A Ribosome-associated Inhibitor of the Digestion of Polyadenylate-containing Ribonucleic Acid*

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SUMMARY

An inhibitor associated with guinea pig adrenal ribosomes inhibits the digestion of polyribosome-associated poly(A)-rich messenger RNA purified from human peripheral lymphocytes by two ribonucleases: (a) a ribonuclease which is co-purified with the poly(A)-rich RNA and which initially cleaves the poly(A) tract and (b) an endoribonuclease (poly(A)ribonuclease) purified from bovine adrenal cortex cytoplasm which cleaves and digests the poly(A) tract of poly(A)-rich RNA synthesized poly(A). The inhibitor has been purified 150- to 200-fold with respect to specific activity. It is rapidly inactivated by heat, freeze-thawing, or by incubation with trypsin, but treatment with DNase or RNase has no effect on its activity. Glycerol gradient density centrifugation indicates an S of 1.5 to 2.0 for the inhibitor. Kinetic studies suggest a competitive mode of inhibition. The inhibitor fails to prevent digestion of synthetic poly(A) by poly(A) ribonuclease and appears to be ineffective in preventing digestion of poly(A)-rich RNA by several other ribonucleases.

A region rich in polyriboadenylate has been identified in heterogeneous nuclear and rapidly labeled polyribosome-associated RNA in eukaryotic cells (1-6), in specific eukaryotic mRNAs (7-12) and in viral mRNAs (13-21). It has been suggested that most or all mRNAs in eukaryotic cells, with the exception of histone mRNA, contain a poly(A) tract (22) at the 3' terminus (23-37). It has been suggested that the poly(A) tract is added post-transcriptionally (28, 29), and several nuclear and cytoplasmic enzymes capable of this function have been identified and purified (30-36); however, other evidence suggests that a portion of the poly(A) tract is transcribed (37). Several eukaryotic ribonucleases which can digest poly(A) have been identified, including a processive nuclear exoribonuclease purified from several tissues (36, 39) and an endoribonuclease first purified from rat liver nuclei (40).

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It appears that mRNA consists of two portions: one which serves an information function, and the other, the poly(A) tract, a regulatory function. This regulatory function of the poly(A) tract remains undefined. Drug and hybridization studies have suggested a role in transport of mRNA from nucleus to cytoplasm (4); however, the presence of a poly(A) tract in viruses whose life cycle is confined to the cytoplasm (19, 21) and the demonstration of cytoplasmic polyadenylation of transcripts in sea urchin embryos (42) suggest other or additional roles.

We have previously reported that polyribosome-associated poly(A)-rich RNA isolated from cultured lymphocytes using the nitrocellulose filter technique (6) is cleaved by a putative ribonuclease co-purifying with the RNA, resulting in the removal of a segment which sediments at 4 to 6 S in linear SDS-sucrose density gradients (40). The base composition of this 4 to 6 S endoribonuclease digestion product is identical to that of the isolated poly(A) tract (44). We have subsequently identified and partially purified a cytoplasmic ribonuclease from bovine adrenal cortex that digests synthetic poly(A) and cleaves the poly(A) tract of the poly(A)-rich RNA. Many properties of this enzyme are similar to those described for a ribonuclease isolated from pig liver nuclei (40). A ribosome-associated factor inhibits digestion of poly(A)-rich RNA by both the RNA-associated ribonuclease and the partially purified cytoplasmic ribonucleases. We report here the purification of this inhibitor and the initial definition of its properties. The identification of a specific inhibitor of ribonucleases which digest the poly(A) tract suggests that regulation of the digestion of the poly(A) tract can occur in the cytoplasm of the eukaryotic cells.

EXPERIMENTAL PROCEDURES

Materials

[3H]Adenosine (15.5 Ci per mmole), [3H]uridine (15 Ci per mmole), and Escherichia coli [3H]tRNA used as a marker for gradient analysis were purchased from Schwarz-Mann, and [35P]orthophosphate in water from New England Nuclear. Phosphate-free pancreatic RNase was obtained from Sigma Chemical Co. and was treated by heating at 60° for 30 min at pH 5 prior to additional use. Insoluble pancreatic RNase attached to Sepharose beads (enzyme concentration 6.7 units per ml) was purchased from Miles Laboratories, Inc. Takadiastase RNase T1 (Sankyo prepara-

