Full-length sequence and expression of the 42 kDa 2–5A synthetase induced by human interferon

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Interferon-induced 2–5A synthetases are probably involved in some antiviral actions of interferon. In human cells two different mRNAs (1.6, 1.8 kb long) coding for this protein are transcribed from the same gene and are produced by differential splicing. The relationship between the two mRNAs of different size and the active enzyme is not clear, nor is the cellular localization of the latter known. We have cloned a cDNA corresponding to the 1.6 kb RNA. This cDNA was sequenced and its complete coding region was subcloned into pSP64. The resulting plasmid was used to direct the synthesis of micrograms of capped RNA transcript after linearization in the 3′-non-coding region. A 39 kDa protein was synthesized when this RNA was translated in rabbit reticulocyte lysate. When this capped RNA was introduced by microinjection into *Xenopus* oocytes, production of 2–5A synthetase was clearly observed in the cytoplasm and 10–30% of the enzyme accumulated with time in the nucleoplasm. Analysis of cytoplasmic homogenates of these oocytes on a glycerol gradient revealed that the enzyme is fully active in the monomeric form.

Interferon induction *cDNA* *Cloning* *Transcription* *Translation*

1. INTRODUCTION

Interferons (IFN) are a class of proteins and glycoproteins secreted by various cells in response to viral infection or to other inducers such as double-stranded RNA (dsRNA) [1–4]. A wide range of biological effects, including inhibition of virus replication, changes in cell membrane, inhibition of cell growth and modification of immune response, occur when interferons interact with discrete plasma membrane receptors [5]. Some of these effects at least are thought to be mediated by proteins whose synthesis is induced in cells after interferon treatment [6,7]. Among these proteins, the 2–5A synthetase has been shown to be involved in some antiviral actions of interferon. In the presence of dsRNA, this enzyme catalyzes the synthesis of 2′–5′ linked oligomers of adenosine, ppp(A2′p)$_n$A. Viral growth is inhibited through mRNA degradation mediated by a latent endoribonuclease which is activated by these 2–5A oligomers. This effect is transient. The 2–5A oligomers are probably rapidly degraded by a 2′-phosphodiesterase [4]. In addition to this antiviral role, the 2–5A system seems to be involved in the regulation of cell growth and differentiation [5]. 2–5A synthetase activities associated with proteins of different sizes were detected in the cytoplasm and nucleoplasm [8,9]. Two functional mRNAs (1.6 and 1.8 kb in human cells [10,11] and 1.8 and 3.6 kb in mouse cells [9]) were shown to encode synthetase activity. The relation between the different mRNAs, which seem to derive from the same gene by differential splicing [10,11], and the different proteins, as well as the cellular localization of the latter, is still not clear. In human cells a cytoplasmic IFN-inducible 2–5A synthetase activity mediated by a 80–105 kDa polypeptide as detected in SDS-polyacrylamide gels has been described [12,13]. This observation is difficult to reconcile with the molecular mass and coding capacity described for the two 1.6 and

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1.8 kb translatable forms of human 2–5A synthetase mRNAs described up until now [10,11,14]. So far no mRNA has been found which codes for the 105 kDa 2–5A synthetase described in human cells. Studies on the regulation, gene organization, transcriptional processing and functional characterization through expression would be greatly facilitated if full-length cDNA clones, corresponding to the various differentially spliced transcripts from the 2–5A synthetase gene, became available. Here we have selected a 2–5A synthetase cDNA clone by screening our human cDNA library prepared from IFN-α treated cells with an oligodeoxyribonucleotide probe derived from the 522 nucleotide sequence described by Merlin et al. [14]. From the 1378 nucleotide sequence of this new cDNA clone, we have derived the complete amino acid sequence of the 42 kDa 2–5A synthetase encoded by the 1.6 kb RNA. Furthermore, the coding region has been subcloned into an SP6 vector which allows cell-free transcription. The recombinant plasmid was used to direct the synthesis of microgram amounts of capped RNA in an 'in vitro' transcription system. When injected into *Xenopus* oocytes, this RNA directs the synthesis of a fully active enzyme, which is found in both the nucleus and cytoplasm, and is presumably active in its monomeric form.

