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Possible involvement of poly(A) in protein synthesis

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ABSTRACT

The experiments of this paper have re-evaluated the possibility that poly(A) is involved in protein synthesis by testing whether purified poly(A) might competitively inhibit in vitro protein synthesis in rabbit reticulocyte extracts. We have found that poly(A) inhibits the rate of translation of many different poly(A)$^+$ mRNAs and that comparable inhibition is not observed with other ribopolymers. Inhibition by poly(A) preferentially affects the translation of adenylated mRNAs and can be overcome by increased mRNA concentrations or by translating mRNPs instead of mRNA. The extent of inhibition is dependent on the size of the competitor poly(A) as well as on the translation activity which a lysate has for poly(A)$^+$ RNA. In light of our results and numerous experiments in the literature, we propose that poly(A) has a function in protein synthesis and that any role in the determination of mRNA stability is indirect.

INTRODUCTION

In the past decade considerable efforts have been directed toward elucidating the functions of the poly(A) tracts found at the 3'-termini of most eukaryotic mRNAs (for reviews see 1,2). In the course of such work three general hypotheses for poly(A) function have emerged. These hypotheses respectively implicate a role for poly(A) in: 1) mRNA stability, 2) mRNA processing and transport or 3) protein synthesis. The possibility that poly(A) sequences might stabilize mRNAs was originally suggested by studies in which globin mRNAs possessing different poly(A) tail lengths were microinjected into Xenopus oocytes (3-6). Globin mRNAs with poly(A) tracts of approximately 30 nucleotides or more were found to be stable, whereas those with shorter poly(A) tracts were found to be unstable. Additional support for the stability hypothesis has been derived from experiments in which poly(A)$^-$ histone mRNAs were rendered more stable in the oocyte system by prior polyadenylation in vitro (7) and from experiments which demonstrated that adenovirus mRNAs synthesized in cells treated with 3'-deoxyadenosine were poly(A)$^-$ and had shortened half-lives (8,9).

However, there is also a body of data which suggests that poly(A) does not regulate mRNA stability. This includes experiments demonstrating that deadenylated forms of interferon and α-2u-globulin mRNAs did not differ in stability from their adenylated counterparts when microinjected into Xenopus oocytes (10,11) and experiments from this laboratory which showed that, for at least 66 different Dictyostelium mRNAs that possessed poly(A) tracts ranging in size from 60-115 nucleotides, there was no correlation between the stability of a messenger RNA and the size of its poly(A) tail (12). An additional complication derives from the fact that poly(A) tracts normally shorten with age, but reach a steady-state size of 40-65 nucleotides (12-17). This steady-state size is significantly larger than the minimum required in the microinjection experiments, suggesting that oocytes do not shorten poly(A) below the steady-state size and that in vitro "trimming" of poly(A) below this size may affect some other property which, in turn, makes mRNA less stable.

A role for polyadenylation in mRNA processing or transport is unlikely. First of all, a cytoplasmic function for poly(A) is suggested by the fact that there are a number of mammalian viruses that totally lack a nuclear component in their life cycles yet have poly(A) on their mRNAs (18-22). In addition, a number of mRNAs which are naturally poly(A)$^-$ or which become poly(A)$^-$ in 3'-deoxyadenosine-treated cells have been shown to be both efficiently transported to the cytoplasm (8,9) and, in those cases examined, properly spliced (8).

The possibility that poly(A) might function in protein synthesis was the first of the models to be extensively tested. Numerous investigators assayed the translational capacity of various poly(A)$^+$ mRNAs and their deadenylated counterparts (23-27). In a related study (28), the poly(A) tail of mRNA was first annealed to poly(U) and then the translational activity of the mRNA: poly(U) complex was assayed. In general, it was concluded that artificial deadenylation or blockage of the poly(A) tail with poly(U) did not significantly reduce the efficiency with which these mRNAs were translated in vitro. However, as noted by Doel and Carey (29), the assays used in these experiments were relatively insensitive because the translation systems used reinitiated poorly. Indeed, Doel and Carey (29) were able to show that native poly(A)$^+$ chicken ovalbumin mRNA was translated more efficiently than deadenylated ovalbumin mRNA in highly active reticulocyte extracts, but no difference was observed in less active wheat germ extracts. This translational discrimination appeared to be due

to a reduced rate of initiation on deadenylated mRNA since such mRNA was present on smaller polysomes and participated in fewer rounds of translation.