1 The abbreviations used are: SDS, sodium dodecyl sulfate; MeSO, dimethylsulfoxide; [3H]uridine or [3H]adenylic, poly(A)-rich RNA prepared from cultured lymphocytes labeled in the presence of [3H]uridine or [3H]adenosine.
tion), RNase T<sub>1</sub> essentially free of RNase T<sub>2</sub> activity, and actinomycin D were purchased from Sigma Chemical Co. Polyribouridylic acid and polyriboadenyllic acid were purchased from Schwarz-Mann. [32P]Polyriboadenyllic acid (0.91 mCi per mmole of phosphorus) and [3H]polyriboadenyllic acid (12.5 mCi per mmole of phosphorus) were obtained from Miles Laboratories, Inc. Trypsin immobilized on Sepharose beads (enzyme concentration 5.9 units per ml) was obtained from Miles Laboratories, Inc. Electrotophoretically purified DNase I was obtained from Worthington Biochemical Corp. Spermine, spermidine, and lysine- and arginine-histones were purchased from Sigma.

**Methods**

**Preparation of Radiolabeled Poly(A)-rich RNA and Isolated Poly(A)-rich RNA**—rapidly labeled polyribosomal-associated poly(A)-rich RNA was isolated from cultures of highly purified human peripheral lymphocytes by the nitrocellulose binding method described by Lee et al. (1). Lymphocyte cultures were prepared and incubated with phytohemagglutinin for 36 hours as previously described (6). Following incubation for 30 min with actinomycin D (0.05 μg per ml) to inhibit RNA synthesis, [3H]adenosine (10 μCi per ml) or [3H]polyriboadenyllic acid (10 μCi per ml) was added to the cultures and incubation was continued for an additional 90 min. Polyribosome poly(A)-rich RNA was isolated using serial pH 7.6 and pH 9.0 extractions as previously described (6). In all procedures, acid- and alkaline-washed autoclaved glassware and autoclaved buffers were used. Poly(A)-rich RNA was eluted from the nitrocellulose filter (Millipore, 0.45 μm) in 10 mM Tris-HCl (pH 7.6) and washed with an excess of the same buffer, and then eluted with 10 mM Tris-HCl (pH 6.0), 0.1% SDS. The eluate was extracted twice with phenol-chloroform (1:1) and ether. The poly(A) tracts sedimented at 4 to 6 S on linear SDS sucrose density gradients.

**Purification of Poly(A) Ribonuclease**—an endonucleosome which digests synthetic polyriboadenyllic acid and poly(A)-rich RNA has been purified 500-fold with respect to specific activity from bovine adrenal cortex cytoplasm, but the enzyme has not been purified to homogeneity. A detailed description of the properties of the bovine adrenal cortex ribonuclease referred to as poly(A) ribonuclease and its purification will be reported elsewhere. At a final concentration of 1 μg per ml, 0.1 μg of this enzyme preparation was added to 0.270 mg of RNA which was incubated for 30 min at 37°C in 0.1 M KCl, 1 mM MgCl<sub>2</sub>, and 0.05% Triton X-100. Following incubation, a 40-fold excess of 10 mM Tris-HCl (pH 7.6), 10 mM KCl, 1 mM MgCl<sub>2</sub>, with 0.88 mM K<sub>2</sub>HPO<sub>4</sub> (pH 7.5), 1 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 10% glycerol (Buffer C), dialyzed extensively against a 1000-fold excess in Buffer C for 12 hours with three changes of buffer. The RNA was then dialyzed against the same buffer, which was previously equilibrated with Buffer C. The volume was washed with 10 bed volumes of Buffer C and then washed with 3 bed volumes of Buffer C containing 0.5 M potassium phosphate (pH 7.5). Final elution was with 1 bed volume of Buffer C containing 0.5 M potassium phosphate (pH 6.5). The eluates were extensively dialyzed overnight against Buffer C without potassium phosphate with three changes of buffer. When additional purification was desired, peak fraction was applied to a 5 to 20% glycerol gradient containing 10 nm Tris-HCl (pH 7.5), 500 mM KCl, 1 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 1 mM dithiothreitol, and centrifuged for 18 hours at 40,000 rpm at 4°C in a 60 Ti rotor. The ribosomal pellet had an A<sub>260</sub>:A<sub>415</sub> ratio of 1.5 to 1.6. The ribosomal pellet was resuspended in a buffer containing 10 mM Tris-HCl (pH 7.6), 0.15 mM KCl, 1 mM MgCl<sub>2</sub>, 0.88 M sucrose, 1% Triton X-100 and centrifuged at 40,000 rpm in a 50 Ti rotor. The supernatant was removed and adjusted to 90% ammonium sulfate, agitated gently for 60 min, and then centrifuged for 20 min at 30,000 rpm. The pellet was resuspended in 10 mM Tris-HCl (pH 7.5), 0.5 mM KCl, 10 mM KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> (pH 7.5), 1 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 10% glycerol (Buffer C), and dialyzed extensively against a 1000-fold excess in Buffer C for 12 hours with three changes of buffer. The RNA was then dialyzed against the same buffer, which was previously equilibrated with Buffer C. The column was washed with 10 bed volumes of Buffer C and then washed with 3 bed volumes of Buffer C containing 0.5 M potassium phosphate (pH 7.5). Elution was with 1 bed volume of Buffer C containing 0.5 M potassium phosphate (pH 6.5). The eluates were extensively dialyzed overnight against Buffer C without potassium phosphate with three changes of buffer.
Protection of poly(A) rich RNA from digestion by an associated ribonuclease and by poly(A) ribonuclease by ribosomal eluate

