INTERFERON ACTION: ISOLATION OF NUCLEASE F, A TRANSLATION INHIBITOR ACTIVATED BY INTERFERON-INDUCED (2′–5′) OLIGO-ISOADENYLATE

A. SCHMIDT, A. ZILBERSTEIN, L. SHULMAN, P. FEDERMAN, H. BERISSI and M. REVEL
Department of Virology, Weizmann Institute of Science, Rehovot, Israel

Received 5 July 1978
Revised version received 22 August 1978

1. Introduction

Cell cultures treated by interferon become unable to support viral replication. At least one of interferon's antiviral effects is to inhibit viral protein synthesis [1–3]. Interferon induces several biochemical mechanisms which could mediate this inhibition:

(i) A double-stranded RNA (dsRNA) and ATP-dependent phosphorylation of initiation factor eIF2 and possibly other ribosome-associated proteins [4–7];
(ii) The dsRNA ATP-dependent synthesis of an unusual oligo-adenylate isomer with 2′–5′ phosphodiester bonds [7–9];
(iii) A mechanism which affects polypeptide chain elongation, that does not require dsRNA and is reversed by tRNAs [10–12].

We have reported [3,9] the separation and isolation, from extracts of interferon-treated L cells, of the dsRNA ATP-activated protein kinase PKi, and of the (2′–5′) oligo-isoadenylate synthetase E. Here, we demonstrate that the oligonucleotide produced by E requires, to inhibit mRNA translation, an additional protein F already present constitutively in untreated cells. The purified F is shown to be an oligo-isoadenylate-dependent ribonuclease which degrades the mRNA template. Our data show that the dsRNA ATP-dependent nuclease activity reported [13,14] to be increased in extracts of interferon-treated cells, can be explained as the activation of a constitutive ribonuclease, F, by the product of the interferon-induced and dsRNA-dependent oligo-isoadenylate synthetase E. This is in line with the recent report that the dsRNA ATP-dependent nuclease is composed of two macromolecular components [15]. The isolation of nuclease F also explains why addition of the interferon-induced oligonucleotide caused mRNA degradation in cell lysates [16,17]. In addition, the present work demonstrates the existence of an enzymatic activity which splits 2′–5′ nucleotide bonds and degrades the activator of nuclease F.

2. Materials and methods

2.1. *Preparation of oligo-isoadenylate (iso-A)*

The iso-A synthetase E was isolated as in [9], from cell sap (150 000 × *g* supernatant) of L cell cultures treated 24 h with 200 U/ml mouse interferon. In short, E was not retained on DEAE-cellulose (pH 7.5), 25 mM KCl, and eluted from phosphocellulose (pH 6.7) at 450 mM KCl. E was incubated with poly(rI:rC) 0.5 μg/ml, and 1 mM [α-^{32}P]ATP (150 mCi/mmol) as detailed in [9]. The nucleotides were purified either by paper high-voltage electrophoresis, at pH 3.5 [9] and water elution, or by DEAE-cellulose chromatography in urea [18], desalting on DEAE-cellulose [18], and lyophilization before dissolving in water. The concentrations of iso-A were calculated from the amount of ^{32}P incorporated/phosphate residue. Synthetic 2′–5′ and 3′–5′ oligo-adenylate were generous gifts from Dr Y. Lapidot (Jerusalem).

2.2. *Isolation and assay of F*

Cell sap from L cells, untreated or interferon-treated (360 mg protein; 75 ml) was loaded on a

