INTERACTION OF INTERFERON WITH tRNA

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1. Introduction

The binding affinity of interferon for the chromophore Cibacron blue F3GA [1,2] can be attributed, as reported recently [3], to an interaction of the dye with interferon through a polynucleotide attachment site present on the interferon molecule. Indeed, when retained on a blue dextran–Sepharose column, mouse interferon can be desorbed by various polynucleotides, such as poly(I) and poly(U), but is not displaced by nucleoside phosphate monomers [3], in contrast to other proteins having a dinucleotide fold [4]. This observation suggested that interferon might also have an affinity for other types of RNA. The interaction of interferon with tRNA of various origins is reported in this paper.

2. Materials and methods

2.1. *Origin of interferon*

The interferon employed throughout this work had been prepared in C243 cells induced with Newcastle disease virus, according to the method of Tovey et al. [5]. The protein content of the crude material was 100 μg/ml; interferon titer was 1.3×10^6 units/ml and the specific activity was 1.3×10^7 units/mg protein.

2.2. *Interferon titrations*

Interferon titrations were performed using a microtiter assay (0.2 ml medium/well) in mouse L cells with Vesicular stomatitis virus (Indiana strain) as challenge virus. One microtiter unit/0.2 ml corresponds to 10 reference units/ml. All results are expressed in terms of reference units.

2.3. *Blue dextran–Sepharose columns*

Blue dextran–Sepharose (BDS) columns were prepared by coupling blue dextran 2000 to CNBr activated Sepharose 6B (Pharmacia, Uppsala, Sweden) as described previously [1]. All chromatographies were carried out at room temperature; when not in use, the columns were stored at + 4°C and protected against infection by addition of 0.02% sodium azide to the eluant.

2.4. *Origin of RNAs*

Escherichia coli total tRNA was obtained from the Laboratoire d'Extraction et de Fermentation du CNRS (Gif-sur-Yvette, France). It had been purified from phenol extracted RNA by chromatography on a DEAE-cellulose column followed by isopropanol purification; the preparation was devoid of rRNA or of DNA.

Wheat germ tRNA was obtained from the Laboratoires Choay (Paris, France); it had been prepared from the 100 000 × *g* supernatant of a cell-free extract chromatographed on DEAE-cellulose.

Yeast tRNA was purchased from Boehringer (Mannheim, FRG); yeast rRNA, prepared from washed ribosomes of brewer's yeast, was a generous gift from

Dr H. Fukuhara, Institut du Radium (Orsay, France).

E. coli 16 S and 23 S rRNA and wheat germ 18 S and 28 S rRNA obtained from the Laboratoires Choay (Paris, France) had been prepared from washed ribosomes after removal of proteins and DNA by treatment with 8 M urea and 4 M LiCl.

C243 cell tRNA and rRNA were purified by phenol–chloroform extraction. After treatment of the total nucleic acid fraction with DNAase (RNAase free grade, Worthington, USA), the tRNA was separated from rRNA by filtration on a Sephadex G-100 column; the purity of the respective RNA species obtained was verified by electrophoresis on a 0.5–2.5% agarose–polyacrylamide gel.

Yeast $tRNA_3^{Leu}$ was purified according to Chang et al. [6]. It was in renatured form and had an acceptant activity of 1250 pmol leucine/A_{260} unit. Brewers yeast specific tRNAs, $tRNA^{Phe}$ [7], $tRNA^{Asp}$, $tRNA^{Trp}$ [8], $tRNA_{II}^{Val}$ and $tRNA^{Tyr}$ [9] were a generous gift from Dr G. Dirheimer and his coworkers (Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France). The degree of purity of these tRNAs was higher than 90% as verified by their charging capacity and/or the sequence analysis directly after their purification.

2.5. *Preparation of tRNA–agarose columns*

The columns of tRNA–agarose were prepared by binding yeast and *E. coli* total tRNA through the oxidized 3′-terminal end to hydrazide agarose, according to the method of Robberson and Davidson [11].

3. Results

3.1. *Desorption by tRNA preparations of various origin of interferon retained on blue dextran–Sepharose columns*

tRNA preparations of various origin were examined for their capacity to desorb mouse interferon from BDS columns as described in the legend of table 1. Results show that whereas total tRNAs from wheat germ, calf liver or rat liver were unable to significantly desorb interferon, 23% of BDS-bound interferon was desorbed with *E. coli* tRNA, and virtually 100% with yeast tRNA. Furthermore, C243 cell tRNA was relatively efficient in desorbing interferon from BDS. For comparison, rRNAs of the same origin as the active tRNAs were tested for their ability to desorb interferon from BDS. Results illustrated in fig.1A and fig.1B show that rRNAs had little or no effect, which indicates that the desorption due to the corresponding tRNAs cannot be attributed to a mere effect of charge of the polynucleotide chain.