Additional evidence implicating a role for poly(A) in protein synthesis is available: Deshpande et al (11) have shown that, in Xenopus oocytes, α-2u-globulin mRNA with a short poly(A) tract is translated less efficiently than the same mRNA possessing a long poly(A) tract. Numerous workers, using different experimental systems, have also observed a correlation between polysome size in vivo and the degree of polyadenylation of the corresponding mRNAs (30-32). Finally, experiments from this lab have shown that, in early development of Dictyostelium discoideum, the translational apparatus discriminates against pre-existing vegetative mRNAs and that this correlates with a rapid shortening of the poly(A) tails of these mRNAs (33).

In this paper we have used an independent approach to assess the possibility that poly(A) might be involved in protein synthesis. We reasoned that if the poly(A) tract of mRNA did participate in some step in translation then purified poly(A) might competitively inhibit the in vitro translation of poly(A)$^+$ mRNAs, much in the same way as purified 5'-CAP structures competitively inhibit in vitro translation of capped mRNAs (34,35). Experiments by Lodish and Nathan (36) have previously shown that many polynucleotides, including poly(A), can inhibit the initiation of protein synthesis in reticulocyte lysates. Although different polymers varied greatly in their inhibitory activity, the fact that many different polymers did inhibit translation led these authors to suggest that their inhibitory activity was due to a generalized interaction between polyanions and the ribosome which led to an overall reduction in initiation efficiency. The experiments of this paper have focused on poly(A)-mediated inhibition. Our results are not consistent with a generalized effect on the ribosome, but rather suggest that the poly(A) tract of mRNA has a function in protein synthesis that is competitively inhibited by exogenously added poly(A).

MATERIALS AND METHODS

a) In vitro Protein Synthesis

mRNA-dependent reticulocyte lysates were prepared and utilized for in vitro translation as described previously (37,38). To ensure linearity of the assays, reactions were incubated at 37^{o}C for only 20 minutes and were programmed with subsaturating concentrations of mRNA and mRNPs. Reticulocyte lysate was always the last reaction component added prior to

incubation. Protein synthesis was measured as the incorporation of ^{35}S-methionine into alkali-resistant, acid-precipitable material. All measurements of incorporation were the average of duplicate determinations (2 ul aliquots) corrected for the incorporation observed in reactions lacking added RNA. Reactions programmed with RNA, but not supplemented with poly(A) (or other polymers) were considered control reactions and incorporation in polymer-supplemented reactions was expressed as a percentage of the incorporation in these controls. Incorporation in control reactions was routinely in the range of 15-50,000 cpm per 2 ul aliquot. Analysis of translation products by SDS-polyacrylamide gel electrophoresis employed the gel procedures of Laemmli (39) and Studier (40) and the fluorographic methods of Laskey and Mills (41).

b) mRNA and mRNPs

Unless noted otherwise, Dictyostelium whole cell RNA, isolated as described previously (38), was used as the source of poly(A)$^+$ RNA. As we have shown elsewhere, greater than 95% of the activity of this RNA is identical to that of purified poly(A)$^+$ RNA and it is thus a suitable substitute (38). Poly(A)$^-$ Dictyostelium RNA was the flow-through fraction from a poly(U)-Sepharose fractionation (38) of whole cell RNA. As we have previously shown (38), this RNA codes predominantly for histones and is devoid of poly(A). Vesicular stomatitis virus (VSV) (Indiana serotype) mRNAs were a generous gift from Dr. Jack Rose (Salk Institute) and were synthesized in vitro from viral cores incubated in the presence of cell extract and S-adenosyl methionine (42). These mRNAs are full-length and properly capped and methylated in vitro, but only about half of them are polyadenylated (60 and J. Rose, personal communication). By gel electrophoresis the respective poly(A)$^+$ and poly(A)$^-$ species of each mRNA can be visualized as discrete bands differing only in the size of their poly(A) tracts (J. Rose, personal communication). Poly(A)$^+$ and poly(A)$^-$ VSV mRNAs were separated by poly(U)-Sepharose chromatography (38). Reoviral mRNAs were synthesized in vitro from purified viral cores and were the generous gift of Dr. Dennis Drayna (Harvard Medical School). Chinese hamster ovary (CHO) cell mRNA was the total RNA recovered from phenol-chloroform extraction (38) of purified mRNPs. The latter were obtained by purifying CHO polysomes through discontinuous sucrose gradients and subsequently fractionating washed, EDTA-dissociated polysomes on 15-40% linear sucrose gradients. Fractions containing mRNPs were selected according to the procedures of Kumar and Pederson (43) and concentrated by

ultracentrifugation. CHO mRNPs and mRNA were a generous gift from Dr. Richard Manrow (University of Massachusetts Medical Center).