Each incubation mixture containing 40 ng of [1H]uridy late lymphocyte poly(A)-rich RNA (150 cpm per 10 ng) was incubated for 10 min at 37° in 100 ml of Buffer A as described under "Methods." Where indicated 0.5 m KCl ribosomal eluate (90 μg/ml) was added. An equal volume of eluate buffer was added to the control tubes. In incubations where poly(A) ribonuclease (0.08 μg/ml) was added, the poly(A)-rich RNA was pretreated to prevent self-digestion as described under "Methods." Assay for digestion was by retention on a Millipore filter as described under "Methods." Results are the average of triplicate determinations differing by less than 3%. Digestion of 3 ng of RNA is the smallest amount that can be detected with a confidence greater than two standard deviations. Similar results were obtained when [3H]adenylate or [3H]uridy late poly(A)-rich RNA was used in eight other experiments of identical design.

| Complete assay mixture | Poly(A)-rich RNA digested (ng × 10^4) |
|------------------------|--------------------------------------|
| Not incubated          | <0.3                                 |
| Incubated              | 8.8                                  |
| + Ribosomal eluate     | <0.3                                 |
| + Ribosomal eluate added postincubation | 8.2       |
| + Poly(A) ribonuclease | 25.8                                 |
| + Poly(A) ribonuclease + ribosomal eluate | <0.3 |

from the phosphocellulose chromatography eluates during this purification technique as assayed by digestion of synthetic [3H]-polyriboadenyllic acid.

RESULTS

Identification and Purification of Inhibitor—Addition of a 0.5 m KCl extract of guinea pig adrenal ribosomes entirely prevented the digestion of poly(A)-rich RNA by its associated ribonuclease or by poly(A) ribonuclease as assayed by the retention on Millipore filters (Table I) or by retention on GF/A filters impregnated with poly(U) immobilized by ultraviolet radiation (48) (data not shown). Addition of eluate following the digestion did not alter retention on the filter (Table I).

The inhibitory factor was purified from the 0.5 m KCl eluate of guinea pig adrenal ribosomes. Purification resulted in a 110- to 100-fold increase in specific activity of the inhibitor (Table II). The increase in specific activity would be much greater if the digestion did not alter retention on the filter (Table I).

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Properties of Inhibitor—The inhibitor was inactivated by heating to 90° for 5 min (Table III). It withstood freeze-thawing poorly with 90 to 100% loss of activity. Storage in buffers with low salt concentration was associated with rapid loss of activity. The inhibitor was inactivated by digestion for 15 min at 37° with pronase (50 μg/ml). Incubation of the purified inhibitor with trypsin (10 μg/ml) for 30 min at 37° in Buffer A, followed by addition of trypsin inhibitor (11 μg/ml) abolished the ability to inhibit self-digestion of poly(A)-rich RNA or digestion by poly(A) ribonuclease. Similarly, preincubation for 20 min at 37° with trypsin immobilized on Sepharose beads abolished inhibitory activity (Table III). Incubation with DNase or with pancreatic RNase A immobilized on Sepharose beads had no effect on inhibitor activity (Table III).

