Cytoplasmic processing events in the polyadenylate region of Physarum messenger RNA

ARTICLE *in* MOLECULAR BIOLOGY REPORTS · JUNE 1981

Impact Factor: 2.02 · DOI: 10.1007/BF00778735 · Source: PubMed

3 AUTHORS, INCLUDING:

William R Jeffery
University of Maryland, College Park
195 PUBLICATIONS **7,415** CITATIONS
SEE PROFILE

Daniel J Noonan
University of Kentucky
69 PUBLICATIONS **4,626** CITATIONS
SEE PROFILE

Cytoplasmic processing events in the polyadenylate region of Physarum messenger RNA

W. R. Jeffery*, D. S. Adams** & D. Noonan
Department of Zoology, University of Texas, Austin, TX 78712, USA

Abstract

Cytoplasmic processing events in the poly(A) region of mRNA from *Physarum polycephalum* are reviewed. Two classes of poly-containing RNA [poly(A)$^+$ RNA] exist in the cytoplasm. One contains very short poly(A) sequences, averaging about 15 adenylate residues, while the other contains relatively long poly(A) sequences, averaging about 60 residues. Molecules with short poly(A) sequences are found exclusively in the polysomes while those with long poly(A) sequences are restricted to the free cytoplasmic mRNP. Since proteins are associated with only the long poly(A) sequences the poly(A) · protein complex is also restricted to the free mRNP. The long poly(A) sequences are relatively short-lived. They are degraded by two distinct processes, a shortening process in which 15–20 residues are gradually removed and a turnover process in which long poly(A) tracts are rapidly converted to the short sequences. This process, along with the dissociation of the poly(A) · protein complex, occurs when poly(A)$^+$ RNA molecules located in free mRNP are transferred to the polysomes. Poly(A) · protein complex dissociation appears to preceed poly(A) turnover during translational selection. The significance of these processing events in relation to mRNA maturation is discussed.

Introduction

During the last decade considerable progress has been made in understanding the structure of mRNA and the process of its synthesis and maturation in eukaryotes. Most eukaryotic mRNA molecules contain extensive non-coding sequences which are present on either side of the codogenic region. The non-coding regions include the methylated cap structure at the 5′ terminus (1), internal sequences located adjacent to the 5′ and 3′ terminal regions (2, 3), and the 3′ terminal poly(A) tail (4). Eukaryotic mRNA molecules are also associated with specific proteins, presumably located in the non-coding regions, forming mRNP particles (5). Messenger RNA-protein complexes exist in polysomes or particles which sediment more slowly, the so-called free cytoplasmic mRNP. Messenger RNA molecules are thought to be formed from larger nuclear precursors by splicing reactions which ligate originally non-adjacent sequences to specific break points in the molecule yielding a mosaic product (6). Some of the non-coding sequences of the mRNA, namely the cap and poly(A) tail, are also added post-transcriptionally within the nucleus (1, 4). Although information is now becoming available concerning the processing of pre-mRNA molecules in the nucleus very little is currently known about the continued maturation of mRNA in the cytoplasm. For instance, the fundamental question of why mRNAs are found both in the polysomes and free mRNP has not been answered. The only cytoplasmic processing step which has been reported is the shortening process in which

* To whom correspondence should be addressed.
** Present address: The Rockefeller University, New York, N.Y. 10021 USA.

Molec. Biol. Rep. 7, 63–70 (1981). 0301-4851/81/0072-0063 $1.60.

adenylate residues are gradually removed from the poly(A) tail as the message ages (7–9). The purpose of this article is to review our recent investigations on cytoplasmic processing events in the poly(A) region of mRNA from the acellular slime mold *Physarum polycephalum*.

The biological system

There are a number of reasons why vegetative plasmodia of *Physarum* are particularly favorable subjects for studying processing events in cytoplasmic mRNA. First, they can be grown in large quantities and are a rich source of the various forms of mRNP. Second the free mRNP from this creature are smaller than those of higher eukaryotes and consequently can be completely separated from the polysomes by differential centrifugation. Finally, and perhaps most significantly, the plasmodia are macroscopic. This attribute allows the fate of an mRNP type to be directly tested by microinjection. Due to the syncytial nature of the protoplasm and the occurance of cytoplasmic streaming the microinjected mRNP readily diffuse through the plasmodium.