3.2. *Binding of interferon to tRNAs covalently attached to agarose beads*

The observation that certain tRNAs were able to desorb interferon retained on BDS columns strongly

Table 1

Desorption by transfer and ribosomal RNAs of mouse interferon retained on blue dextran–Sepharose columns

Origin of tRNA:	Wheat germ	Calf liver	Rat liver		*E. coli*	C243 cells	Yeast	
			Expt. 1	Expt. 2			Expt. 1	Expt. 2
Percent of interferon desorbed by tRNA	4	4	6	8	23	54	90	91
Percent of interferon desorbed by rRNA	5	ND	ND		10	11	5	

For each experiment 1×10^7 units of crude interferon were adsorbed to a BDS column. The interferon preparation had been dialyzed overnight against Tris–HCl, 10 mM buffer, pH 7.5. After adsorption, the column was rinsed with the same buffer until the optical density at 280 nm was 0; it was then washed with at least two more bed volumes. For desorption, 3 ml or 4 ml of a tRNA solution at a concentration of 100 μg/ml in 10 mM Tris–HCl were applied to the column. Subsequently, a second desorption step was carried out by adding 1 M NaCl to the Tris–HCl buffer in order to elute remaining interferon, if any. Total recovery was then calculated and compared to input, in order to ascertain that all activity had been recovered

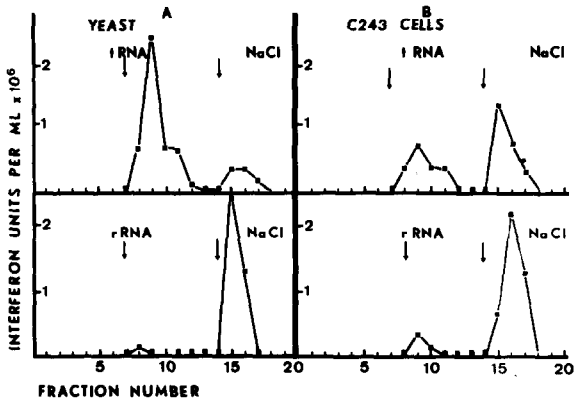

Fig.1. This figure summarizes four experiments. For each of them, 7.7 × 10^6 units of interferon were fixed to a BDS column (fractions 1–6). Desorption was then attempted either with yeast tRNA, or rRNA (fig.1A) or with C243 cell tRNA or rRNA (fig.1B). In each case the RNA was suspended in 1 ml (one bed volume) of Tris–HCl, pH 7.5, at a concentration of 2 *A* units/ml. After this first desorption step (fractions 7–11) the column was washed with 6 bed volumes of 10 mM Tris–HCl buffer, and a second desorption was carried out by adding 1 M NaCl to the buffer in order to recover the remaining interferon. 1 ml fractions were collected throughout, and interferon content was determined for each fraction.

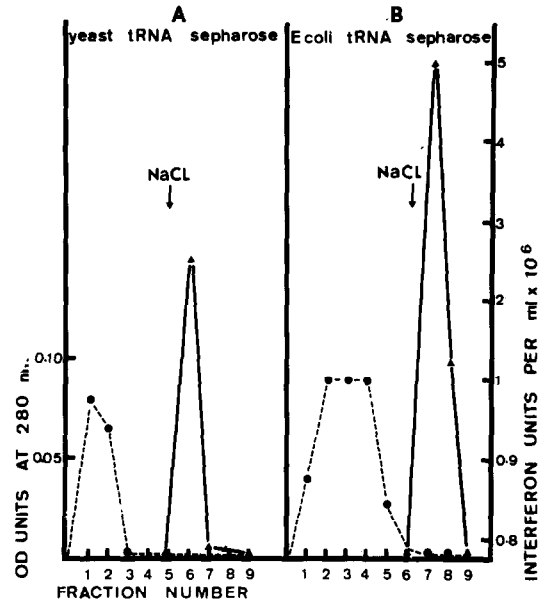

Fig.2A. A total of 29 *A* units of total yeast tRNA were covalently linked to 1.6 ml agarose beads as described in Materials and methods. A total of 5.1 × 10^6 units of interferon were applied to the column (fractions 1 and 2) which was then washed with 6 bed volume of Tris–HCl 10 mM, pH 7.5 (fractions 3–5). Desorption was carried out by 3 bed volumes of NaCl 1 M in Tris–HCl 10 mM, pH 7.5 (fractions 6–8). 2 ml fractions were collected throughout and interferon titered for each fraction. The titer of the peak fraction was 2.5 × 10^6 units/ml. Recovery was 109% of theoretical input. Fig.2B. 70 *A* units of total *E. coli* tRNA were covalently linked to 1 ml agarose gel as described in Materials and methods. 1.3 × 10^7 units of interferon were put on the column (fractions 1–4) which was then washed with 5 bed volume of Tris–HCl 10 mM, pH 7.5 (fractions 5–6). Subsequently, 6 bed volumes of 10 mM Tris–HCl 1 M NaCl were applied (fractions 7–9). 2 ml fractions were collected throughout and interferon was titered for each fraction. The titer of the peak fraction was 5.1 × 10^6 units/ml. Recovery was 105% of theoretical input. (●- - - -●) *A*. (▲——▲) Interferon.