2. MATERIALS AND METHODS

2.1. *Preparation and screening of a cDNA library from α-IFN treated cells*

Total cellular RNA was prepared by extraction in 8 M guanidine hydrochloride [15] of human amniotic U cells (UAC) that had been grown in roller bottles and treated for 6 h at 37°C with 200 U/ml of partially purified α-IFN. After oligo(dT) cellulose chromatography, this RNA was fractionated on a 5–20% sucrose/50% formamide gradient as described [16] and the 16–25 S region of the gradient was used for cDNA synthesis. We used the method described in [17] with the following modifications: in the first strand cDNA synthesis, Na·pyrophosphate was omitted, and 'Super' reverse transcriptase from avian myeloblastosis virus was used (from Anglian Biotechnology-Stehelin); for the second strand synthesis, T_4 DNA ligase (100 U/ml) was used instead of *E. coli* DNA ligase and the incubation was carried out overnight at 14°C; 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 [18]. About 10–30000 clones were replicated from each 82 mm nitrocellulose filter. Duplicate replicas were screened by hybridization with an oligodeoxyribonucleotide (5′-CTAAGGACCTTGTCTCACAGAGTTC-3′) that was synthesized following the procedure of Sproat and Bannwarth [19], following the sequence published for the human 2–5A synthetase cDNA [14]. This oligonucleotide was labeled by kination with [γ-^{32}P]ATP to 2–5 × 10^8 cpm/μg [20]. Hybridization was performed as described in [18] at 52°C except that the medium contained heparin (50 U/ml) and 6 × SSC instead of 6 × SET (SET is 0.15 M NaCl, 30 mM Tris, pH 8, 1 mM EDTA). After hybridization, the filters were washed 4 times in 6 × SSC, 0.5% SDS at 20°C and for 5 min at 52°C. Two clones (p2–5A-1 and p2–5A-2) were selected. After two rounds of purification and hybridization at intermediate and low density, the clones were amplified and the CsCl purified plasmid DNA characterized by restriction analysis.

2.2. *DNA sequence analysis*

First, p2–5A-1 was digested by *Hin*dIII, *Hin*dIII and *Pvu*II, *Pst*I and *Pvu*II, and *Pst*I and *Sac*I. The corresponding fragments deriving from the cDNA insert were resolved by agarose gel electrophoresis, electroeluted and subcloned into M13mp10 and mp11 and used to transform *E. coli* JM101. Second, a 0.7 kb *Pst*I fragment from p2–5A-1 was digested by *Alu*I or *Sau*3AI and the resulting fragments were cloned in M13mp10 and mp11. Single-stranded DNA from the resulting plaques was hybridized to a 17 bp M13 sequencing primer and the sequence of the DNA was analyzed by the dideoxy-chain termination method using ^{35}S-labeled dATP as the labeled nucleotide [21,22]. The 5′-*Pst*I-*Pvu*II fragment, containing the GC tail, was labeled with [α-^{32}P]dideoxyadenosine 5′-triphosphate using terminal transferase (BRL kit). The fragment was then digested with *Sph*I, resolved on a polyacrylamide gel and the eluted *Pst*I-*Sph*I fragment was sequenced according to Maxam and Gilbert [23].

2.3. *Subcloning of p2–5A-1 into pSP64*

As indicated on the restriction map of p2–5A-1 (fig.1), the entire amino acid coding region could not be excised from the pBR322 vector by a combination of unique proximal and distal restriction endonuclease sites. Therefore subcloning of the p2–5A-1 coding region into pSP64 was done in several steps. First, a 1.5 kb fragment of a partial *Pst*I digest was subcloned into pSP64; from the resulting construct, a 1.1 kb *Sph*I-*Eco*RI fragment was excised, corresponding to almost the entire coding region. Then a 0.65 kb *Pst*I fragment of the p2–5A-1 clone was purified and digested by *Alu*I and *Sph*I. The 150 bp *Alu*I-*Sph*I fragment was purified and ligated with the 1.1 kb *Sph*I-*Eco*RI fragment into a *Sma*I-*Eco*RI digested pSP64 vector, downstream of the SP6 promoter.