DEAE-cellulose column (Whatman DE52; 6 × 28 cm), equilibrated with 25 mM KCl in Hepes buffer 20 mM (pH 7.5), Mg acetate 5 mM, dithiothreitol (DTT) 1 mM, glycerol 10%. Non-adsorbed proteins were removed and elution performed with a 1 L linear gradient of 25–500 mM KCl in the same buffer. Fractions of 6 ml were collected at 1.2 ml/min, dialyzed against the above buffer with 120 mM KCl and stored in liquid air. F was assayed by its inhibition of mRNA translation in the presence of oligo-isoadenylate, but not in the absence of the oligonucleotide, using L cell S10 without dsRNA (fig.2). F eluted from DEAE-cellulose between 110–130 mM KCl (F-DE). F-DE was dialyzed against Hepes buffer (pH 6.7), 20 mM, KCl 25 mM, DTT 1 mM, glycerol 5% and applied (30 mg protein, 85 ml) to a phosphocellulose column (Whatman P11; 1.5 × 10 cm) equilibrated in the same conditions. Fractions (2 ml; 0.3 ml/min) were eluted with a 90 ml linear gradient of 25–500 mM KCl in the same buffer. F activity eluted at ~400 mM KCl (F-PC1); it was dialyzed against Hepes buffer (pH 7.9), 20 mM, KCl 25 mM, DTT 1 mM and glycerol 5%, before loading (7 mg protein, 35 ml) on phosphocellulose (1 × 9 cm) in the same buffer. A 12 ml 25–500 mM KCl gradient was used for elution and F was recovered at about 200 mM KCl (F-PC2). This fraction (1 mg protein; 4 ml) was finally dialyzed against potassium phosphate buffer 25 mM (pH 7.2), KCl 120 mM, DTT 1 mM and adsorbed on hydroxylapatite (0.5 × 2 cm). By stepwise elution, F was recovered in the 100 mM potassium phosphate fraction (F-HAP). Protein F-HAP (0.1 μg) was needed for 10 nM oligo-isoadenylate to inhibit Mengo RNA translation by 50% in an S10 cell-free system (0.025 ml). This procedure was used to purify F from interferon-treated and from untreated L cells and gave an overall spec. act. 450 increase over the crude cell sap.

2.3. *Translation assays*

S10 extracts of L cells were used to measure [^{35}S]methionine (600 Ci/mmol) incorporation directed by Mengo virus RNA or globin mRNA as in [9,10]. Globin mRNA was prepared by passage through oligo(DT)-cellulose of rabbit reticulocyte polysomal RNA, and translated with crude reticulocyte initiation factors [19]. Iodination of mRNA was carried out as in [20].

3. Results

3.1. *Oligo-isoadenylate formation by iso-A synthetase E*

Enzyme E was isolated from interferon-treated L cells, as a dsRNA and ATP-dependent activity producing a thermostable translational inhibitor [3,9]. In the presence of dsRNA, this enzyme polymerizes ATP into a mixture of oligonucleotides with the general structure ppp(A2′p)$_n$5′A [8,9], called oligo-isoadenylate (iso-A). The oligonucleotide produced can be separated on DEAE-cellulose (pH 7.5) in urea (fig.1A) into dimers pppA2′p5′A(×2) eluting at charge −4, trimers, (×3) and tetramers (×4). Because of their triphosphate end, the oligomers all migrate to the same position on paper electrophoresis, at pH 3.5, slightly ahead of ADP (fig.1B). When synthesized with [α-^{32}P]ATP, the nucleotides yield after treatment with bacterial alkaline phosphatase (BAP), radioactive cores that can be identified by paper electrophoresis, at pH 3.5 (fig.1B). The ×2, ×3, ×4 cores comigrated with synthetic 2′–5′ (Ap)$_n$A markers with n=1–3, well separated from their 3′–5′ isomers in this electrophoretic system. Enzyme E is efficient and converts about 20% of the ATP input into iso-A nucleotides. Even with nearly saturating concentrations of E however, the polymerization reaction remains slow and reaches a maximum only after 12 h at 30°C. Table 1 shows the molar yield of the different oligonucleotides measured as a function of incubation time: first dimers are formed, trimers accumulating later, while longer oligonucleotides are made in smaller amounts.

3.2. *Effect of oligo-isoadenylate on translation: requirement for factor F*

The inhibitory effects of oligo-isoadenylate on mRNA translation can be shown to require a protein factor F, different from enzyme E which synthesizes these oligonucleotides. Figure 2 shows that purified iso-A trimers inhibit Mengo RNA translation only to a small extent, unless factor F is added. In the presence of F, nM concentrations of iso-A trimer become efficient inhibitors. Dimers have no activity by themselves, but can give some inhibition with F. Tetramers were as active as trimers; no inhibition was seen when F was supplemented with ATP or ADP (not shown).