c) Ribopolymers

Poly(A), poly(C) and poly(U) were obtained from Miles Laboratories and poly(G) was obtained from Collaborative Research. Sigma was a suitable alternative source of poly(A). All ribopolymers were dissolved in sterile distilled water and stored at -20°C. Extinction coefficients provided by the respective manufacturers were used to obtain accurate measurements of polymer concentration. Poly(A) samples of reduced size were prepared by limited alkaline hydrolysis of the Miles product. $Poly(A)_{200}$ was prepared by incubating a 1 mg/ml solution of poly(A) in 0.18 N NaOH at 15°C for 5 minutes and $poly(A)_{30}$ was prepared by incubation of the original solution in 0.18 N NaOH at 37°C for 60 minutes. In both cases hydrolysis was terminated by neutralizing the solutions with HCl. Size estimates for all polymers were determined by electrophoresis in denaturing formaldehyde-agarose gels (44) after labeling 5'-ends with polynucleotide kinase and γ-$AT^{32}P$ (45). Molar ratios of free poly(A) to poly(A) on mRNA were derived from these size determinations and knowledge of the percentage of mRNA in a preparation of Dictyostelium whole cell RNA (5%) (38), the average size of a Dictyostelium mRNA (1200 nucleotides) (46) and the steady-state size of Dictyostelium poly(A) tracts (60-65 nucleotides) (38).

RESULTS

To test the hypothesis that poly(A) might be involved in protein synthesis we have incubated rabbit reticulocyte extracts with heterologous mRNAs and examined the consequences of also adding purified poly(A) to such extracts. Protein synthesis was monitored by following the incorporation of ^{35}S-methionine. The experiments of Figure 1 assess the effects of various ribopolymers on protein synthesis directed by total $poly(A)^+$ mRNA from Dictyostelium discoideum. Figure 1 shows that there is a dose-dependent inhibition of protein synthesis by exogenously added poly(A) that is not observed with poly(G). Poly(U) and poly(C) also inhibit protein synthesis to some extent, but are much less effective than poly(A). With poly(A), 50% inhibition is achieved at a molar ratio of competitor poly(A) to poly(A) in mRNA of approximately 1:1. Inhibition by poly(A) results from a reduction in the overall rate of protein synthesis (Figure 2) and this reduction affects virtually all of the major translation products from Dictyostelium $poly(A)^+$ mRNA (Figure 3, lanes H and I). Figure 3 also shows that

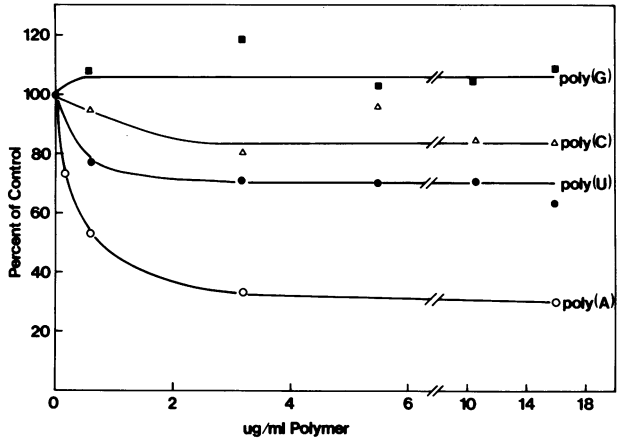

Figure 1. Inhibition of Protein Synthesis by Ribopolymers.
Translation in mRNA-dependent extracts was programmed by Dictyostelium mRNA (3 ug whole cell RNA/25 ul reaction). All reactions were identical except for their ribopolymer content. Each point is the average of duplicate determinations.

poly(A)-mediated inhibition does not affect the average size of the polypeptides synthesized in vitro. This suggests that poly(A) inhibits the initiation of protein synthesis, a conclusion consistent with previous studies (36).