Two classes of poly(A) exist in cytoplasmic RNA

Two classes of poly(A)$^+$ RNA, distinguishable on the basis of the size of their poly(A) sequences, exist in *Physarum* cytoplasmic RNA. As shown in Figure 1A, if the total cytoplasmic RNA obtained

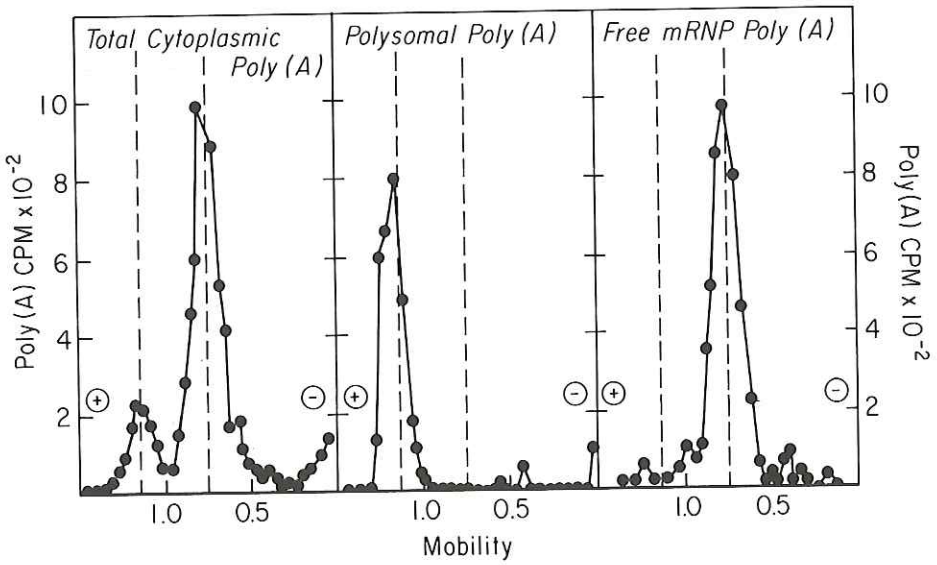

Fig. 1. Polyacrylamide gel electrophoresis of poly(A) sequences in cytoplasmic RNA. Left) Total cytoplasmic RNA, Center) Polysomal RNA, Right) RNA from free mRNP. Cultures were labeled for 2 hrs. with [^{3}H]-adenosine, harvested, and homogenized as described previously (10). The post-mitochondrial supernatant (total cytoplasmic fraction) was fractionated by differential centrifugation into a pellet containing the polysomes and another supernatant fraction containing the free mRNP. This was accomplished by layering the post-mitochondrial supernatant over 15 ml of 20% sucrose dissolved in 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 100 mM $MgCl_2$ and centrifuging at 27000 rpm in a Beckman T30 rotor at 4° for 150 min. The free mRNP which remains in the supernatant was recovered by another cycle of centrifugation as given above but for 16 hr. RNA was isolated from the total cytoplasmic fraction, the polysomal fraction, and the free mRNP by phenol extraction (10). Poly(A) sequences were prepared from the RNA by treatment with a mixture of 5 μg/ml pancreatic RNase A and 1 μg/ml T_1 RNase for 1 hr in the presence of 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 1 mM $MgCl_2$ (on ice). The poly(A) sequences were recovered by oligo (dT)-cellulose chromatography, concentrated by Millipore filtration, and loaded on polyacrylamide gels (12). Following electrophoresis the gels were sliced, RNA was eluted from the slices, and the eluate was passed through glass fiber filters impregnated with poly(U) in order to measure the radioactivity in poly(A). In each panel of the figure the left-hand vertical, dashed line represents the average mobility of the smaller poly(A) sequence class while its right-hand counterpart represents that of the larger poly(A) sequence class. The mass average poly(A) size was determined by a comparison to the mobility of poly(A) standards on separate gels. A mobility of 1.0 represents a migration distance of 4.8 ± 0.4 cm through the gel toward the anode.