2.4. *Cell-free transcription*

The pSP64 2–5A DNA construct was used for the synthesis of non-polyadenylated 2–5A synthetase mRNA after linearization with *Eco*RI which cleaves at a unique site, 150 nucleotides beyond the termination UGA (fig.1). The conditions for RNA transcription were as described in [24] except that the concentration of GTP was lowered to 200 μM and that 1 mM $m^7G(5')ppp(5')G$ (PL-Biochemicals) was present during transcription. Human placental ribonuclease inhibitor (Amersham) and SP6 RNA polymerase (Boehringer Mannheim) were used for transcription. Such a reaction usually yields 1 μg homogenous ~ 16 S RNA on agarose gel per μg linearized plasmid DNA transcribed. The RNA was isolated from the transcription reaction after DNase treatment, phenol extraction and ethanol precipitation under the conditions described [24].

2.5. *Cell-free translation*

The capped RNA was subjected to cell-free translation in a reticulocyte lysate mixture at a final concentration of 100 μg/ml during 90 min at 33°C in the presence of [^{35}S]methionine and [^{35}S]cysteine. The conditions for translation in the rabbit reticulocyte lysate and for analysis of the translated products by polyacrylamide gel electrophoresis were as described in [25] and fluorography of the gel was done with Amplify (Amersham).

2.6. *Microinjection of RNA into* Xenopus *oocytes*

Groups of 30–60 oocytes were injected with 50 nl/oocyte of synthetic 2–5A synthetase mRNA at a concentration of 0.05–0.5 mg/ml. They were incubated for 22–24 h (or other periods of time as specified) at 18°C in 1 ml Barth's medium. Total homogenates were prepared by disrupting 30 oocytes in 150 μl buffer B [9] containing 20 mM Hepes, 120 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 0.2% Triton X-100, 10% glycerol, pH 8.0, in a teflon glass homogenizer. After centrifugation for 2 min at 9000 × *g* the supernatant (with the exception for the superficial fat layer) was collected and frozen in liquid nitrogen until used. The nuclei were isolated by incision of the oocytes at the animal pole with a 26G needle, while applying a slight pressure on the oocyte, with forceps. The nuclei were suspended in a minimal volume of buffer B (100 μl/10 oocytes) and frozen at −70°C. The remaining enucleated oocytes were homogenized as described above for the total oocytes.

2.7. *2–5A synthetase assay*

Binding of the enzyme to poly(I)·poly(C)-agarose beads (P.L.), washing and incubation for 16 h at 30°C with [α-^{32}P]ATP were exactly as described [9,11]. 10 μl of each incubation was then made in 30 mM Tris, pH 9, and incubated with 1 U calf intestine alkaline phosphatase for 2 h at 37°C and spotted onto polyethylene imine cellulose TLC plates (Macherey Nagel) [26]. Chromatography in 1 M acetic acid, followed by autoradiography and counting of the radioactive spots was as described [26].

2.8. *Velocity gradient analysis*

Extracts of oocytes prepared as described above were centrifuged in 15–30% glycerol gradients in buffer B containing 10^{-5} M phenylmethanesulfonyl fluoride. Samples of 100 μl were layered onto the gradient and centrifuged for 16 h at 60000 rpm and 4°C in an SW60 Beckman rotor. Fractions of 200 μl were collected and analyzed for enzyme content as described above. Markers (50000 cpm) of ^{125}I-goat-IgG were analyzed in the same gradient. The ~ 160 kDa protein dissociates under partially reducing conditions into light chain (25 kDa), heavy chains (52 kDa average value) and a heavy-light chain complex (77 kDa average value).