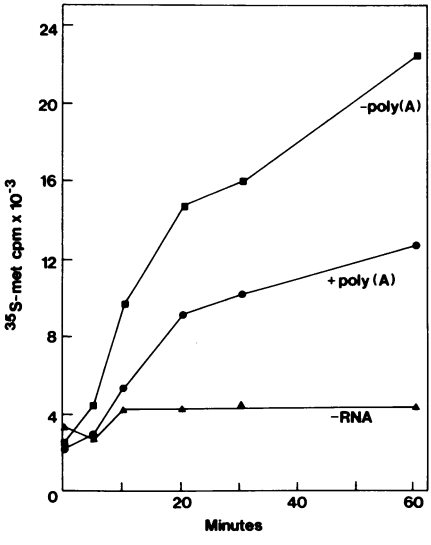

Figure 2. Rate of Protein Synthesis in the Presence and Absence of Poly(A).
Three 50 ul translation reactions respectively contained 4 ug Dictyostelium RNA ("-poly(A)"), 4 ug Dictyostelium RNA + 0.4 ug poly(A) ("+poly(A)") and no added RNA or poly(A) ("-RNA"). At the designated intervals 2 ul aliquots were withdrawn and processed to determine incorporation of ^{35}S-methionine. Each point is an average of duplicate determinations.

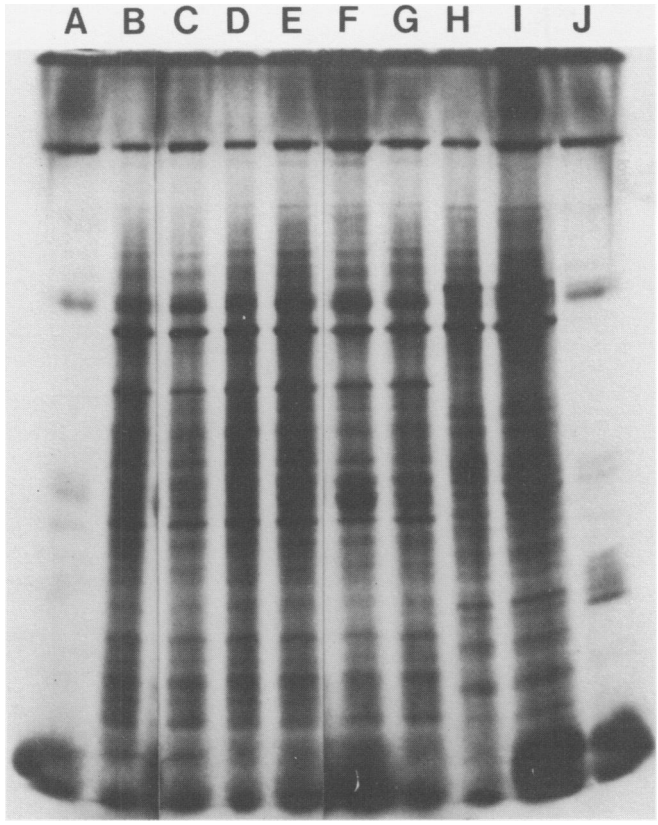

Figure 3. Proteins Synthesized in the Presence and Absence of Poly(A).
Aliquots of the translation reactions depicted in Figure 5 were fractionated on an SDS-polyacrylamide gel which was subsequently fixed and fluorographed. Lanes B-I contain approximately equivalent amounts of acid-precipitable ^{35}S-methionine whereas lanes A and J contain approximately half the number of cpm of lanes B-I. Lane A - no added RNA; Lane B - CHO mRNA, no poly(A); Lane C - CHO mRNA + poly(A) at 3.2 ug/ml; Lane D - CHO mRNA + poly(A) at 0.13 ug/ml; Lane E - CHO mRNP, no poly(A); Lane F - CHO mRNP + poly(A) at 16 ug/ml; Lane G - CHO mRNP + poly(A) at 3.2 ug/ml; Lane H - Dictyostelium mRNA - no poly(A); Lane I - Dictyostelium mRNA + poly(A) at 16 ug/ml; Lane J - no added RNA.