by phenol extraction from a culture labelled for 2 hours with [^{3}H]-adenosine is digested with pancreatic RNase A and the resistant fragments are analyzed by polyacrylamide gel electrophoresis, two classes of poly(A) sequences can be detected. The longer class showed a mass average size of about 65 nucleotides and a size range of about 35–100 nucleotides while the shorter class averaged about 15 nucleotides and ranged from about 5 to 25 nucleotides. The long and short poly(A) sequences are associated with different RNA molecules since they first appear in the cytoplasm at different times after the beginning of the [^{3}H]-adenosine labelling period. The RNA molecules containing long poly(A) sequences appear in the cytoplasm after 20 min of labelling while those containing short poly(A) appear gradually between 20 and 60 min of labeling (10).

In order to determine the subcellular distribution of RNA molecules containing long and short poly(A) sequences the polysomes and free mRNP were separated by differential centrifugation, poly(A) sequences were prepared from RNA extracted from each class of cytoplasmic particle, and poly(A) size was measured using polyacrylamide gels (Fig. 1B, C). Poly(A) prepared from the polysomes comigrated exclusively with the short poly(A) sequences whereas that derived from the free mRNP showed the same electrophoretic mobility as the longer poly(A) sequences. In order to determine the relative proportion of cytoplasmic poly(A)$^+$ RNA containing long and short poly(A) sequences the poly(A) titer of polysomes and free mRNP was measured by the [^{3}H]-poly(U) binding assay (11). This allows the proportion of poly(A)$^+$ RNA in a particular fraction to be estimated at steady state if the average poly(A) tract length is known and it is assumed that only one poly(A) segment exists per molecule. By this method it was demonstrated that about 30% of the poly(A)$^+$ RNA molecules of the cytoplasm contain the long poly(A) sequences (12). This proportion is in general agreement with the proportion of mRNA which commonly exists as free cytoplasmic mRNP in other eukaryotic cells (13, 14).

Table 1. Micrococcal nuclease sensitivity of poly(A)-containing components derived from the polysomes and free mRNP.

Poly(A)$^+$ RNA fraction	Total	RNase-released components	Phenol-extracted poly(A)
Polysomes	6%	6%	0%
Free mRNP	77%	78%	0%

The polysomal and free RNP was isolated from cultures labeled with [^{3}H] adenosine for 2 hr. The poly(A)-containing components derived from this material by RNase treatment or their phenol-extracted RNA counterparts were divided into two 0.5-ml fractions, one of which was incubated with 200 μg/ml micrococcal nuclease in the presence of 20 mM $CaCl_2$ while the other remained untreated. At various intervals during an incubation period at 4 °C, aliquots were removed from each fraction, the digestion was terminated by the addition of 20 mM EGTA, and the radioactivity remaining in poly(A) was determined by the proteinase K-poly(U) filtration assay (see the legend of Figure 2).

The restriction of the poly(A) · protein complex to cytoplasmic RNA with long poly(A)

Poly(A) sequences in eukaryotic mRNP are associated with a specific set of proteins forming a subparticle at the 3′ terminus of the message. This poly(A) · protein complex can be isolated, usually as a particle which sediments at about 8–15S, after the mRNP is treated with RNase (11, 15). In order to determine whether both classes of cytoplasmic poly(A)$^+$ RNA molecules contain a poly(A) · protein complex, poly(A)-associated components released from the polysomes and free mRNP were exhaustively digested with micrococcal nuclease. Free poly(A) tracts are hydrolyzed under the standard conditions selected while protein associated poly(A) sequences should show a great degree of resistance. The poly(A)-containing fragments released from the polysomes, like pure poly(A) tracts, were completely digested by micrococcal nuclease while about 80% of the sequence derived from the free mRNP was resistant to the enzyme. The partial resistance of the sequences from free mRNP was not due to any special property of their polynucleotide moiety since poly(A) prepared from this fraction by phenol extraction could be entirely digested (Table 1). It is possible that poly(A) · protein complexes cannot be released from the polysomes by RNase due to conformational differences within the free cytoplasmic mRNP. In order to test this possibility, the polysomes and free mRNP were directly treated with micrococcal nuclease and the proportion of resistant poly(A) was measured. The same results were obtained (Table 1); polysomal