Factor F was first detected during fractionation

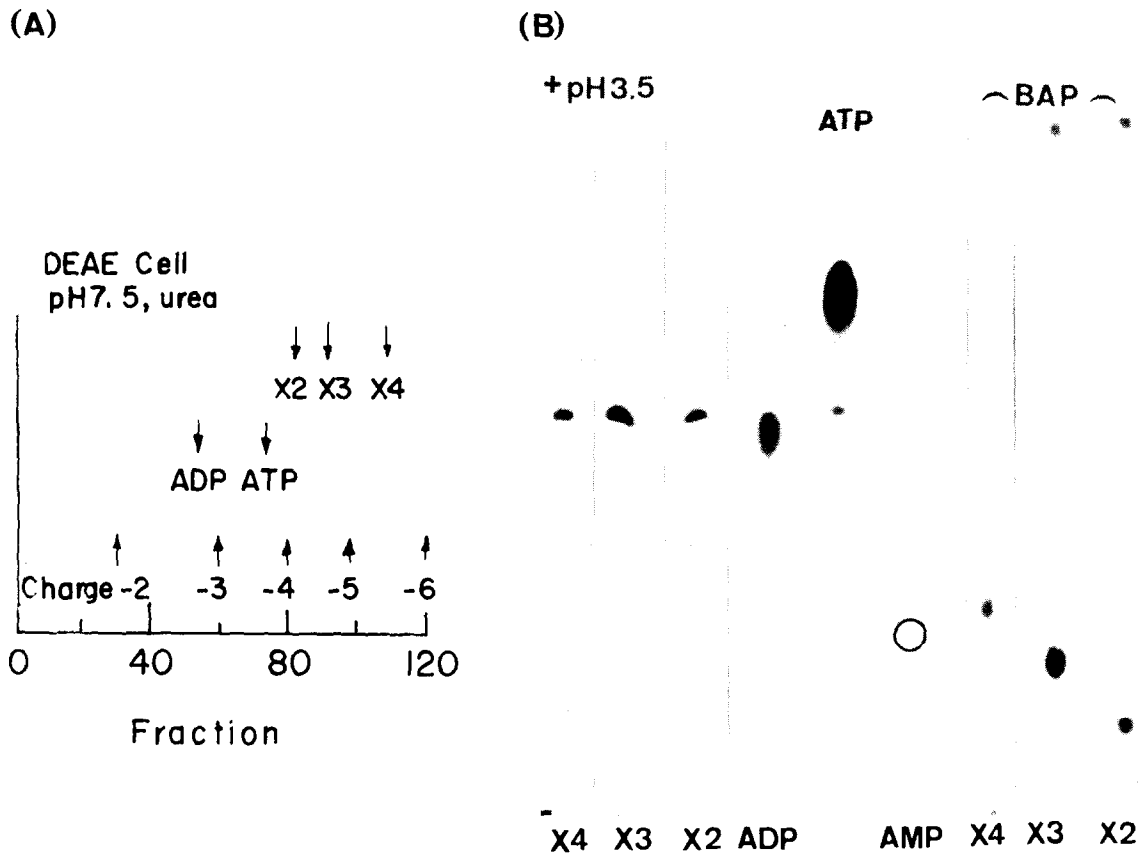

Fig.1. Separation of iso-adenylate dimers (X2), trimers (X3) and tetramers (X4). Iso-A synthetase E, 3.7 μg (see section 2) was incubated in 0.03 ml with [α-^{32}P]ATP and poly(rI:rC) for 8.5 h at 30°C. The reaction mixture was loaded on a DEAE-cellulose column (0.6 × 40 cm) in Tris 50 mM (pH 7.6), NaCl 50 mM, urea 7 M and eluted in 1 ml fractions (0.1 ml/min) with a 150 ml 50–200 mM NaCl gradient (A). The position of ^{32}P peaks is indicated by arrows in relation to nucleotide markers $(Xp)_n$ of known charge (lower arrows). Fractions eluting at positions X2, X3, X4 in (A) were electrophoresed on paper at pH 3.5 (3 kV, 4 h) together with ADP, ATP or a 5′-AMP unlabelled marker (○). The electrophoresis is shown in (B). Aliquots of X2, X3, X4 were treated by bacterial alkaline phosphatase (BAP) [9] and electrophoresed in the indicated slots in (B). After drying, the paper was exposed to RP54 X-ray film with an intensifying screen.

