TWO FORMS OF MOUSE INTERFERON MESSENGER RNA

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SUMMARY - Two forms of biologically active mouse interferon mRNA have been characterized: one is retained by oligo(dT)cellulose, has an S value exceeding 18 S, is destroyed by small doses of pancreatic RNase and resists RNase III; the other form has a lower S value, is not retained by oligo(dT)cellulose, is relatively resistant to pancreatic RNase and is destroyed by RNase III; this latter form may be double-stranded.

We have previously described a biological assay for interferon messenger RNA (1). It is based on the species specificity of interferon and on the ability of cells from a given vertebrate species to translate the messenger RNA of interferon from another unrelated species. Thus, RNA extracted from interferon producing mouse cells can be translated in chick cells, which will therefore produce mouse interferon. This procedure eliminates any inductive effect of the RNA, which would result in production of chick interferon. Taking advantage of the high sensitivity of this assay, we have attempted to characterize mouse interferon messenger RNA. While a fraction seems to exhibit features of other messenger RNAs, another fraction shows some unusual properties, best explained by a double-stranded structure.

MATERIALS AND METHODS

Two mouse cell systems were selected, yielding enough interferon mRNA to allow quantitative studies:

- C-243 cells, a cell line transformed by mouse sarcoma virus (2) which yields high interferon titers (5×10^5 units/ml) about 10 hours after induction with UV-irradiated Newcastle disease virus. For each experiment, 15 to 20 petri dishes (150 mm diameter) were used; 8 hours after induction, the cells were scraped off with a rubber policeman, centrifuged and stored at -70°C until RNA extraction
- L cells, a cell line yielding low interferon titers (100 units/ml)

upon induction with poly (I·C). The latter was left for 2 hours on the cells, at a concentration of 10 µg/ml, in the presence of 10 µg/ml of DEAE dextran; it was then washed off and the cultures were further incubated to be collected as described above. In one experiment, L cells were induced by UV-irradiated NDV, as stated for C-243 cells; under such conditions, L cells produce high levels of interferon (1×10^4 units/ml).

RNA extraction was performed as previously described (1). In some experiments, a 1:1 chloroform phenol mixture was used instead of phenol. Unless stated otherwise, only A-phase RNA, the fraction extracted by phenol and detergent, was tested.

Pancreatic RNase A (electrophoretically purified, 2800 units/mg) was purchased from Worthington (U.S.A.).

RNase III was prepared from E.coli; it had a specific activity of 30,000 nM/mg/hr, as measured on poly (A·U) and was free of RNase A activity.

Oligo (dT)cellulose was purchased from Searle Laboratories, U.K. and from Collaborative Research Inc., U.S.A. Chromatography on oligo(dT)cellulose columns was performed according to Aviv and Leder (3). To control the ability of the column to bind poly A containing RNA, use was made of H^3-labelled early vaccinia virus mRNA produced *in vitro*. Usually, 70% of this RNA was retained and could be eluted by 0.01M Tris buffer.

Titration of interferon mRNA translated in chick cells pretreated with actinomycin D and interferon titrations on mouse embryo fibroblasts have been described previously (1).

RESULTS

I. In C-243 cells induced with NDV

1°) Fractionation by oligo(dT)cellulose chromatography of two interferon mRNA components (Table 1): the total RNA extracted by phenol and detergent was applied to an oligo(dT)cellulose column (11 x 0.5 cm). As expected, most of the cellular RNA was not retained by this column and appeared in the wash buffer (0.01M Tris, 0.5M KCl). This fraction had messenger activity in chick cells. An intermediate elution step by Tris - 0.1M KCl did not yield any material detectable by optical density, radioactive labelling or mRNA activity, but the last fraction eluted by Tris buffer only, again was active; this fraction consists of poly A containing messenger RNAs (3) (Table 1).

TABLE 1

Properties of Interferon mRNA from NDV-UV induced C-243 cells

Fraction	RNA* concentration	Mouse Interferon Units/ml			
		RNase A**		RNase III***	
		Control	Treated	Control	Treated
RNA not bound to oligo(dT) cellulose	11 µg/ml	6	6	9	0
	7 µg/ml	7	4	6	0
RNA bound to oligo(dT) cellulose	3.5 µg/ml	7	0	7	6
	0.7 µg/ml	3	0	N.D.	N.D.

N.D. : not done

* Final concentration in chick cell plates

** 0.1 µg/ml of RNase A 30 min. at 20°C in NaCl 0.15M Tris 10^{-2}M pH 7.0. Control: RNA dilution in the same buffer without the enzyme.