poly(A) was hydrolyzed while poly(A) sequences in the free mRNP were largely protected. These results suggest that the polysomal poly(A)$^+$ RNA does not contain a poly(A) · protein complex.

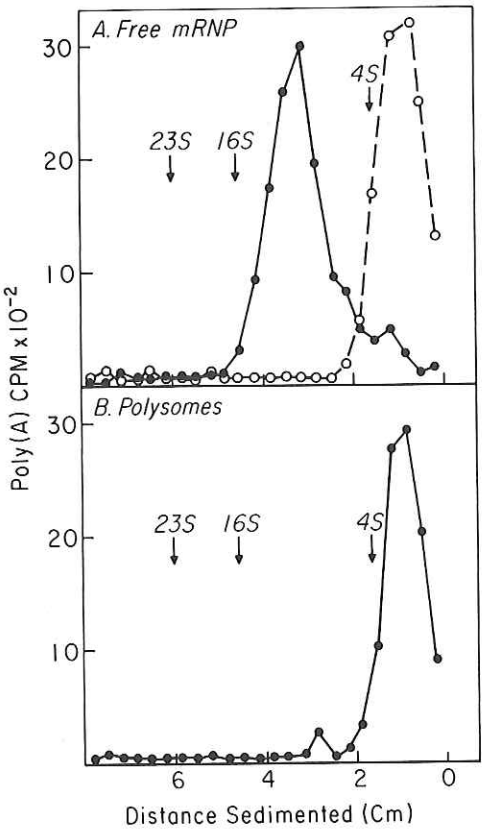

Fig. 2. Zone sedimentation of poly(A)-containing components derived from A) the free mRNP and B) the polysomes. Polysomes and free mRNP fractions were isolated from exponentially growing cultures labeled for 2 hr with [^{3}H]-adenosine by differential centrifugation as described in the legend of Figure 1. For the preparation of poly(A)-containing components each fraction was brought to 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, 1 mM $MgCl_2$, 20 mM EDTA and treated with a mixture of 5 μg/ml pancreatic RNase A and 1 μg/ml T_1 RNase for 1 hr on ice. Ribosomes were removed from the digest by centrifugation (12) and the supernatants (●—●) were centrifuged through 20–30% linear sucrose density gradients dissolved in 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 1 mM $MgCl_2$ for 16 hrs at 35000 rpm. In some gradients the supernatant was treated with 500 μg/ml proteinase K for 1 hr at 37 °C prior to sedimentation (O---O). The position of poly(A)-containing components in the gradient was determined by treating each fraction with proteinase K (4°; 12–16 hr) and then passing the digests through poly(U)-glass fiber filters. In the figure the markers used (arrows) represent *E. coli* rRNA and tRNA centrifuged on parallel gradients.

Further evidence for the absence of a poly(A) · protein complex in the polysomes was obtained by zone sedimentation (Figure 2). The components released from the free mRNP by RNase treatment sedimented as 8–15S particles. The presence of proteins in these particles was confirmed by treating them with proteinase K prior to sedimentation. This converted the 10–15S complex to a 2–4S component, similar in sedimentation velocity to pure poly(A) (Fig. 2A). In contrast, RNase treatment of the polysomes did not yield a large, protein containing particle (Fig. 2B). Instead a component which co-sedimented with uncomplexed poly(A) was obtained. We conclude from this that poly(A) · protein complexes are restricted to poly(A)$^+$ RNA molecules bearing the larger poly(A) sequences found only in the free mRNP.