Table 1
Oligo-isoadenylate synthesis

Incubation (h)	ATP incorp./μg protein (nmol)	Molar distribution of oligomers (%)			
		Dimer	Trimer	Tetramer	Larger
0.5	0.3	94	4	0.6	0.5
1	0.4	85	12	0.5	1.5
8	1.0	73	22	3	1.5
16	1.5	56	34	6	3

Synthesis carried out as in section 2. Separation of the BAP-cores by electrophoresis, at pH 3.5, as in fig.1

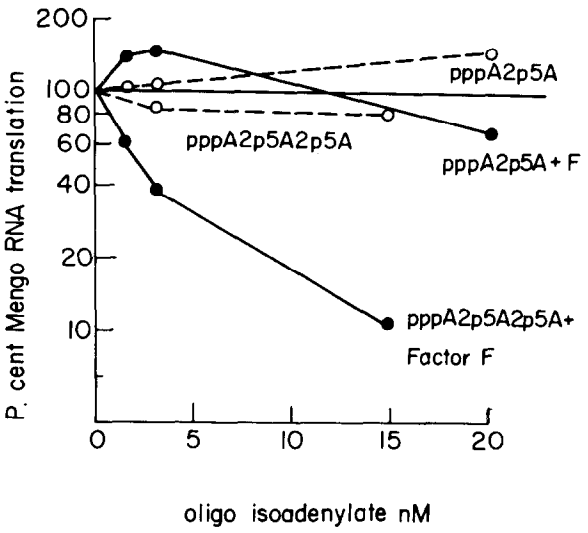

Fig.2. F requirement for protein synthesis inhibition by oligo-isoadenylate. Mengo RNA translation in L cell S10 crude extract was carried out without dsRNA, as in section 2. [^{35}S]Methionine incorporation was 4.2 × 10^5 cpm, and 5.1 × 10^5 cpm with 2.5 μg F (F-DE from interferon-treated cells). These values were taken as 100%. A background without mRNA of 3 × 10^4 cpm was subtracted. Preparation of iso-A dimers and trimers as in fig.1.

of cell sap on DEAE-cellulose, as an activity which inhibits translation when added to extracts of interferon-treated cells in the presence of dsRNA, but not without dsRNA [21]. In extracts from untreated L cells, factor F did not inhibit unless the oligo-isoadenylate synthetase E purified from interferon-treated cells was added. The dsRNA-dependent protein kinase obtained from these cells [9] had no effect. Factor F is found in untreated control L cells, although the activity of a similar preparation from interferon-treated cells was higher by 1.5–2-fold: in an experiment as in fig.2, 6 nM iso-A trimers gave 55% inhibition with F-control against 72% for F-interferon. The amount of F present in extracts from both our interferon-treated or untreated cultures is much below saturation, which allows to use either type of extracts to measure the iso-A-dependent F activity. Partial purification of F is described in section 2. Crude F (F-DE) stimulates Mengo RNA translation when added without iso-A; most of the stimulator can be separated during purification. Heating F, 10 min to 50°C, destroys its iso-A-dependent inhibitory activity, while the stimulation remains.

The inhibitory effect of F + iso-A was much less pronounced with globin mRNA as messenger (table 2)

Table 2
Loss of mRNA activity upon preincubation with F and iso-A

Exp.	mRNA	Preincubation	Incubation	[^{35}S]Methionine incorp. during incubation (cpm)
1.	Globin mRNA	–	mRNA + S10	140 530
		–	mRNA + S10 + F + iso-A	122 630
		mRNA + F + iso-A	S10	29 325
		mRNA + F	S10	112 940
		mRNA + iso-A	S10	117 980
2.	Globin mRNA	mRNA	S10 + F	138 980
		mRNA + F + iso-A	S10 + F	45 350
		(F + iso-A)* then mRNA	S10 + F	114 100
3.	Mengo RNA	–	mRNA + S10 + F + iso-A	41 000
		mRNA + F + iso-A	S10	4525
		mRNA + F	S10 + iso-A	39 510
		S10 + F + iso-A	mRNA	58 720