Kinetic Studies—The kinetics of the inhibition of poly(A)-rich RNA digestion was studied by assaying digestion of varying amounts of lymphocyte poly(A)-rich RNA pretreated to prevent self-digestion in mixtures containing constant amounts of poly(A) ribonuclease and inhibitor. Two concentrations of inhibitor found to confer incomplete protection against digestion of poly(A)-rich RNA by poly(A) ribonuclease were selected. A Lineweaver-Burk analysis of the inhibitor suggests a competitive mode of inhibition (Fig. 4).

Neither the enzyme nor inhibitor has been purified to homogeneity; therefore, the $K_i$ cannot be accurately stated.
inhibitor did not prevent digestion of synthetic poly(A) even by poly(A) ribonuclease was tested. As shown in Table IV, the inhibitor to prevent digestion of synthetic polyriboadenylic acid substrate requirements for the inhibitory activity, the ability of the preparation of inhibitor and poly(A)-rich RNA.

were obtained in an experiment of identical design using a different fraction of eluate from the phosphocellulose column with peak inhibitory activity was dialyzed against Buffer C and sedimented through a linear 5 to 30% glycerol gradient (see "Methods"). Inhibitor activity was assayed using 20-μl aliquots in duplicate determinations with 50 ng of [3H]uridylate lymphocyte poly(A)-rich RNA as described in the legend to Table I. Radiolabeled mRNA retained reflects inhibitor activity. Migration of myoglobin and ovalbumin markers are indicated by arrows. Similar results were obtained using three separate preparations of inhibitor (purified through ammonium sulfate fractionation in A; purified through glycerol gradient in B), O--O. Similar results were obtained in only two of the four preparations.

Results are the average of duplicates differing by less than 3%.

FIG. 1 (left). Sedimentation profile of inhibitor. Inhibitor was prepared from 25 g of guinea pig adrenal cortex (Table I). The fraction of eluate from the phosphocellulose column with peak inhibitory activity was dialyzed against Buffer C and sedimented through a linear 5 to 30% glycerol gradient (see "Methods"). Inhibitor activity was assayed using 20-μl aliquots in duplicate determinations with 50 ng of [3H]uridylate lymphocyte poly(A)-rich RNA as described in the legend to Table I. Radiolabeled mRNA retained reflects inhibitor activity. Migration of myoglobin and ovalbumin markers are indicated by arrows. Similar results were obtained using three separate preparations of inhibitor (purified through ammonium sulfate fractionation in A; purified through glycerol gradient in B), O--O. Similar results were obtained in only two of the four preparations.

Effects of physical and enzymatic pretreatment on inhibitor activity

Table III

| Pretreatment of inhibitor | Poly(A)-rich RNA digested (ng x 10^-1) |
|--------------------------|---------------------------------------|
| No inhibitor added        | 17.2                                  |
| Preincubution             |                                       |
| At 90°                   | 16.8                                  |
| At 37°                   | <0.3                                  |
| + Trypsin                 | 16.5                                  |
| + Pancreatic RNase        | 0.3                                   |
| + DNase I                 | 0.7                                   |

Substrate Specificity of Inhibitor—In order to clarify the substrate requirements for the inhibitory activity, the ability of the inhibitor to prevent digestion of synthetic polyriboadenylc acid by poly(A) ribonuclease was tested. As shown in Table IV, the inhibitor did not prevent digestion of synthetic poly(A) even when the concentration of inhibitor used was 10-fold higher than that required for complete inhibition of poly(A)-rich RNA digestion. Inhibitor activity was also tested by using isolated radiolabeled poly(A) tracts prepared from lymphocyte poly(A)-rich.
under "Methods." When the deproteinized mRNA was used as a substrate for poly(A) ribonuclease, the purified inhibitor was able to inhibit the digestion of the RNA at concentrations similar to those required for inhibition with nondeproteinized poly(A)-rich RNA (Table V). Kinetic studies revealed quantitatively similar inhibition of poly(A) RNA digestion of deproteinized or nondeproteinized poly(A)-rich RNA by purified inhibitor (data not shown).