The turnover of large poly(A) sequences in cytoplasmic poly(A)$^+$ RNA

Pulse-chase labeling experiments were conducted in order to determine whether age-related modifications occur in the poly(A) sequences of poly-somal and free mRNP. In these experiments cultures were labelled for two hours with [^{3}H]-adenosine, chased, and the size of poly(A) sequences at various intervals during the chase period was determined by gel electrophoresis. As shown in Figure 3 both the average size and the total amount of the large poly(A) sequences gradually decreased during the chase period. We estimate that the large sequences were shortened by 15–20 nucleotides and that about 95% of their total radioactivity disappeared from the cytoplasm after 8 hr (10). The smaller poly(A) sequences, in contrast, did not appear to be altered in size or quantity during the same time interval. Since translational repression has been reported to suppress poly(A) shortening in mammalian cells (8, 16) it was of interest to determine if this would also affect the metabolism of large poly(A) in *Physarum* RNA. Cycloheximide and a 37° heat shock were used to inhibit protein synthesis. As shown in Figure 3 neither cycloheximide treatment nor the application of a heat shock during the case prevented shortening. The loss of poly(A) radioactivity, however, behaved differently. It was suppressed in cycloheximide-treated cultures but not in those exposed to a heat shock (Fig. 3). The results suggest

Table 2. Micrococcal nuclease sensitivity of poly(A)-containing components after microinjection of labeled free mRNP into host plasmodia.

	Original injected free mRNP	Polysomes	Endogeneous free mRNP
Percent micrococcal nuclease resistant poly(A)	76.2	13.3	87.6

Micrococcal nuclease digestions were conducted on poly(A)-containing components released from the various fractions by RNase treatment as described in Table 1.

that two independent degradation processes occur in the large poly(A) sequences (10). The first, a shortening process, is responsible for the gradual shortening of the large poly(A) by 15–20 nucleotides. As also shown by others this process is independent of concomitant protein synthesis (7, 9). The second process is a more extensive one in which large poly(A) sequences rapidly disappear from the cytoplasm without any detectable products larger than the short poly(A). The resistance of this process to heat shock, a manipulation which blocks translation in *Physarum* (17), suggests it does not