Translation assay as in section 2, in 0.025 ml for 60 min, 30°C. Preincubation was carried out for 30 min at 30°C in 0.01 ml under same salt conditions, with 0.5 μg globin mRNA or 0.3 μg Mengo RNA, 1 μg F (PC1) and 0.16 pmol iso-A trimers. In exp. 2, (F + iso-A)* indicates that these were first incubated 30 min together, before being added to mRNA preincubation

than with Mengo RNA (fig.2). However preincubation of globin mRNA with F + iso-A, prior to addition to the translation system, strongly potentiated the inhibition observed (table 2). Omission of either factor F or of the oligonucleotide prevented this effect. A stronger inhibition was also seen when Mengo RNA was preincubated with F + iso-A before translation; this treatment abolished the reported [22] lag period preceding inhibition by oligo-isoadenylate. In contrast, preincubation of F + iso-A with the cell extract S10 prior to addition of mRNA, produced less inhibition than when all components were added simultaneously. The iso-A-triggered translation inhibitor F appears, therefore, to act better on the free mRNA template, than when mRNA is associated with the translation system.

3.3. *Oligo-isoadenylate-dependent nuclease activity of factor F*

The fate of mRNA during incubation with F and iso-A was investigated. Table 3 shows that after incubation of ^{125}I-labelled Mengo RNA with F from either control or interferon-treated cells, together with nM concentrations of iso-A trimers, a significant amount of the mRNA is made acid-soluble. The nucleolytic activity of F was stimulated 3–4-fold by the presence of iso-A. This contrasts with the behaviour of known ribonucleases which would be inhibited by 2′–5′ nucleotides [23].

The process of mRNA degradation by F + iso-A was further analyzed by electrophoresis on polyacrylamide gels in urea. Figure 3A shows that preincubation of ^{125}I-labelled globin mRNA with pppApApA (2′–5′) and our purified protein F preparation, produces a complete loss of intact 9 S molecules. The iodinated mRNA was not completely stable during incubation, but incubation with iso-A alone, or even with F alone, produced no such drastic degradation. The globin mRNA fragments produced by the iso-A-dependent F nuclease showed some half-size molecules but mainly material migrating with the front of electrophoresis. To avoid problems due to iodination, we have more recently used an assay based on agarose gel electrophoresis of unlabelled globin mRNA followed by staining with ethidium bromide [24]. This rapid assay shows clearly (fig.3B) the F + iso-A trimer-dependent degradation of the 9 S RNA, in parallel with the loss of template activity in the translation system (table 2). Iso-A dimer had very little activity (fig.3C); ATP or ApApA did not stimulate nuclease F. The small nucleolytic activity of F alone, which is seen even with F-HAP, could be either a contaminating activity or the basal level of the nuclease which is activated by iso-A. The mechanism of activation is yet unclear. When ^{32}P-labelled oligo-isoadenylate was incubated with F, bound radioactivity eluted with the proteins in the void volume of a Biogel P60 column (unpublished data). F was, however, not permanently activated after preincubation with iso-A. On the contrary, preincubation of partially-purified F with the oligonucleotide prior to addition to mRNA, leads to a loss of the translation inhibitory activity (table 2, line 8). As shown below, F up to the phosphocellulose step contains an

Table 3
Degradation of Mengo RNA by oligo-isoadenylate-dependent nuclease F

Exp.	Factor F	Oligo-isoadenylate trimer	^{125}I-Labelled Mengo RNA (cpm) made trichloroacetic acid-soluble
1.	None	+	150
	F-INT, 3.5 μg	–	940
	F-INT, 3.5 μg	+	2840
2.	F-CONT, 5 μg	–	1470
	F-CONT, 5 μg	+	5810

^{125}I-Labelled Mengo RNA (0.3 μg; 18 000 cpm) was incubated with F (F-DE from interferon-treated or control cells) and 10 nM iso-A trimers as in fig.3. Reaction was stopped with 0.5 ml ice-cold trichloroacetic acid 10%, 0.5 mg bovine serum albumin was added and after 15 min in the cold, supernatant was counted for acid soluble radioactivity

activity which degrades the oligo-isoadenylate itself. Most of this activity was separated from F in the hydroxylapatite step, without loss of the mRNA-degrading function and the iso-A-dependent translation inhibition.