Table V

| Effect of deproteinization of poly(A)-rich RNA on inhibitor activity |
|---------------------------------------------------------------|
| Poly(A)-rich RNA | Deproteinized poly(A)-rich RNA |
| Complete assay mixture | ng x 10^-1 | ng x 10^-1 |
| No addition | <0.2 | <0.2 |
| + Poly(A) ribonuclease | 14.3 | 12.2 |
| + Inhibitor | <0.2 | <0.2 |
| + Poly(A) ribonuclease + inhibitor | <0.2 | <0.2 |

Fig. 4. Kinetics of inhibitor action. Varying concentrations (0.03 to 0.3 μg) of [3H]uridylyl lymphocyte poly(A)-rich RNA (100 cpm per 10 ng) pretreated to prevent self-digestion were added to 100 μl of Buffer A containing poly(A) RNAse (0.08 μg per ml) and, when indicated, inhibitor at two concentrations providing partial protection (0.75 or 2 μg per ml). Following incubations at 37° for 20 min, assay for digestion was as described under "Methods." Each point of Lineweaver-Burk analysis of the data represents the average of duplicate determinations differing by less than 5%. [S] is expressed as μg x 10^-1 of poly(A)-rich RNA and V as μg x 10^-1 of poly(A)-rich RNA digested. Similar results were obtained in six separate experiments of identical design, and the results of all experiments were then subjected to least squares analysis to calculate the best x and y intercepts.

Table IV

Effect of inhibitor on synthetic poly(rA)

| Additions to incubation | Poly(A) digested ng x 10^-1 |
|-------------------------|---------------------------|
| None                    | <0.2                      |
| + Inhibitor (40 μg per ml) | <0.2                     |
| + Poly(A) RNAse (0.8 μg per ml) | 10.4                     |
| + Poly(A) RNAse (0.8 μg per ml) + inhibitor (40 μg per ml) | 10.6                      |
| + Poly(A) RNAse (0.08 μg per ml) | 1.3                       |
| + Poly(A) RNAse (0.08 μg per ml) + inhibitor (4 μg per ml) | 1.4                       |

RNA (see "Methods") as substrate. The purified inhibitor did not prevent digestion of these tracts by poly(A) ribonuclease.

Since poly(A)-rich RNA prepared by the method of Lee et al. (1) has been shown to have protein associated with the RNA despite the SDS-phenol treatment (27), it is possible that the presence of specific proteins associated with the mRNA was necessary for the action of the inhibitor. In order to evaluate this possibility, deproteinized lymphocyte poly(A)-rich RNA was prepared by pronase digestion of the mRNA as described under "Methods." When the deproteinized mRNA was used as a substrate for poly(A) ribonuclease, the purified inhibitor was able to inhibit the digestion of the RNA at concentrations similar to those required for inhibition with nondeproteinized poly(A)-rich RNA (Table V). Kinetic studies revealed quantitatively similar inhibition of poly(A) RNAase digestion of deproteinized or nondeproteinized poly(A)-rich RNA by purified inhibitor (data not shown).

Studies of Identity of Inhibitor—The experiments demonstrating lability of inhibitor suggested that it was not a polyamine or histone. Purified spermine, spermidine, or lysine- or arginine-rich histone were unable to inhibit digestion of mRNA by poly(A) RNAse at concentrations effective for the inhibitor described above. At much higher concentrations, they partly inhibited digestion of synthetic poly(A) by poly(A) RNAse, another characteristic distinguishing them from the ribosome-associated inhibitor. The kinetics of this inhibition revealed noncompetitive inhibition (data not shown). For these reasons, it is concluded that the ribosome-associated inhibitor described above is not a polyamine. Since the existence of a prokaryotic RNA ligase has recently been reported (49) and since such an enzymatic activity would explain much of the data presented here on "inhibition" of RNAse, the inhibitor was tested for possible activity as an RNA ligase. Various concentrations of synthetic [3H]polyriboadenylate acid (average size 4 to 5 S) were added to 100 μl of Buffer A containing [3H]poly(A)-rich RNA or 60 ng of...
TABLE VI
Effect of inhibitor on digestion of poly(A)-rich RNA by several ribonuclease inhibitors