The effects of poly(A) are not limited to proteins synthesized by Dictyostelium poly(A)$^+$ mRNAs. We have observed comparable inhibition of protein synthesis directed by poly(A)$^+$ mRNAs isolated from Chinese hamster ovary (CHO) cells (Figure 4B and Figure 3, lanes B-D) and poly(A)$^+$ mRNAs of vesicular stomatitis virus (Figure 5). However, the effects of adding

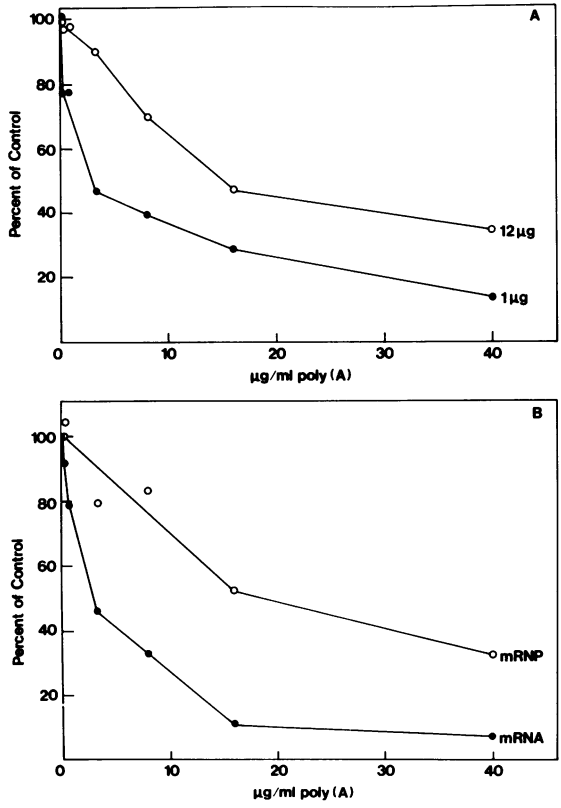

Figure 4.
A. Effects of Poly(A) on the Translation of Different Amounts of RNA.
In vitro translation was carried out as in the experiments of Figure 1. The two reactions differed only in their content of Dictyostelium RNA: "1 ug" - 1 ug of RNA/25 ul reaction; "12 ug" - 12 ug of RNA/25 ul reaction.
B. Effects of Poly(A) on the Translation of CHO mRNA and mRNPs.
In vitro translation was carried out as in the experiments of Figure 1 except that CHO mRNA and mRNPs replaced the Dictyostelium mRNA. "mRNA" - 2 ug CHO RNA added to each 25 ul reaction; "mRNP" - that amount of CHO mRNPs containing 2 ug RNA was added to each 25 ul reaction.

poly(A) to reticulocyte lysates are significantly different when the extracts are programmed by poly(A)$^-$ mRNAs. Translation of Dictyostelium poly(A)$^-$ mRNAs (which code predominantly for histones (38)) is stimulated by low concentrations of poly(A) and inhibited only by considerably higher doses of poly(A) than required for adenylated mRNAs (Figure 6). To control against the possibility that such results are due to intrinsic differences

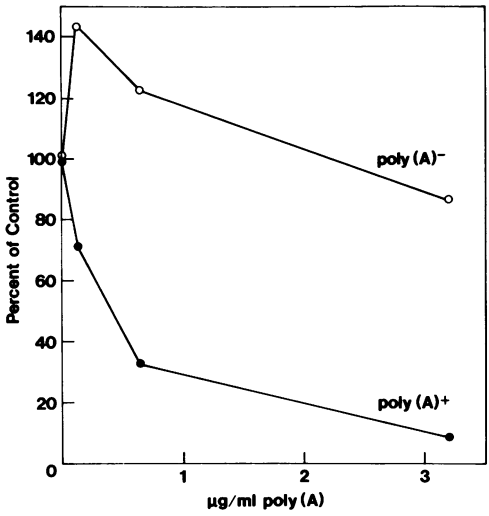

Figure 5. Comparison of the Effects of Poly(A) on the Translation of Poly(A)$^+$ and Poly(A)$^-$ Vesicular Stomatitis Virus mRNAs.
In vitro translation was carried out as in the experiments of Figure 1. The poly(A)$^+$ reactions contained 0.15 ug of VSV poly(A)$^+$ mRNA and the poly(A)$^-$ reactions contained 0.44 ug of VSV poly(A)$^-$ mRNA. In the absence of inhibitors, these represent RNA concentrations of equivalent translation activity. The data represent the average of two independent experiments.