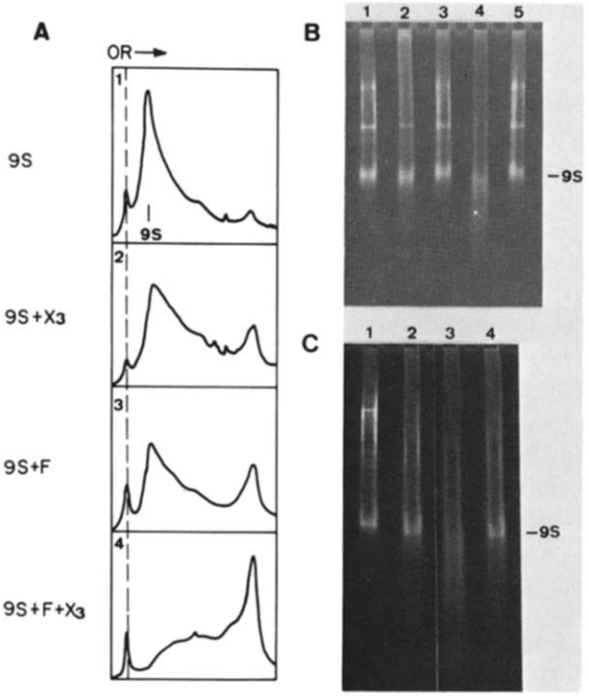

Fig.3. Oligo-isoadenylate-dependent globin mRNA degradation by nuclease F. (A) ^{125}I-Labelled globin mRNA (0.5 μg; 10^5 cpm) was incubated for 30 min at 30°C in 0.01 ml with 0.3 μg F (F-PC2, section 2) and 0.16 pmol iso-A trimers in Hepes buffer 13 mM (pH 7.5), KCl 80 mM, Mg acetate 3 mM, DTT 0.6 mM and glycerol 6%, and applied to a polyacrylamide slab-gel in Tris–borate 0.1 M (pH 8.3), EDTA 2.5 mM, urea 7 M. Electrophoresis was at 10 V/cm, 18°C; an overnight autoradiography of the gel on Kodak RP54 was scanned at 600 nm in a Gilford photometer. (1) Non-incubated globin mRNA; (2) globin mRNA incubated with iso-A (X3) alone; (3) with F alone; (4) with F + iso-A. (B) Unlabelled globin mRNA (1 μg) incubated as in A with 2.5 μg F (F-DE interferon) and 0.4 pmol iso-A trimers, 20 min at 30°C, then made 0.5% in SDS with 20% sucrose and applied to a pre-run 2% agarose slab gel in Tris–HCl 40 mM (pH 7.3), Na acetate 10 mM, EDTA 1 mM, glycerol 10%. After electrophoresis at 10 V/cm for 1.5 h, 18°C, gels were stained in 0.5 μg/ml ethidium bromide 30 min and photographed under ultraviolet light [24]. Slot (1): mRNA alone; slot (2): with F alone; slot (3): with iso-A alone; slot (4): with F + iso-A; slot (5): mRNA alone. (C) Similar experiment as B, but mRNA incubated with F + iso-A dimers (slot 4) as well as with F + iso-A trimers (slot 3), F alone (slot 2), or without addition (slot 1).

3.4. *Oligo-isoadenylate degradation activity*

Iso-A dimers and trimers (α-^{32}P-labelled) were incubated with F-PC under conditions which lead to loss of translational inhibition (table 2, line 8). Analysis by paper electrophoresis at pH 3.5 (fig.4) shows extensive degradation of the native nucleo-