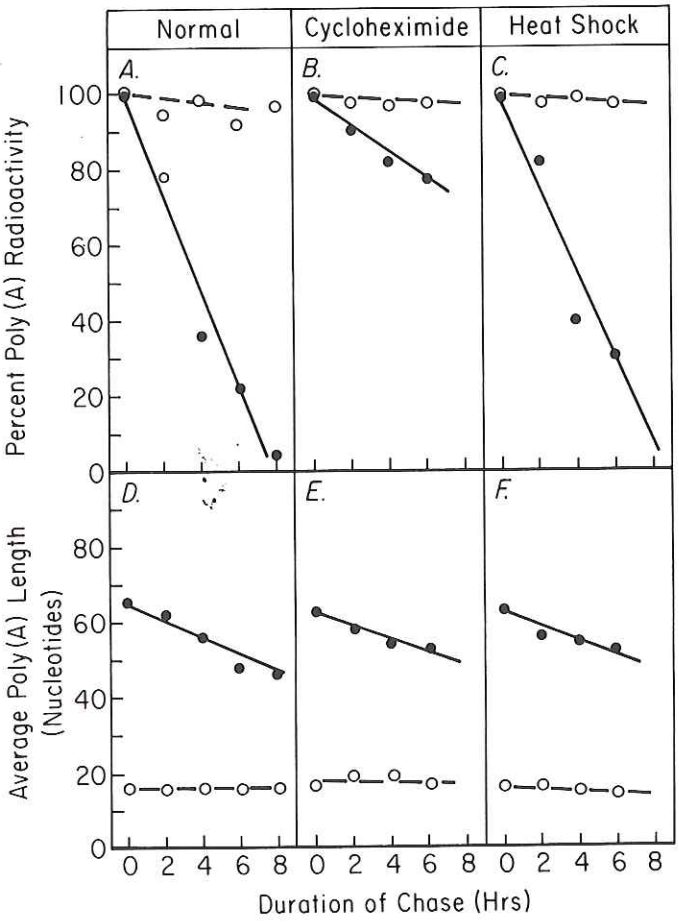

Fig. 3. The effects of translational inhibition on size reduction and turnover of large and small poly(A) in the cytoplasmic RNA. Cultures were labeled for 2 hrs with [^{3}H]-adenosine and then chased as described previously (10). A) Normal chase conditions, B) A chase conducted in the presence of 20 μg/ml cycloheximide, and C) A chase conducted during a 37° heat shock. Poly(A) preparation and gel electrophoresis were conducted as described as in the legend of Figure 1. The original gels may be seen in Reference 10. A-C) Total poly(A) radioactivity as estimated from the area of each poly(A) peak on the gels. D-F) Mass average poly(A) size. Open circles represent the smaller poly(A) class while closed circles represent the large poly(A) class.

depend on the synthesis of new proteins *per se*. On the other hand, its sensitivity to cycloheximide suggests it requires polypeptide translocation.

The metabolic relationship between long and short poly(A) in cytoplasmic RNA

The rapid turnover of the large poly(A) sequence and its exclusive presence in RNA molecules associated with the free mRNP suggest that it may be a precursor to the small poly(A) observed in polysomal RNA. It is also possible, of course, that the free mRNP is not a precursor to polysomal mRNA and that it, along with its large poly(A) sequence, is degraded in the cytoplasm without translational function. In order to decide between these possibilities we have developed a method in which isolated free mRNP, labeled *in vivo*, can be microinjected into an unlabeled host plasmodium and structural alterations in its poly(A) region can be directly monitored. About 60–80% of the microinjected poly(A)$^+$ RNA can be detected in the host plasmodium an hour after microinjection. A substantial proportion of this material (about 70%) is transferred to the host polysomal fraction by the normal translational steps (18). When the size of the labeled poly(A) sequences which entered the polysomes was determined it was discovered that most of them showed a number average size similar to that of the endogenous polysomal poly(A) (Fig. 4). Since the microinjected components originally contained large poly(A) sequences this experiment shows very clearly that the large poly(A) sequences of free mRNP are converted to small poly(A) sequences when they enter the polysomes. It also demonstrates that a large proportion of the free mRNP are the potential precursors to polysomal mRNA.

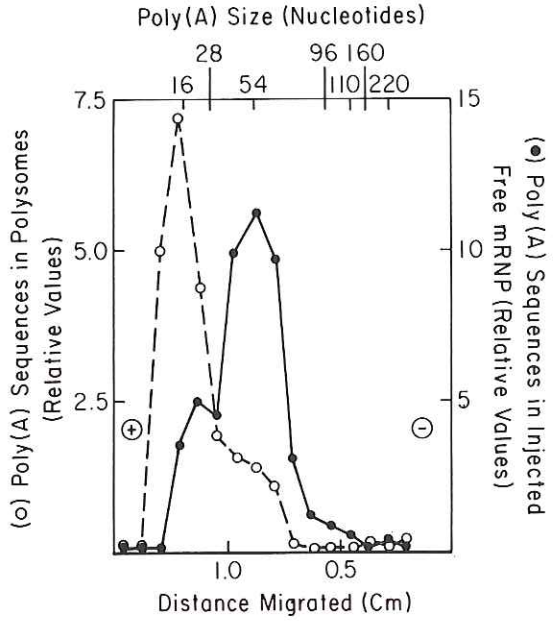

Fig. 4. Reduction of poly(A) size following the incorporation of microinjected free mRNP into the polysomes of host plasmodia. Free mRNP, prepared by differential centrifugation from plasmodia labeled with [^{3}H]-adenosine as described in the legend of Figure 1, was microinjected into unlabeled host plasmodia. The hosts were incubated for 1 hr. at 25° after which the polysomes were isolated (18), the size of labeled polysomal poly(A) was determined by gel electrophoresis, and the position of the labeled poly(A) on the gels was determined by poly(U) filtration (see the legend of Figure 1 for details). In the figure the closed circles (•—•) represent the number average size of poly(A) tracts in the microinjected free mRNP while the open circles (O—O) represent the number average size of poly(A) tracts in the microinjected RNA which entered the polysomes.

