cells during their growth or differentiation (Yarden *et al.*, 1984; Revel, 1984). Identification of the complete promoter of the (2'-5') oligo A synthetase gene and its functional analysis should, therefore, reveal some of the fundamental aspects of IFN-dependent gene activation.

Materials and methods

Lambda gt10 cDNA clones of the (2'-5') oligo A synthetase

A λgt10 cDNA library prepared from poly A^+ RNA of human SV80 cells (Wolf and Rotter, 1985) was screened using as probe the *PstI-PstI* insert of the E1 cDNA plasmid described previously (Merlin *et al.*, 1983). The insert corresponding to the 3' end of the 1.6-kb E RNA (Benech *et al.*, 1985) was purified by agarose gel electrophoresis and nick-translated (Rigby *et al.*, 1977). Plaques were repeatedly picked from 9 cm plates (10^5 phages), and small scale λ DNA preparations were analyzed by restriction mapping using routine procedures (Maniatis *et al.*, 1982). Fifteen λ gt10 cDNA clones containing the E1 cDNA fragment were isolated and phages 9-2 and 5-2 with the longest inserts were cut with *Eco*RI and the inserts subcloned in pBR322 to obtain E16 cDNA clones 9-21 and 5-21 of Figure 1A. The same library was rescreened with a human genomic *PstI-PstI* 0.9-kb fragment from phage λchE1 (Benech *et al.*, 1985), a fragment which specifically hybridizes to the 1.8-kbRNA. We thereby isolated λgt10 cDNA clone 48-1 of Figure 1B, along with another cDNA clone representing a partially-spliced E RNA. Sequencing was carried out according to Maxam and Gilbert (1980). Restriction enzymes were from New England Biolabs and Boehringer. Homology-matrix and hydropathy-plot computer programs of Pustell and Kafatos (1982a,b) were run on an IBM PC. Three-base periodicity to locate protein-coding frames was computed according to Trifonov (1984).

Genomic DNA clones containing the (2'-5') oligo A synthetase gene

Three overlapping genomic clones were isolated as previously described (Benech *et al.*, 1985): λchE1 from a partial *Eco*RI-cut DNA library (Mory *et al.*, 1981) and λchE2 and E3 from a partial *AluI/HaeIII* DNA library (Maniatis *et al.*, 1978). The genomic *Eco*RI fragments of these phages were subcloned in pBR322. Exon mapping was done (i) by Southern-blot hybridization of restriction digests of subcloned genomic fragments to various cDNA probes, (ii) by hybridization of genomic DNA restriction fragments to Northern blots of poly A^+ RNA from IFN-treated and untreated cells as described (Benech *et al.*, 1985) and (iii) by sequencing of intron-exon boundaries in comparison to cDNA.

The internal *SphI-SphI* 0.87-kb segment of the genomic 4.2-kb *Eco*RI fragment containing the 5' end of the mRNA, was subcloned in the *SphI* site of pBR322 before sequencing. Primer extensions using synthetic oligodeoxyribonucleotides of 18−20 bases complementary to the mRNA (gift of Dr. D. Segev, InterYeda) were done as before (Rosa *et al.*, 1983a). Riboprobe synthesis after subcloning in the SP6 vector was carried out according to instructions of Promega Biotec. DNA from Daudi lymphoblastoid cells and from FS11 foreskin fibroblasts was prepared according to Wigler *et al.* (1979) and Southern-blot analysis was done on Gene-Screen Plus nylon-fiber sheets using hybridization procedure B recommended by the manufacturer (New England Nuclear).

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