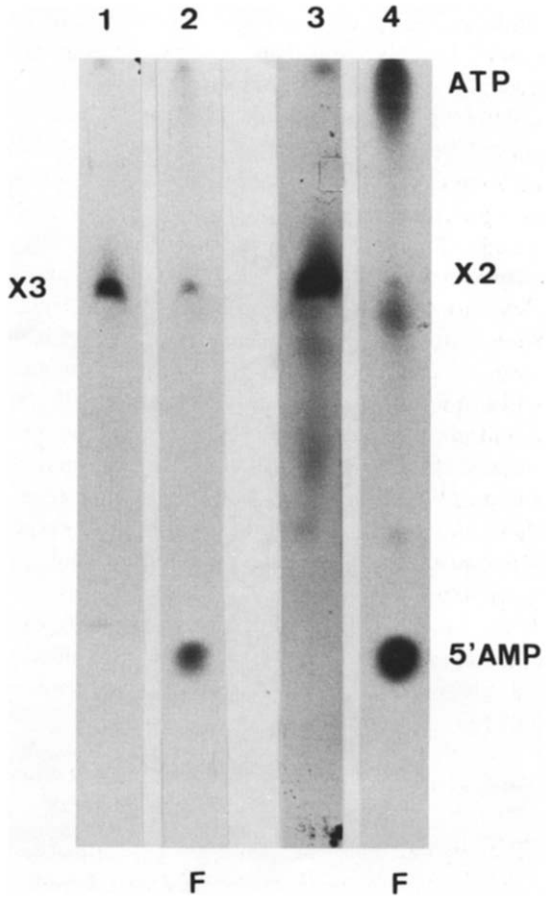

Fig.4. Degradation of oligo-isoadenylate by F. Oligo-isoadenylate trimers (X3, 30 pmol, 1350 cpm) or dimers (X2, 190 pmol, 5740 cpm) prepared as in section 2 from [α-^{32}P]-ATP were incubated in 0.01 ml with 0.6 μg F (F-PC2) in Hepes buffer 16 mM (pH 7.5), Mg acetate 4 mM, KCl 100 mM, DTT 0.8 mM and glycerol 8% for 60 min, 30°C. The reaction was analyzed by paper electrophoresis, at pH 3.5, with 5'-AMP, (2', 3')AMP, ADP and ATP markers. After autoradiography spots were counted (see text). Slot (1): iso-A trimers alone; slot (2): same with F; slot (3): iso-A dimers alone; slot (4): same with F.

tide to products which were identified as 5′-AMP, ATP and very small amounts of ADP. From the dimers (×2), the AMP : ATP ratio formed was 1.2 and from the trimer (×3), 2.1, as expected. The cleavage therefore takes place at the 2′-phosphate bond of the molecule: pppA2′/p5′A2′/p5′A. Longer oligomers could also be degraded, but less than the shorter ones. F-PC could also readily degrade the alkaline phosphatase resistant cores of iso-A dimers and trimers, as well as synthetic 2′–5′ApA, to 5′AMP and adenosine. At 37°C, 2′–5′ ApA was degraded at 0.2 nmol/min; the 3′–5′ApA was degraded more slowly (not shown). The 2′–5′ phosphodiester bond is known to be resistant to most nucleases, but sensitive to snake venom phosphodiesterase [8]. The 2′-phosphodiesterase activity accompanying factor F had no effect on DNA. Heating to 55–60°C destroyed the activity.

The co-purification of a 2′-phosphodiesterase activity degrading iso-A and the iso-A-stimulated ribonuclease, over several steps, is intriguing. The association may be functionally significant, providing both endonuclease and exonuclease activities. More likely, the association plays a role in the regulation of nuclease F. The data strongly suggest that the translation inhibitory function of F is regulated not only by the synthesis of oligo-isoadenylate but also by its degradation. The F nuclease activity would be only transiently enhanced by iso-A, its effect on mRNA translation requiring the continued presence of the oligonucleotide and being shut-off when the 2′–5′ bond of the activator is split.

4. Discussion

The oligo-isoadenylate, synthesized by the interferon-induced enzyme E, appears to exert at least one of its functions by triggering a specific nuclease F. This nuclease is found constitutively in cells not treated by interferon; although some increase may occur after interferon, this change is small compared to the large increase in oligo-isoadenylate synthesis [9]. The main variable appears therefore to be the concentration of the oligonucleotide effector, and the degradation of iso-A provides in addition an autoregulatory feed-back on the level of F translational inhibitory activity. F is clearly a key component of the dsRNA-dependent translational inhibition: its concentration is limiting in L cell extracts, and we have observed that some interferon-treated extracts are not inhibited by dsRNA unless more F is added. There is no evidence, however, that F is also involved in the dsRNA-dependent translation inhibition mediated by the interferon-induced eIF-2 protein kinase [9].