in the efficiency of initiation by the two mRNA populations (47) we have also examined the effect of poly(A) on the translation of poly(A)$^+$ and poly(A)$^-$ vesicular stomatitis virus (VSV) mRNAs. These two mRNA samples are identical except for their poly(A) content and direct the in vitro translation of the same VSV polypeptides, although the poly(A)$^+$ mRNAs are translated approximately 2.5-3.0 times more efficiently than the poly(A)$^-$ mRNAs in reticulocyte lysates (60; Favreau and Jacobson, unpublished observations; J. Rose, personal communication.). The difference in relative translatability of the two mRNA samples is consistent with the experiments

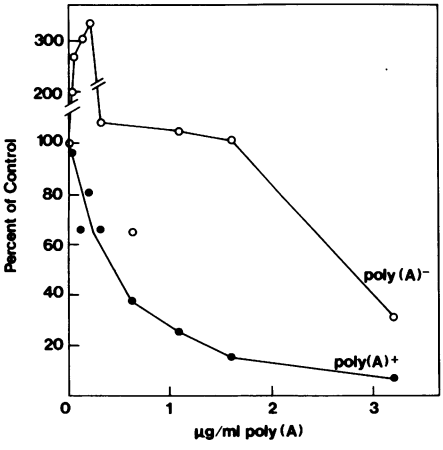

Figure 6. Comparison of the Effects of Poly(A) on the Translation of Poly(A)$^+$ and Poly(A)$^-$ Dictyostelium mRNAs.
In vitro translation was carried out as in the experiments of Figure 1 except that the two reactions respectively contained 1 ug of Dictyostelium whole cell RNA ("poly (A)$^+$") or 1 ug of a poly(U)-Sepharose flow-through of that same RNA ("poly(A)$^-$").

of Doel and Carey (29). Figure 5 shows that translation of VSV poly(A)$^+$ and poly(A)$^-$ mRNAs is affected by exogenously added poly(A) in much the same way as the translation of Dictyostelium poly(A)$^+$ and poly(A)$^-$ mRNAs. The only significant differences are that translation of VSV poly(A)$^-$ mRNAs is stimulated less by low poly(A) concentrations than is the translation of Dictyostelium poly(A)$^-$ mRNAs and that VSV poly(A)$^-$ mRNAs appear to be more resistant to inhibition by added poly(A). However, this latter difference is undoubtedly due to the large difference in the concentration of the respective poly(A)$^-$ mRNAs in the two experiments. The experiment depicted in Figure 5 used equivalent translation activities of poly(A)$^+$ and poly(A)$^-$VSV mRNAs, which amounted to 2.9 times more poly(A)$^-$ mRNA than poly(A)$^+$ mRNA. The difference in dose response to exogenously added poly(A) by the VSV poly(A)$^+$ and poly(A)$^-$ mRNAs (Figure 5) can only be partially accounted for by differences in the respective concentrations of the two mRNAs (see below) since there was approximately a 20-fold difference in the poly(A) concentration required to achieve 50% inhibition of translation of the two mRNA samples (data not shown).

The inhibition of translation mediated by poly(A) can be overcome by increasing the relative concentration of poly(A)$^+$ mRNA, by translating mRNPs rather than mRNAs and by reducing the size of the exogenously added poly(A). Figure 4A shows that a twelve-fold increase in mRNA concentration leads to a comparable shift in the poly(A) dose required for 50% inhibition of protein synthesis. Figure 4B shows that CHO mRNPs are considerably more resistant to poly(A) mediated inhibition of translation than the equivalent amount of deproteinized RNA. Figure 3 (lanes B and E) shows that the CHO mRNAs and mRNPs are making the same proteins and that the mRNPs are not more resistant to poly(A) treatment by virtue of the selective translation of a subset of the mRNAs (lanes E-G).