*** 1 µg/ml of RNase III 30 min. at 20°C in Tris HCl pH 7.9, 2.10^{-2}M, $MgCl_2$ 10^{-2}M, NaCl 0.15M, EDTA 10^{-4}M, β-mercaptoéthanol 6.10^{-2}M. Control incubated in the same buffer.

It should be noted that with some preparations, high concentrations of RNA (more than 20 µg/ml for RNA not bound to column, 5 µg/ml for RNA bound to column) were less active than dilutions.

2°) Respective size of these active components : the two active components eluted from oligo(dT)cellulose column were sedimented on a sucrose gradient. As shown in Fig. B, the poly A containing mRNA sedimented as a discrete peak in the 25 S region. This activity was totally destroyed by pancreatic RNase (1 µg/ml). In contrast, the activity of the RNA which was not retained by oligo(dT)cellulose was dispersed on sucrose gradient and could be found in fractions ranging from 6 S up to over 30 S. The activity was resistant to

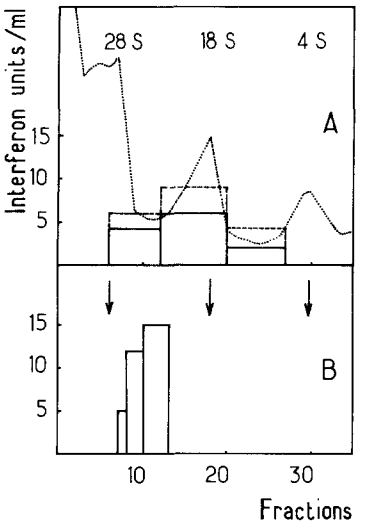

Fig. A - 6.5 mg of RNA not bound to oligo(dT)cellulose, concentrated by ethanol precipitation, were layered on top of a 5-20% sucrose gradient and centrifuged for 18 hrs at 23,000 r.p.m. in the rotor SW 27/2 at 15°C. The collected fractions were pooled in 5 regions, precipitated by ethanol and redissolved in 0.15M NaCl, 0.015 Na Citrate. One half of each pool was directly used for determination of mRNA activity (full line). The other half was first submitted to RNase A treatment (1 µg/ml, 30 mins at 20°C) and then titrated (dotted line). In pointed line, optical density profiles at 260 nM.

Fig. B - The fractions eluted from oligo(dT)cellulose at 0.01M Tris buffer were concentrated by ethanol precipitation and sedimented as described in Fig. A. The resulting fractions were pooled two by two and tested for mRNA activity. The arrows show the position of the cellular RNA markers sedimented in a parallel gradient.

mild pancreatic RNase treatment (Fig. A). If the RNA was treated by 1 µg/ml of pancreatic RNase (30 min, at 20°C) before being applied to the gradient, the activity was restricted to the 6 S fractions (not shown on figure).

3°) Effect of RNase III on the two components : this was exactly the opposite of pancreatic RNase: it destroyed the messenger activity of the fraction not retained by the oligo(dT)cellulose but did not affect the poly A containing mRNA fraction (Table 1).

II. In L cells induced by poly (I·C)

Studies in this system gave a somewhat different picture.

1°) No activity was found associated with the fraction retained

by the oligo(dT) column, either in the RNA fraction extracted by phenol and detergent or in the interface reextracted with pronase. Since phenol extraction may remove poly A from mRNAs, a mixture of phenol:chloroform was also used for extraction, with similar results; the messenger activity was exclusively associated with the fraction not retained by the column.

2°) Further evidence for the absence of poly A in this interferon mRNA was brought by experiments using cordycepin, which is believed to prevent addition of poly A to nuclear mRNA (4). Incubation of L cells, induced or not induced by poly (I·C) with 20 µg/ml of cordycepin for 1 hour inhibited by 85% appearance of labelled RNA bound to oligo(dT)cellulose. However, production of interferon was not impaired, even when cordycepin was present during the whole period of induction.

3°) RNase A resistance, sensitivity to RNase III of the interferon mRNA present in L cells induced by poly (I·C) were similar to those of the fraction devoid of poly A sequence described in C-243 cells (Table 2). In sucrose gradient sedimentation, the activity was localized in the 6-14 S region and was reduced to 6 S if the RNA was treated by RNase before sedimentation. It was also found that this fraction, in contrast to the poly A containing fraction of C-243 cells, is mostly soluble in 0.15 $MgCl_2$, a characteristic of RNA molecules with highly ordered secondary structure (6) (Table 2).

III. In L cells induced by NDV

Finally an experiment was made in which L cells were induced to produce interferon by UV inactivated NDV. Interferon mRNA activity was found both in fractions retained and not retained by oligo(dT) cellulose. Thus, characteristics of interferon mRNA produced in the same cells depend on the type of induction.