suggested that these RNAs were competing with the blue dextran ligand for interferon binding. Direct evidence for binding of interferon to tRNA was obtained by using tRNA–agarose columns. As shown in fig.2A and 2B, when a crude interferon preparation is applied to a column of total yeast tRNA–agarose or total *E. coli* tRNA–agarose, 100% of the interferon activity is retained. Desorption of interferon molecules is achieved by increasing the molarity of the eluant (1 M NaCl).

3.3. *Desorption by various specific yeast tRNAs of interferon retained on BDS columns*

Although all tRNAs have a rather similar secondary and tertiary structure, among various tRNA preparations tested for their capacity to desorb interferon from BDS columns, only a few were active, as shown in table 1. It was therefore envisaged that a total yeast tRNA preparation might contain a mixture of active and inactive tRNAs species. This point was examined by comparing the relative efficiencies of desorption of 7 highly purified specific yeast tRNA species.

From table 2, it can be seen that $tRNA^{Phe}$, $tRNA^{Trp}$, $tRNA^{Val}$ and $tRNA^{Asp}$ were capable of desorbing interferon with relative efficiency, whereas $tRNA^{Lys}$, $tRNA^{Tyr}$ and the renatured $tRNA_3^{Leu}$ were totally inactive.

Table 2
Desorption by yeast specific tRNAs of mouse interferon retained on blue-dextran-sepharose columns

tRNA species	$tRNA^{Lys}$	$tRNA^{Tyr}$	$tRNA_3^{Leu}$	$tRNA^{Asp}$	$tRNA^{Phe}$	$tRNA^{Val}$	$tRNA^{Trp}$
Percent interferon desorbed	0	0	0.6	23	25	30	32

3.9×10^6 units of interferon in 3 ml were bound to a 1 ml BDS column. The column was washed with 6 bed volumes of Tris–HCl 10 mM, pH 7.5 and 3 ml of a tRNA preparation ($A_{260\,nm}$ 1) were then applied. Subsequently, a washing step was carried out with 6 bed volumes of 10 mM Tris. The final desorption was obtained with 3 ml 1 M NaCl in Tris–HCl buffer. 1 ml fractions were collected throughout and the interferon titer of each fraction was determined. For each experiment, the sum of total units desorbed by the tRNA and of total units desorbed by 1 M NaCl were added and compared to input. This sum varied between experiments from 75–180% of input, and this was considered as total recovery, in view of the relative accuracy of the micro assay used. The percent of desorption by the respective tRNA is calculated against total recovery

3.4. *Thermoinactivation curves of interferon in the presence of tRNAs*

Mouse C243 interferon is thermolabile and has a half-life of less than 3 min at 60°C in Tris–HCl, 10 mM, pH 7.5, buffer. It can be seen from fig.3 that significant protection from thermal inactivation was conferred by adding tRNA to the interferon solution before heating. Moreover those tRNA species most active in desorbing interferon from BDS were also found to be the most efficient in protecting interferon against thermal denaturation.

4. Discussion

Among the various tRNAs tested for their capacity to desorb mouse interferon from blue dextran–Sepharose, only yeast, *E. coli* and C243 cell total