| Addition to incubation mixture | Poly(A)-rich RNA digestion (ng x 10⁻³) |
|-------------------------------|---------------------------------------|
| None                          | <0.2                                  |
| Inhibitor                     | <0.2                                  |
| Poly(A) RNase                 | 26.3                                  |
| Poly(A) RNase + inhibitor     | <0.2                                  |
| Pancreatic RNase (0.08 μg per ml) | 11.2                               |
| Pancreatic RNase (0.08 μg per ml) + inhibitor | 10.5                               |
| Pancreatic RNase (0.008 μg per ml) | 0.9                                |
| Pancreatic RNase (0.008 μg per ml) + inhibitor | 0.5                                |
| T₁ RNase                      | 15.8                                  |
| T₁ RNase + inhibitor          | 15.2                                  |
| T₂ RNase                      | 1.3                                   |
| T₂ RNase + inhibitor          | 1.2                                   |
| E. coli RNase II              | 14.3                                  |
| E. coli RNase II + inhibitor  | 14.8                                  |

[3H]poly(A)-rich RNA pretreated to prevent self-digestion, 0.08 μg of poly(A) ribonuclease, and various concentrations of inhibitor. The molar concentration of the [3H]poly(A) was varied from 0.5- to 10-fold that of the [3H]poly(A)-rich RNA. Following incubation for 15 min at 37°C, the samples were adjusted to 0.5% SDS and analyzed by sedimentation through linear SDS sucrose density gradients. No alteration of the sedimentation of [3H]-labeled material from 4 to 5 S to heavier moieties characteristic of the lymphocyte poly(A)-rich RNA was observed. Addition of various concentrations of ATP (10⁻⁸ to 10⁻⁶ M), GTP (10⁻⁴ to 10⁻³ M), or MgCl₂ (1 to 10 mm) had no effect on this result nor did use of poly(A) tracts prepared from lymphocyte poly(A)-rich RNA.

DISCUSSION

It appears that mRNA consists of two portions: one which serves an informational function, and the other, the poly(A) tract, a regulatory function. If this is correct, the synthesis and cleavage of the poly(A) tract could regulate mRNA function at one or more of several post-transcriptional levels. The results presented above describe a factor dissociated from ribosomes by 0.5 M KCl which inhibits digestion of poly(A)-rich RNA by two specific, recently described RNases (43).

Extensive purification of this inhibitor by salt fractionation, phosphocellulose chromatography, and rate-zonal centrifugation have permitted some definition of its properties. The inhibitor was eluted from the polyribosome fraction of guinea pig adrenal cortex with no inhibitor activity detectable in the 100,000 × g supernatant fraction. However, the large amount of other ribonuclease activities in the cytosol may well have obscured inhibitor activity. Hence, the association of inhibitor with the ribosomal fraction does not prove conclusively that it is a true ribosome-associated protein.

The inhibitor is a small (1.5 to 2 S) protein which specifically inhibits two ribonuclease activities which digest the poly(A) tract of mRNA. The first of these, isolated with lymphocyte poly(A)-rich RNA, is specific for the poly(A) tract (44); the second is a partially purified cytoplasmic endoribonuclease from bovine adrenal cortex which digests synthetic poly(A) as well as the poly(A) tract of lymphocyte mRNA. The latter ribonuclease shares many properties of an endoribonuclease identified in pig liver nuclei (40). Kinetic studies suggest a competitive mode of inhibition. The inhibitor does not appear to represent an altered form or subunit of the poly(A) ribonuclease since it has extremely different physical properties and sediments at a markedly different Svedberg or linear glycerol density gradients. The purified inhibitor preparation does not contain RNA ligase activity nor can ribonuclease activity be demonstrated once the inhibitory activity is lost upon storage or freeze-thawing.

The inhibitor failed to prevent the digestion of synthetic poly(A) by poly(A) ribonuclease although it inhibited digestion of the poly(A) tract of poly(A)-rich RNA. Since the method of isolation of poly(A)-rich RNA may fail to remove all associated proteins (27), one possible explanation is that these proteins are, in some way, required for inhibition. However, deproteinization did not alter the ability of the inhibitor to prevent digestion by poly(A) ribonuclease. This suggests that a portion of the mRNA other than the poly(A) tract may be required for expression of the inhibitor activity against poly(A) ribonuclease. Apparent enzyme specificity of the inhibitor is suggested by the failure of the purified inhibitor to prevent digestion of poly(A)-rich RNA by a number of ribonucleases other than the associated RNase or poly(A) RNase. However, since (a) cleavage of the poly(A) tract is detected with an amplified sensitivity compared to digestion of other portions of the RNA because the assay method is based on retention of RNA on a nitrocellulose filter due to the properties of the poly(A) tract, and (b) since the poly(A) ribonuclease has not been purified to homogeneity, the inhibitor may be present in relatively greater concentration with respect to actual poly(A) ribonuclease concentration compared to other ribonucleases tested. At very low concentrations of pancreatic ribonuclease, producing less than 3% digestion of radiolabeled mRNA, addition of inhibitor did prevent release of radiolabeled RNA from the nitrocellulose filter.