DISCUSSION

One fraction of the mRNA for mouse interferon has the properties described for other mRNAs: it is retained by oligo(dT)cellulose and readily destroyed by pancreatic RNase. This is in agreement with the results obtained by P.M. Pitha and F.H. Reynolds for human interferon mRNA isolated from poly (I·C) induced fibroblasts (personal communication). The characteristics found for the fraction of mRNA activity not bound to oligo(dT)cellulose, suggest that it is associa-

TABLE 2

Properties of Interferon mRNA from poly (I·C) induced L cells

Fraction	RNA concentration added to chick cells	Mouse Interferon Units/ml
- RNA sedimented in sucrose gradient, fractions 6 - 14 S	25 µg/ml	100
	10 µg/ml	48
- RNA, not bound to oligo(dT)cellulose	50 µg/ml	30
	25 µg/ml	76
	10 µg/ml	80
- RNA, bound to oligo(dT)cellulose	4.8 µg/ml	0
	2.4 µg/ml	0
- RNA 6 - 14 S precipitated by 0.15M $MgCl_2$	15 µg/ml	3
	5 µg/ml	2
- Supernatant of $MgCl_2$ precipitation*	15 µg/ml	20
	5 µg/ml	15
- RNA 6 - 14 S**	20 µg/ml	43
- treated by RNase A 0.1 µg/ml	20 µg/ml	14
" " " 1 µg/ml	20 µg/ml	22
" " " 10 µg/ml	20 µg/ml	0
- RNA 6 - 14 S,*** fraction soluble in $MgCl_2$ 0.15M	10 µg/ml	8
- treated by RNase A 1 µg/ml	10 µg/ml	6
- treated by RNase III 10 µg/ml	10 µg/ml	0

* Dialyzed against buffer used for mRNA titration (1)
** Other preparation; for detailed conditions of RNase treatment, see Table 1.
*** Other preparation; conditions for RNase A and III treatment are the same as in Table 1.

ted with a double-stranded structure. Its resistance to RNase A together with its sensitivity to RNase III, an enzyme specifically active on double-stranded RNA (5), are striking. Preliminary experiments indicate that this form has a lower density in caesium sulfate than single-

stranded RNA, is resistant to diethyl pyrocarbonate and can be made RNase A sensitive by heat denaturation.

All these criteria however do not distinguish between RNA molecules folded back on themselves such as the double-stranded loop present in T4 lysozyme mRNA (7) and a real duplex of two complementary strands.

In any case this structure seems to be associated in intact cells with single-stranded molecules which are not essential for activity; this is shown by the decrease in sedimentation value of active molecules after RNase A treatment able to remove all single-stranded chains. This association is reminiscent of that found in viral RNA replication (replicative intermediate).

Since we have as yet no data on the kinetics of appearance of the two forms of mRNA after induction, it is too early to speculate on their respective function and mutual relationship. It is however clear that the abundance of one form relative to the other depends on the mechanism of interferon induction and production. When cells are induced to produce large amounts of interferon, a poly A containing interferon mRNA is detectable as well as a double-stranded form and investigations in progress show that there is also a nuclear precursor of the former mRNA. When cells produce a low level of interferon, as is the case for poly (I·C) induced L cells, only the double-stranded form is present.

It remains to be explained how this form can be translated efficiently in recipient embryo cells. A mechanism may exist to read or to open the double-stranded structure, as postulated for the infectious replicative form of viral RNAs (8).

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REFERENCES

1. De Maeyer-Guignard, J., De Maeyer, E., and Montagnier, L. (1972) Proc. Nat Acad. Sci. USA 69, 1203-1207.
2. Bassin, R.H., Philips, L.A., Kramer, M.J., Haapala, D.K.,

Peebles, P.T., Nomura, S., and Fischinger, P.J. (1971) Proc. Nat. Acad. Sci. USA 68, 1520-1524.
3. Aviv, H., and Leder, P. (1972) Proc. Nat. Acad. Sci. USA 69, 1408-1412.
4. Darnell, J.E., Philipson, L., Wall, R., and Adesnik, M. (1971) Science 174, 507-514.
5. Robertson, H.D., Webster, R.E. and Zinder, N.D. (1968) J. Biol. Chem. 243, 82-91.
6. Harel, L., and Montagnier, L. (1971) Nature New Biol. 229, 106-108.
7. Ricard, B., and Salser, W. (1974) Nature 248, 314-317.
8. Montagnier, L. (1968) The Molecular Biology of Viruses, 18th Symp. Soc. Gen. Microbiol., pp. 125-148, Cambridge, Academic Press.