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 -94
  A ACT GAA ACC AAC AGC AGT CCA AGC TCA GTC AGC AGA AGA GAT AAA AGC AAA CAG GTC
 -36                                                1                      (8)
TGG GAG GCA GTT CTG TTG CCA CTC TCT CTC CTG TCA ATG ATG GAT CTC AGA AAT ACC CCA
                                                Met Met Asp Leu Arg Asn Thr Pro
  25                                                                       (20)
GCC AAA TCT CTG GAC AAG TTC ATT GAA GAC TAT CTC TTG CCA GAC ACG TGT TTC CGC ATG
Ala Lys Ser Leu Asp Lys Phe Ile Glu Asp Tyr Leu Leu Pro Asp Thr Cys Phe Arg Met
  85                                                                       (48)
CAA ATC AAC CAT GCC ATT GAC ATC ATC TGT GGG TTC CTG AAG GAA AGG TGC TTC CGA GGT
Gln Ile Asn His Ala Ile Asp Ile Ile Cys Gly Phe Leu Lys Glu Arg Cys Phe Arg Gly
 145                                                                       (68)
AGC TCC TAC CCT GTG TGT GTG TCC AAG GTG GTA AAG GGT GGC TCC TCA GGC AAG GGC ACC
Ser Ser Tyr Pro Val Cys Val Ser Lys Val Val Lys Gly Gly Ser Ser Gly Lys Gly Thr
 205                                                                       (88)
ACC CTC AGA GGC CGA TCT GAC GCT GAC CTG GTT GTC TTC CTC AGT CCT CTC ACC ACT TTT
Thr Leu Arg Gly Arg Ser Asp Ala Asp Leu Val Val Phe Leu Ser Pro Leu Thr Thr Phe
 265                                                                      (108)
CAG GAT CAG TTA AAT CGC CGG GGA GAG TTC ATC CAG GAA ATT AGG AGA CAG CTG GAA GCC
Gln Asp Gln Leu Asn Arg Arg Gly Glu Phe Ile Gln Glu Ile Arg Arg Gln Leu Glu Ala
 325                                                                      (128)
TGT CAA AGA GAG AGA GCA TTT TCC GTG AAG TTT GAG GTC CAG GCT CCA CGC TGG GGC AAC
Cys Gln Arg Glu Arg Ala Phe Ser Val Lys Phe Glu Val Gln Ala Pro Arg Trp Gly Asn
 385                                                                      (148)
CCC CGT GCG CTC AGC TTC GTA CTG AGT TCG CTC CAG CTC GGG GAG GGG GTG GAG TTC GAT
Pro Arg Ala Leu Ser Phe Val Leu Ser Ser Leu Gln Leu Gly Glu Gly Val Glu Phe Asp
 445                                                                      (168)
GTG CTG CCT GCC TTT GAT GCC CTG GGT CAG TTG ACT GGC AGC TAT AAA CCT AAC CCC CAA
Val Leu Pro Ala Phe Asp Ala Leu Gly Gln Leu Thr Gly Ser Tyr Lys Pro Asn Pro Gln
 505                                                                      (188)
ATC TAT GTC AAG CTC ATC GAG GAG TGC ACC GAC CTG CAG AAA GAG GGC GAG TTC TCC ACC
Ile Tyr Val Lys Leu Ile Glu Glu Cys Thr Asp Leu Gln Lys Glu Gly Glu Phe Ser Thr