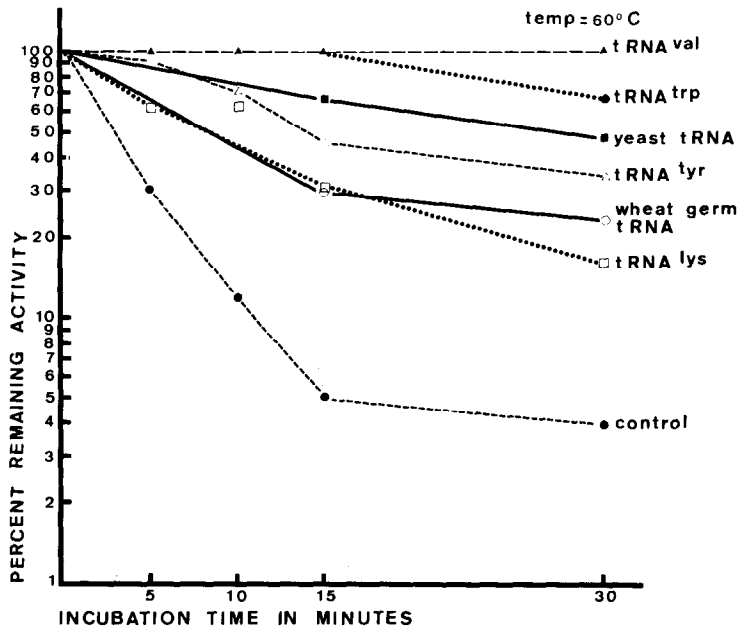

Fig.3. 0.2 ml aliquots containing 3.9×10^5 units interferon in Tris–HCl 10 mM buffer, at pH 7.5, were incubated at 60°C in a waterbath, in the presence of the tRNA preparation to be tested; the latter was at a concentration of 10 μg/ml. At the indicated time intervals, 20 μl aliquots were removed, diluted one-hundred fold in culture medium (minimum essential medium, Gibco, enriched with 3% calf serum) and cooled in an ice bath. Titrations were performed immediately at the end of the experiment.

tRNAs were active, whereas wheat germ, rat and calf liver total tRNAs had no significant effect. This strongly suggests that the displacement of interferon from BDS cannot be ascribed to a mere effect of charge but requires a more specific interaction between interferon and tRNA molecules. The higher content of modified bases in yeast tRNA as compared to *E. coli* tRNA cannot explain the better efficiency of desorption of yeast tRNA. Indeed, among other eucaryotic RNAs tested, C243 cell tRNA probably does not have a significant higher content of modified bases than rat or calf liver tRNA, yet C243 cell tRNA can desorb about 50% of interferon bound to blue dextran, while the rat and calf liver tRNA have no activity. Still more surprising are the results with purified specific yeast tRNAs: seven of them, chosen among the class I, II and III clover leaf structure [12] were tested for their ability to displace interferon from BDS columns; four only, $tRNA^{Asp}$, $tRNA^{Phe}$, $tRNA^{Trp}$ and $tRNA^{Val}$, had enough affinity to displace interferon from the dye ligand. No correlation can be found between the class of clover leaf structure and the ability to desorb interferon, Indeed, $tRNA^{Asp}$, $tRNA^{Phe}$, $tRNA^{Trp}$ belong to class I and $tRNA^{Val}$ to class II, while the other three inactive tRNAs are from class I, II and III. These results suggest that interferon recognizes on the various tRNA molecules a specific region of defined structure rather than the global tertiary structure.

Evidence for a direct binding of interferon to tRNAs is given by the thermal inactivation curves of interferon in the presence of various tRNAs and by attachment to agarose-bound *E. coli* and yeast tRNA. It is noteworthy that those tRNA preparations that were the most active in desorbing interferon from blue dextran–Sepharose were at the same time the most efficient in conferring protection against thermal denaturation.

It is difficult to imagine that the interaction of interferon with tRNAs would be devoid of physiological significance, especially since mouse interferon prepared in C243 cells showed a binding affinity for tRNA derived from the same cells. At the present stage of our knowledge, it is difficult however, to specify the nature or function of this interaction. It has been reported that the addition of certain tRNA species to S10 extracts of interferon treated cells restores the ability of these extracts to translate different mRNAs [13–15]; a correlation between this observation and what we report here seems unlikely since it is now generally accepted that the antiviral activity of interferon is not due to a direct action, but occurs through induction of other factor(s) in the interferon-treated cell. Furthermore, from studies with pure mouse interferon, we have calculated that a few molecules per cell are sufficient to induce the antiviral state, and this would seem to exclude an effect through direct action on tRNA for purely stoichiometrical reasons. However, the possibility remains that interferon is a very active enzyme, modifying some tRNAs so that even a few molecules of interferon per cell would be sufficient to act on a large number of tRNA molecules, especially if a minor tRNA species were also involved.

So called 'spontaneous' interferon production has been observed many times in different cells, and though its presence has usually been ascribed to latent virus infection, it has by no means been proved that this is the case. The possibility that interferon is normally present in the cell in very low amounts and plays some role in the regulation of tRNA function deserves further examination. Plant, bacterial and animal virus genomes have been found to possess tRNA like structures [16–18] and the appearance in the cell of RNA molecules with domains mimicking the interferon binding tRNA domains could result in competition for the interferon molecules present and thus trigger interferon synthesis. If this hypothesis is correct, RNAs able to induce interferon should have an affinity for the interferon molecules; we are now actively investigating this possibility. In this connection we have recently reported the affinity of poly(I) and poly(U) for mouse interferon [3]. Both polynucleotides are part of the well known interferon inducers poly(I)·poly(C) and poly(A)·poly(U). Some preparations of poly(I) have even been found to be highly active interferon inducers in rabbit, mouse and human cells [19,20].

Whatever its implication may be for induction or mode of action of interferon, the affinity of mouse interferon for tRNA can be used from a practical point of view for purification studies.

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