The iso-A activated F-nuclease seems to degrade the mRNA rather than other components of the translational machinery. This hypothesis is in line with the effects of pppApApA(2′–5′) on polysomes in reticulocyte lysates [16]. Nuclease F does not appear very specific, since it degrades a viral and a host mRNA and to some extent even free ribosomal RNA. The role of the oligonucleotide activator is not clear: it binds to the protein but could also interact with the RNA. The in vivo function of nuclease F and of the oligo-isoadenylate synthesizing and degrading enzymes in the antiviral and cell regulatory effects of interferon will now be very interesting to elucidate.

Acknowledgements

We thank Drs Y. Groner and A. Kimchi for helpful discussions, and Ms M. Tarjan for her help. Work supported by NCRD (Israel) and GSF (München, FRG). These data were communicated at the Warwick Meeting, April 12, 1978.

References

[1] Metz, D. H. (1975) Cell 6, 429–439.
[2] Yakobson, E., Prives, C., Hartman, J. R., Winocour, E. and Revel, M. (1977) Cell 12, 73–81.
[3] Revel, M., Gilboa, E., Kimchi, A., Schmidt, A., Shulman, L., Yakobson, E. and Zilberstein, A. (1978) Proc. 11th FEBS Meet. Copenhagen 1977 (Clark, B. F. C. et al. eds) vol. 43, pp. 47–58, Pergamon Press, Oxford.
[4] Zilberstein, A., Federman, P., Shulman, L. and Revel, M. (1976) FEBS Lett. 68, 119–124.
[5] Lebleu, B., Sen, G. C., Shaila, S., Cabrer, B. and Lengyel, P. (1976) Proc. Natl. Acad. Sci. USA 73, 3107–3111.
[6] Cooper, J. and Farrell, P. J. (1977) Biochem. Biophys. Res. Commun. 77, 124–131.
[7] Roberts, W. K., Hovanessian, A., Brown, R. E., Clemens, M. J. and Kerr, I. M. (1976) Nature 264, 477–480.

[8] Kerr, I. M. and Brown, R. E. (1978) Proc. Natl. Acad. Sci. USA 75, 256–260.
[9] Zilberstein, A., Kimchi, A., Schmidt, A. and Revel, M. (1978) Proc. Natl. Acad. Sci. USA, in press.
[10] Content, J., Lebleu, B., Nudel, U., Zilberstein, A., Berissi, H. and Revel, M. (1975) Eur. J. Biochem. 54, 1–10.
[11] Sen, G. C., Gupta, S. L., Brown, G. E., Lebleu, B., Rebello, M. A. and Lengyel, P. (1976) J. Virol. 17, 191–203.
[12] Falcoff, R., Falcoff, E., Sanceau, J. and Lewis, J. A. (1978) Virology 86, 507–515.
[13] Sen, G. C., Lebleu, B., Brown, G. E., Kawakita, M., Slattery, E. and Lengyel, P. (1976) Nature 264, 370–373.
[14] Samuel, C. E., Farris, D. A. and Eppstein, D. A. (1977) Virology 83, 56–71.
[15] Ratner, L., Wiegand, R. C., Farrell, P. J., Sen, G. C., Cabrer, B. and Lengyel, P. (1978) Biochem. Biophys. Res. Commun. 81, 947–954.
[16] Clemens, M. J. and Williams, B. R. G. (1978) Cell 13, 565–572.
[17] Baglioni, C., Minks, M. A. and Maroney, P. A. (1978) Nature 273, 684–687.
[18] Tener, G. M. (1967) Methods Enzymol. 12, 398–404.
[19] Soreq, H., Nudel, U., Salomon, R., Revel, M. and Littauer, U. Z. (1974) J. Mol. Biol. 88, 233–245.
[20] Prensky, W., Steffensen, D. M. and Hughes, W. L. (1973) Proc. Natl. Acad. Sci. USA 70, 1860–1864.
[21] Revel, M., Kimchi, A., Schmidt, A., Shulman, L. and Zilberstein, A. (1978) Proc. 12th FEBS Meet. Dresden 1978, Pergamon Press, Oxford, in press.
[22] Hovanessian, A. G., Brown, R. E. and Kerr, I. M. (1977) Nature 268, 537–540.
[23] White, M. D., Bauer, S. and Lapidot, Y. (1977) Nuc. Acid. Res. 4, 3029–3038.
[24] Hewlett, M. J., Rozenblatt, S., Ambros, V. and Baltimore, D. (1977) Biochemistry 16, 2763–2767.