 565                                                                      (208)
TGC TTC ACA GAA CTA CAG AGA GAC TTC CTG AAG CAG CGC CCC ACC AAG CTC AAG AGC CTC
Cys Phe Thr Glu Leu Gln Arg Asp Phe Leu Lys Gln Arg Pro Thr Lys Leu Lys Ser Leu
 625                                                                      (228)
ATC CGC CTA GTC AAG CAC TGG TAC CAA AAT TGT AAG AAG AAG CTT GGG AAG CTG CCA CCT
Ile Arg Leu Val Lys His Trp Tyr Gln Asn Cys Lys Lys Lys Leu Gly Lys Leu Pro Pro
 685                                                                      (248)
CAG TAT GCC CTG GAG CTC CTG ACG GTC TAT GCT TGG GAG CGA GGG AGC ATG AAA ACA CAT
Gln Tyr Ala Leu Glu Leu Leu Thr Val Tyr Ala Trp Glu Arg Gly Ser Met Lys Thr His
 745                                                                      (268)
TTC AAC ACA GCC CAG GGA TTT CGG ACG GTC TTG GAA TTA GTC ATA AAC TAC CAG CAA CTC
Phe Asn Thr Ala Gln Gly Phe Arg Thr Val Leu Glu Leu Val Ile Asn Tyr Gln Gln Leu
 805                                                                      (288)
TGC ATC TAC TGG ACA AAG TAT TAT GAC TTT AAA AAC CCC ATT ATT GAA AAG TAC CTG AGA
Cys Ile Tyr Trp Thr Lys Tyr Tyr Asp Phe Lys Asn Pro Ile Ile Glu Lys Tyr Leu Arg
 865                                                                      (308)
AGG CAG CTC ACG AAA CCC ACG CCT GTG ATC CTG GAC CCG GCG GAC CCT ACA GGA AAC TTG
Arg Gln Leu Thr Lys Pro Thr Pro Val Ile Leu Asp Pro Ala Asp Pro Thr Gly Asn Leu
 925                                                                      (328)
GGT GGT GGA GAC CCA AAG CGT TGG AGG CAG CTG GCA CAA GAG GCT GAG GCC TGG CTG AAT
Gly Gly Gly Asp Pro Lys Arg Trp Arg Gln Leu Ala Gln Glu Ala Glu Ala Trp Leu Asn
 985                                                                      (348)
TAC CCA TGC TTT AAG AAT TGG GAT GGG TCC CCA GTG AGC TCC TGG ATT CTG CTG GTG AGA
Tyr Pro Cys Phe Lys Asn Trp Asp Gly Ser Pro Val Ser Ser Trp Ile Leu Leu Val Arg
1045                                                                    (364)
CCT CCT GCT TCC TCC CTG CCA TTC ATC CCT GCC CCT CTC CAT GAA GCT TGA GAC ATA TAG
Pro Pro Ala Ser Ser Leu Pro Phe Ile Pro Ala Pro Leu His Glu Ala
1105
CTG GAG ACC ATT CTT TCC AAA GAA CTT ACC TCT TGC CAA AGG CCA TTT ATA TTC ATA TAG
1165
TGA CAG GCT GTG CTC CAT ATT TTA CAG TCA TTT TGG TCA CAA TCG AGG GTT TCT GGA ATT
1225
TTC ACA TCC CTT GTC CAG AAT TCA TTC CCC TAA GAG TAA TAA TAA ATA ATC TCT AAC ACC
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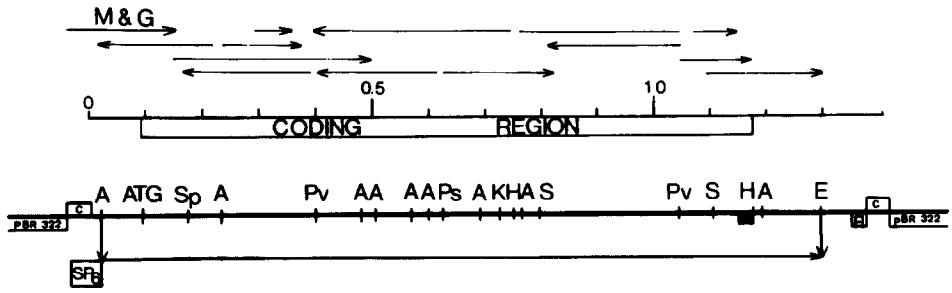