The poly(A) used in the above inhibition experiments is heterogenous in size, ranging primarily from 200-400 residues in length (data not shown). By limited treatment with alkali it was possible to produce preparations which averaged 200 and 30 residues, respectively. Figure 7 shows that poly(A)$_{200}$ is as potent an inhibitor of translation as the poly(A)$_{200}^+$ sample. However, Figure 7 also shows that the shorter poly(A) sample, poly(A)$_{30}$, is inactive as an inhibitor and, in fact, stimulates translation to some extent. Since the polymers are used at equivalent weights, the inactivity of poly(A)$_{30}$ is observed even at 6-7 fold molar excess. It

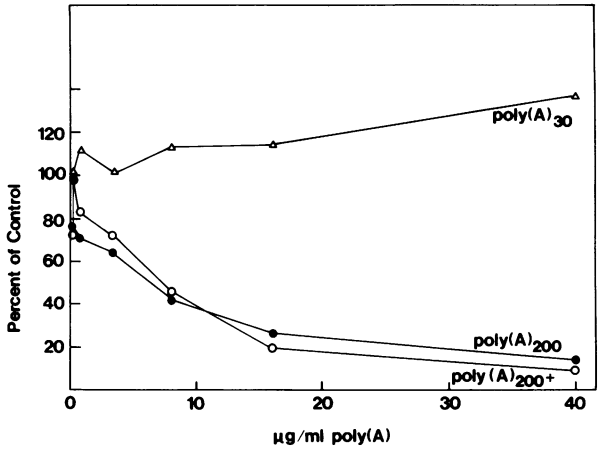

Figure 7. Poly(A) Size Affects its Inhibitory Activity.
Poly(A) was hydrolyzed as described in the Materials and Methods to yield a fraction averaging 200 nucleotides in length and a fraction averaging 30 nucleotides in length. These two poly(A) samples and the original, unhydrolyzed poly(A) ("poly(A)$_{200+}$") were used to inhibit protein synthesis as in Figure 1. Dictyostelium whole cell RNA was used in all reactions at 2 ug/25 ul reaction.

should be noted that the poly(C) and poly(U) samples used in the experiments of Figure 1 also ranged from 200-400 nucleotides in length and that the poly(G) used in Figure 1 was considerably shorter (approximately 30 nucleotides long) (M. Schmidt and A. Jacobson, unpublished observations). However, in other experiments, gel-purified poly(G) samples of approximately 100 and 200 nucleotides in length showed the same lack of inhibitory activity as the shorter poly(G) sample (M. Schmidt and A. Jacobson, unpublished observations). Therefore, the weak inhibitory activity of poly(C), poly(U) and poly(G) must be attributed to properties other than size.

A comparison of Figures 1 and 6 with Figures 2, 4 and 7 suggests an inconsistency in the amount of poly(A) required to achieve 50% inhibition of protein synthesis directed by Dictyostelium mRNA. However, the experiments of Figures 1 and 6 and those of Figures 2, 4 and 7 were performed with different reticulocyte extracts and those extracts consistently differed in their respective extents of inhibition by a given amount of poly(A) (Table 1). Moreover, the extent of inhibition by poly(A) was dependent on the degree of stimulation of the two extracts by poly(A)$^+$ RNA, i.e., the extract which was more resistant to poly(A) treatment also had more

TABLE 1

TRANSLATION ACTIVITY AND POLY(A) INHIBITION OF DIFFERENT RETICULOCYTE LYSATES

Lysate #	Translation Activity (cpm, ^{35}S-methionine) poly(A)$^-$ mRNA	poly(A)$^+$ mRNA	Poly(A) Required for 50% Inhibition of Translation
1	56239	14888	1-2 ug/ml
2	69292	45629	6-7 ug/ml
Lysate 2:Lysate 1	1.23	3.06	3.0-7.0

Two different reticulocyte lysates were tested for their stimulation by either 3 ug of Dictyostelium whole cell RNA ("poly(A)$^+$") or 2 ug of reoviral mRNA ("poly(A)$^-$"). Lysate #1 was used in the experiments of Figures 1 and 6 and lysate #2 was used in the experiments of Figures 2, 4 and 7. The data represent an average of duplicate determinations corrected for incorporation in control samples lacking added RNA.

translation activity (Table 1). This enhanced activity was restricted to translation of poly(A)$^+$ mRNAs; the two extracts had virtually equivalent activity for the translation of poly(A)$^-$ reoviral mRNAs (Table 1).