Following the identification of cytoplasmic RNase inhibitors (50), several studies have been interpreted to suggest that these ribonuclease inhibitors are required to maintain the integrity of polyribosomes (51, 52). The purification and characterization of these inhibitors have been quite difficult due to their characteristic lability, but partial purification has been achieved (53, 54). The partially purified inhibitor stabilized incubated polyribosomes suggesting that mRNA cleavage was entirely prevented (54). The possibility that induction of ribonuclease inhibitor activity is regulated was suggested by increased activity in the regenerating rat liver (55).

The existence of a protein capable of inhibiting the ribonuclease which digests the poly(A) tract suggests that eukaryotic cells could regulate removal of the poly(A) tract from mRNA in the cytoplasm. Complexing of the ribonuclease with an inhibitor may be a mechanism commonly used in eukaryotic cells to control RNase activity; a previously described example is "alkaline RNase" activity (56-58). It has been proposed that mRNA digestion is regulated by partition of the alkaline RNase between...
a soluble RNase inhibitor and a ribosomal site which could bind the enzymatic subunit (56, 58).

The demonstration of a poly(A) tract in viruses whose life cycle occurs entirely in the cytoplasm (19, 21) suggested a cytoplasmic role for the poly(A) tract. This possibility was given additional support by the demonstration that cytoplasmic polyadenylation of pre-existing transcripts occurs in the sea urchin oocyte following fertilization and that this polyadenylation of mRNA to the polyribosomal fraction (42, 57). Therefore, mRNA function or stability in the cytoplasm of eukaryotic cells might be affected by addition or removal of poly(A) tracts with regulation occurring at one or more of several levels.