Fig.1. Restriction map of the 2–5A synthetase cDNA clone (p2–5A-1). The sites represented are those determined by restriction. Those deduced from the sequence are not shown. The C box corresponds to the tail added for cloning. Box A corresponds to the poly(A) segment. The black segment near the 3′-*Hin*dIII site shows the location of the synthetic oligodeoxyribonucleotides used for screening of the cDNA library. The fragment cloned in SP64 (SP6) starts from the *Alu*I site at the 5′-end and extends to the *Eco*RI site at the 3′-end as indicated by the arrows. The sequenced fragments and sequence direction are indicated by arrows. Only one fragment was sequenced by the method of Maxam and Gilbert: it is labeled M&G. A = *Alu*I, Sp = *Sph*I, Pv = *Pvu*II, Ps = *Pst*I, K = *Kpn*I, H = *Hin*dIII, S = *Sac*I, E = *Eco*RI.

3. RESULTS AND DISCUSSION

3.1. *Isolation of the p2–5A-1 cDNA clone*

A cDNA library was prepared from 16–25 S poly(A)$^+$ mRNA obtained from human amniotic U cells that had been treated for 6 h at 37°C with 200 U/ml human IFN-α. After screening the library with a 25-mer oligodeoxyribonucleotide probe derived from the published 522 bp sequence of a human 2–5A synthetase cDNA [14], we obtained 2 positive clones (p2–5A-1 and p2–5A-2) among the 18000 clones screened. Restriction analysis showed that all the restriction sites of the 3′-end of the cDNA clone described before [14] were present, that both clones were identical except that p2–5A-1 possesses a 300 bp longer insert and that the anticipated location of the oligodeoxyribonucleotide probe used for screening was confirmed by hybridizing Southern blots of p2–5A-1 with this oligonucleotide. The restriction map suggests that p2–5A-1 is entirely homologous to the cDNA described before [14] but extends about 800 bp towards the presumed 5′-end of the mRNA. The very large size of the cloned cDNA insert suggested that it could correspond to a full-size cDNA copy of the 2–5A synthetase mRNA. A Northern blot analysis using the 5′-*Pst*I-*Pst*I fragment demonstrated the strong induction by 200 U/ml of α-IFN of 1.6 and 1.8 kb mRNAs and also several larger RNAs in agreement with previous reports [10,14] (not shown).

3.2. *Nucleotide sequence of the 2–5A synthetase cDNA*

M13 subclones of overlapping fragments were isolated and sequenced by the dideoxy-chain termination method [21,22]. Nucleotides −94 to −26 were also determined using the chemical degradation method [23]. The segments sequenced are indicated in fig.1. The cDNA insert of p2–5A-1 contains 1402 bp derived from the 2–5A synthetase mRNA including a poly(A) tail of 24 nucleotides (fig.2). It consists of a segment of 94 nucleotides of the 5′-non-coding region, a coding segment of 1092 nucleotides, a TGA termination triplet and a 3′-non-coding segment of 192 nucleotides, followed by the poly(A) tail. The coding part of this molecule corresponds to a polypeptide of 364 amino acids having a calculated molecular mass of 41905 Da. It should be noted here that the 5′-non-

Fig.2. Nucleotide sequence of human 2–5A synthetase cDNA. The nucleotide sequence of the human 2–5A synthetase cDNA insert p2–5A-1 is presented as well as the amino acid sequence translated from the only long open reading frame. The numbers on the left refer to the nucleotide number starting with the adenosine of the initiating ATG. The numbers in brackets on the right refer to the amino acid number starting with the initiating methionine.

coding region of the cDNA presents an open reading frame. It is however thought that the protein really starts at the indicated ATG since the molecular mass of the protein encoded agrees with the published value [14].