DISCUSSION

It has been previously shown that poly(A) and other polynucleotides can inhibit translation in extracts prepared from wheat germ or rabbit reticulocytes (31,36,48,49). The fact that many different polynucleotides have at least some inhibitory activity led Lodish and Nathan (36) to conclude that inhibition was due to non-specific interactions with the ribosome which reduced the overall efficiency of the initiation of protein synthesis. In the experiments of this paper we have also found that poly(A) inhibits translation in reticulocyte extracts and that such inhibition is partially manifested by other ribopolymers. However, our conclusions differ from those of Lodish and Nathan (36). While our results do not rule out the postulated mechanisms for the general inhibitory effects of polynucleotides at high concentrations they do suggest that poly(A)-mediated inhibition, which occurs at much lower concentrations, might be more suitably explained in terms of competition with the poly(A) tract on mRNA. We have found that inhibition by poly(A) preferentially affects the rate of translation of poly(A)$^+$, rather than poly(A)$^-$, mRNAs and that such inhibition can be overcome by increased concentrations of mRNA or by translating mRNPs rather than mRNA. Moreover, we find that the extent of inhibition observed is

dependent on both the translation activity which a given lysate has for poly(A)$^+$ mRNA as well as the size of the competitor poly(A). This size dependence of the poly(A) effect is further evidence that poly(A) acts as a specific, as opposed to generalized, inhibitor of protein synthesis.

Our interpretation of these results is that the poly(A) tracts of mRNAs are somehow involved in translation and that addition of purified poly(A) to reticulocyte lysates titrates away some component in the lysate which would otherwise have interacted with these mRNA poly(A) tracts. Since the translation of mRNPs is more resistant to poly(A)-mediated inhibition than the translation of mRNAs, it is likely that the component of the lysate which interacts with poly(A) is also present in mRNPs. A good candidate for this component would be the ubiquitous cytoplasmic poly(A)-binding protein (PABP) (50-54). Binding of the PABP to mRNA must require a minimal length of poly(A) (59), consistent with the inhibitory activity of large, but not small poly(A). Successful binding of the PABP to poly(A) could facilitate an interaction with an initiation factor, the ribosome, another mRNP protein or the cytoskeleton.

Several different experimental approaches have previously implicated a role for poly(A) in the determination of mRNA stability (3-9) and it must, therefore, be asked whether such results are compatible with experiments supporting a function for poly(A) in protein synthesis. It seems reasonable to postulate that the two phenomena could be indirectly related, i.e., that mRNA stability is, in part, a consequence of translational efficiency. If efficient translation is dependent on the binding of the PABP to poly(A) and this event, in turn, is dependent on a critical poly(A) length, then mRNAs with little or no poly(A) would be translated less efficiently and degraded more readily than fully adenylated mRNAs. It should be noted, however, that there are several examples of stable mRNAs which are not actively engaged in translation (55,56,61).

A function for poly(A) in protein synthesis is also compatible with previously characterized wholesale changes in the adenylation of different mRNA populations. Thus, the developmentally regulated loss of poly(A) from Dictyostelium, Xenopus or Spisula mRNAs is associated with the exclusion of some of these mRNAs from polysomes (33,55,56) and the addition of poly(A) to stored Xenopus, Spisula sea urchin and cotton seed mRNAs (55-58) is associated with the recruitment of some of these mRNAs onto polysomes. As we have noted elsewhere (33), the ability to add or delete poly(A) in a population of mRNAs offers the cell an opportunity to radically alter its

pattern of protein synthesis without a concomitant alteration in mRNA complexity.

The experiments of Figures 5 and 6 suggest that low concentrations of exogenously added poly(A) stimulate the translation of poly(A)$^-$ mRNAs. We believe that this reflects the fact that nuclease-treated reticulocyte lysates contain residual amounts of active, polyadenylated mRNAs and the fact that poly(A)$^+$ mRNAs are more efficiently translated than poly(A)$^-$ mRNAs in these extracts (29,60). In the absence of exogenously added poly(A) the residual poly(A)$^+$ RNA would, thus, be a competitor of the translation of the poly(A)$^-$ mRNA in question. However, addition of poly(A) to the extract would preferentially inhibit the translation of the poly(A)$^+$ mRNA, eliminating the competition and yielding an apparent stimulation of the translation of the poly(A)$^-$ mRNA. The differing degrees of stimulation observed in Figures 5 and 6 probably reflect the different amounts of poly(A)$^-$ RNAs in the two experiments. For example, in Figure 5, where the amount of poly(A)$^-$ mRNA is higher than in Figure 6, the initial competition from poly(A)$^+$ mRNA should be less significant and, hence, the stimulatory effect of added poly(A) should also be less.

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