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REFERENCES
1. Lee, S. Y., Mendelk, J., and Brasserman, G. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 1321-1325
2. Darnell, J. E., and Brewer, R. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 1321-1325
3. Edmonds, M., Vaughan, M. H., and Nakazato, H. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 1330-1340
4. Darnell, J. E., Phillipson, L., Wull, R., and Adensin, M. (1971) Science 174, 507-510
5. Bubb, H., and Linder, J. B. (1971) Nature New Biol. 333, 41-43
6. Rosenfeld, M. G., Abbass, I. B., Mendelsohn, J. M., Roos, B. A., Boone, R. F., and Garren, L. D. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 2296-2301
7. Lim, L., and Canellakis, E. S. (1970) Nature 227, 710-712
8. Aviv, H., and Leder, P. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 1408-1412
9. Stevens, R. H., and Williamson, A. R. (1972) Nature New Biol. 333, 143-146
10. Swan, D., Aviv, H., and Leder, P. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 1967-1971
11. Comstock, J. F., Rosenfeld, G. C., O'Malley, B. W., and Means, A. R. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 2877-2880
12. O'Malley, B. W., Rosenfeld, G. C., Comstock, J. F., and Means, A. R. (1972) Nature New Biol. 240, 45-48
13. Green, M., and Caps, M. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 791-794
14. Lai, M. C., and Duesberg, P. H. (1972) Nature New Biol. 335, 383-386
15. Weinberg, R. A., Ben-Israel, Z., and Newbold, J. E. (1972) Nature New Biol. 335, 111-113
16. Prescott, D. M., Katse, J., and Kirsch, J. B. (1971) J. Mol. Biol. 69, 505-508
17. Kates, J. (1970) Cold Spring Harbor Symp. Quant. Biol. 35, 749-752
18. Johnston, R. E., and Bose, H. R. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 1514-1515
19. Armstrong, J. A., Edmonds, M., Nakazato, H., Phillips, B. A., and Vaughan, M. H. (1972) Science 176, 525-528
20. Phillipson, L., Wall, R., Gruen, G., and Darnell, J. E. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 2806-2809
21. Eaton, B. T., Donahue, T. F., and Faelener, P. (1972) Nature New Biol. 330, 103-111
22. Aedesnik, M., Salditt, M., Thomas, W., and Darnell, J. E. (1972) J. Mol. Biol. 71, 21-30
23. Yogo, Y., and Wimmer, E. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 1977-1982
24. Molloy, G. R., Sporn, M. B., Kelley, D. E., and Perry, R. F. (1972) Biochim. Biophys. Acta 11, 3259-3260
25. Sheldon, K. E., Kester, J., Kelley, D. E., and Perry, R. F. (1972) Biochim. Biophys. Acta 11, 3823-3834
26. Nakazato, H., and Edmonds, M. (1972) J. Biol. Chem. 247, 3365-3367
27. Mendelk, J., Lee, S. Y., and Brasserman, G. (1972) Biochim. Biophys. Acta 11, 709-718
28. Darnell, J. E., Felts, W. R., and Molloy, G. R. (1973) Science 181, 1215-1221
29. Marchal, S., and Gillespie, D. (1972) Nature New Biol. 240, 43-45
30. Edmonds, M., and Abrams, R. (1960) J. Biol. Chem. 235, 1142-1149
31. Edmonds, M., and Abrams, R. (1962) J. Biol. Chem. 237, 2636-2642
32. Neisig, J., and Sekeris, C. E. (1972) Fed. Eur. Biochem. Soc. Lett. 22, 83
33. Ohashi, S., and Tsugita, A. (1972) Nature New Biol. 240, 35-38
34. Ohashi, S., Tsugita, A., and Miy, S. (1972) Nature New Biol. 249, 39-41
35. Hardy, S. J. S., and Kurland, C. G. (1966) Biochemistry 5, 3676-3684
36. Tsapalis, C. M., Dobson, J. W., DeCosentino, D. M., and Bollum, F. J. (1973) Biochem. Biophys. Res. Commun. 60, 757-743
37. Jacobson, A., Firtel, R. A., and Lodish, H. F. (1974) Proc. Nat. Acad. Sci. U. S. A. 71, 1607-1611
38. Lazareus, H. M., and Sporn, M. B. (1967) Proc. Nat. Acad. Sci. U. S. A. 67, 1408-1412
39. Miller, H. I., Riggs, A., and Row, New York
40. Deleted in proof
41. Slater, I., Gillespie, D., and Slater, D. W. (1973) Proc. Nat. Acad. Sci. U. S. A. 70, 400-411
42. Rosenfeld, M. G., Abbass, I. B., and Perkins, L. A. (1972) Biochem. Biophys. Res. Commun. 49, 230-238
43. Rosenfeld, M. G., Abbass, I. B., Mendelsohn, J., and Miller, H. I. (1973) Proc. Soc. Exp. Biol. Med. 144, 215-219
44. Bray, G. A. (1960) Anal. Biochem. 1, 279-285
45. Lowy, O. H., Rosebrough, N. J., Farr, A. L., and Randell, R. J. (1961) J. Biol Chem. 195, 265-275
46. Miller, H. I., Richs, 2. D., and Gill, G. N. (1973) J. Biol. Chem. 248, 2621-2624
47. Sheldom, R., Jureid, C., and Kates, J. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 417-421
48. Silber, R., Malahi, V. G., and Hewitt, J. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 3009-3013
49. Roth, J. S. (1964) Biochim. Biophys. Acta 61, 177-180
50. Blobel, G. and Potter, V. R. (1966) Proc. Nat. Acad. Sci. U. S. A. 66, 1283-1288
51. Bont, W. S., Reeler, G., Meisner, L., and Bloemeland, H. (1967) Arch. Biochem. Biophys. 119, 36-40
52. Shortman, K. and Meisner, L., and Blobemelnd, H. (1967) Arch. Biochem. Biophys. 119, 36-40
53. Shortman, K. (1961) Biochim. Biophys. Acta 61, 37-40
54. Gribnau, A. A. M., Schoenmakers, J. G. G., and Bloemeland, H. (1969) Arch. Biochem. Biophys. 130, 48-52
55. Shortman, K. (1962) Biochim. Biophys. Acta 61, 50-55
56. Smith, A. B., and Winkler, H. (1965) Nature 207, 654
57. Utsunomiya, T., and Roth, J. S. (1966) J. Cell Biol. 29, 395-403
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