3.3. *Cell-free translation of the p2–5A-1 cDNA insert*

To verify the molecular mass, enzymatic activity and cellular localization of the protein encoded by the cDNA described above, we have subcloned a portion of this cDNA extending from the 5′-*Alu*I to the *Eco*RI site (see fig.1) into an SP64 vector and obtained a construction named SP64-2–5A (see section 2). By transcribing this plasmid DNA with the SP6 RNA polymerase we obtained a pure capped non-polyadenylated synthetic 2–5A synthetase mRNA. This RNA was efficiently translated into a labeled single protein having an apparent molecular mass of 39 kDa in a SDS-polyacrylamide gel (fig.3) indicating that most likely the first or second AUG indicated in fig.2 is functional. The molecular mass determined experimentally in fig.3 is in good agreement with the calculated molecular mass (42 kDa) based on the sequence described (fig.2). Furthermore we asked whether the protein translated from this synthetic 2–5A synthetase mRNA was functional. This could not be tested easily by translation in rabbit reticulocyte lysates since such extracts contain abundant amounts of endogenous rabbit 2–5A synthetase [13]. For this reason the SP64-2–5A-derived mRNA was injected into *Xenopus* oocytes that were incubated for various periods of time before homogenization and measurement of their content in 2–5A synthetase. A high level of 2–5A synthetase was found in homogenates from oocytes that had been injected with 2–25 ng RNA per oocyte. In the oocyte cytoplasm the 2–5A synthetase activity increased between 24 and 48 h indicating that either the injected synthetic mRNA or the enzyme, or both are stable in the oocyte for this period of time. Interestingly, when the oocyte nuclei were dissected and assayed separately from the cytoplasmic extracts for 2–5A synthetase activity 10–30% of the total enzymatic activity was obtained in the nuclear fraction. Since the 'accessible' nuclear volume is about 12% of the total oocyte volume [27], this indicates that the 42 kDa 2–5A synthetase always migrates to some extent into the nuclei and that in some experiments it

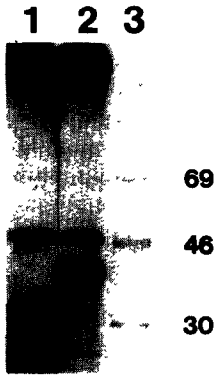

Fig.3. Cell-free translation of the mRNA corresponding to the 2–5A synthetase insert. Rabbit reticulocyte lysate was used for translation of the RNA transcribed in vitro on the SP64-2–5A DNA linearized with *Eco*RI. Lanes: 1, no RNA added; 2, RNA was added at final concentration of 100 μg/ml; 3, molecular mass markers.

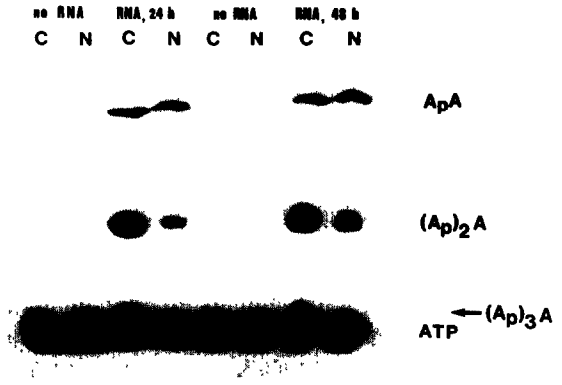

Fig.4. Chromatography on polyethyleneimine-cellulose thin layer sheets of 2–5A molecules synthesized from ATP in the presence of 2–5A synthetase and dsRNA. This enzyme was obtained after purification on polyI-C Sepharose of extracts from frog oocytes microinjected with synthetic 2–5A synthetase mRNA. This RNA was transcribed in vitro on the SP64-2–5A plasmid linearized with *Eco*RI. Production of 2–5A was measured either in the cytoplasmic (C) or the nuclear (N) extract of the oocytes (5 oocytes per lane). As indicated, the oocytes were incubated for 24 or 48 h after injection. ApA corresponds to dimers and $(Ap)_2A$ to trimers of 2–5A. Tetramers are not completely resolved from ATP.

reaches a concentration 2-fold higher than that found in the cytoplasm. On the other hand the 42 kDa 2–5A synthetase could possibly function as an oligomer containing either several subunits or would associate with other polypeptide(s) to constitute an enzymatically active structure, this pattern of association being different in the nuclear and cytoplasmic compartment. In the following experiment, we show that such a quaternary structure certainly does not occur in *Xenopus* oocytes and is not required for binding to double-stranded RNA or for expressing the 2–5A synthetase activity of the oocyte-translated 42 kDa 2–5A synthetase. When homogenates from oocytes that had been injected with 25 ng/oocyte of synthetic 2–5A synthetase mRNA and incubated for 24 h at 18°C were analyzed by sedimentation through glycerol gradients, all the 2–5A synthetase activity was localized in a region of the gradient corresponding to an approximate size of 50 kDa. If these results can be transposed to human cells [27], this would indicate that the 42 kDa 2–5A synthetase could correspond to part or the totality of the IFN-induced nuclear 2–5A synthetase and to the small 2–5A synthetase described in the cytoplasm of human cells [10]. So far it is still not clear which RNA, related or not to the existing clones, codes for the large human cytoplasmic enzyme [12,13]. Thus it remains possible that another gene exists which contains the information for this larger 2–5A synthetase.

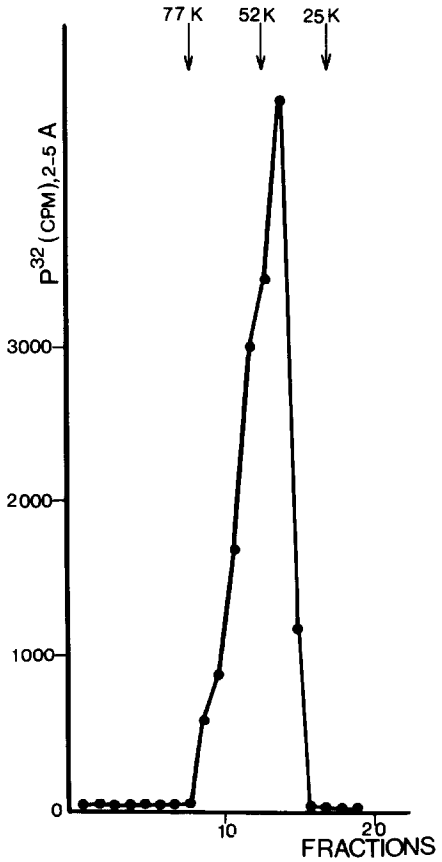

Fig.5. Size determination of the 2–5A synthetase enzymatic activity translated in frog oocytes after injection of the synthetic 2–5A synthetase mRNA. 100 μl of cytoplasmic extract from 40 frog oocytes was fractionated by centrifugation in a glycerol gradient. The 2–5A synthetase activity was assayed on each fraction by measurement on thin layer chromatograms of the [^{32}P]2–5A synthesized as described. Markers were partially reduced, ^{125}I-labeled goat-IgG (see section 2).

ADDENDUM

When this manuscript was ready for publication, the sequence of the same 2–5A synthetase cDNA was published by Benech et al. [28]. The 5′-untranslated region of this cDNA is 58 nucleotides shorter than our p2–5A-1 clone. One also notes in these sequences 2 base differences: at position 91, a guanine instead of an adenine. This means at the level of the protein that aspartic acid is in the place of asparagine. Similarly at position 343 a cytidine is present in the sequence of Benech et al. instead of thymidine, resulting in a triplet coding for leucine rather than phenylalanine. This may be the reflection of polymorphism which may exist in this human 2–5A synthetase gene. It may also be the result of cloning artefacts. It should be stressed here that the protein encoded by our cloned cDNA appears to be fully active (it produces 2–5A tetramers as shown in fig.4). It would be interesting to compare its specific activity with that of the enzyme described by Benech et al. in comparable expression conditions.

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