Since poly(A) · protein complexes are restricted to the free mRNP the turnover of poly(A) sequences demonstrated above brings up the possibility that poly(A)-associated proteins may be dissociated during the transfer of mRNA to the polysomes. In order to resolve this issue the sensitivity of poly(A) from the polysomes and the post-polysomal fraction of microinjected plasmodia to micrococcal nuclease digestion was tested. Labeled poly(A) sequences from the post-polysomal fraction, like those originally present in the microinjected free mRNP, were quite resistant to the enzyme. On the other hand, those poly(A) sequences which entered the polysomes were almost entirely hydrolyzed (Table 2). This shows that two structural changes, extensive poly(A) turnover and poly(A) · protein complex dissociation, occur during the incorporation of mRNA to the polysomes.

Two major possibilities exist for the temporal sequence of these changes. Poly(A) turnover may occur first, thus indirectly causing protein dissociation due to the absence of a sufficiently long protein binding site in the poly(A) tract, or protein dissociation may be the initial step leading to hydrolytic destruction of most of the exposed poly(A). We used the earlier finding that cycloheximide treatment blocks poly(A) turnover (Figure 3) to in-

vestigate the temporal relationship between the structural changes in the 3′ region of mRNA. Cultures were labeled with [^{3}H]-adenosine for 2 hours then chased in the presence of cycloheximide. The cytoplasmic poly(A)-containing components were isolated at various times during the chase and their sensitivity to micrococcal nuclease was tested. The resistance of the large poly(A) sequences gradually decreased during the cycloheximide chase. After about 6 hours only about 30% of its original radioactivity remained unhydrolyzed. No decrease in micrococcal nuclease sensitivity was observed in parallel cultures chased without cycloheximide. The production of micrococcal nuclease sensitive poly(A) sequences while poly(A) turnover was blocked implies that protein dissociation preceeds poly(A) turnover during the incorporation of mRNA into the polysomes.

Processing events in the poly(A) region of cytoplasmic RNA

The experiments discussed here indicate that extensive structural alterations occur in the poly(A) region of *Physarum* mRNA during its cytoplasmic maturation and incorporation into the polysomes. These events are summarized in Figure 5. They include the gradual hydrolysis or shortening of poly(A) tracts previously described in eukaryotic cells (7–9, 19, 20) and two newly discovered phenomena, the dissociation of the poly(A) · protein complex and the rapid turnover of most of the poly(A) sequence. The free mRNP microinjection experiment unambiguously shows that the latter two events occur when mRNA molecules are transferred from the free mRNP to the polysomes. Presumably events like these occur when any form of mRNA containing long poly(A) sequences enters the cytoplasm and is engaged in translation. We have only been able to detect the poly(A) shortening process, as defined by its gradual effects and its insensitivity to protein synthesis inhibitors (10), in the free mRNP. Thus at present it cannot be ascertained whether or not shortening may trigger the more extensive processing events which succeed it.

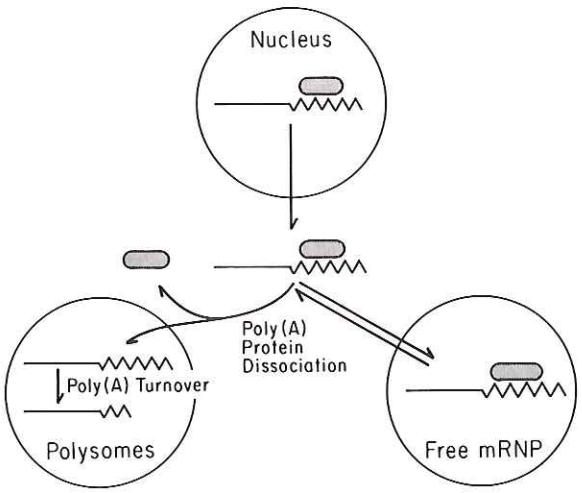

Fig. 5. A model for the maturation of poly(A)$^+$ mRNA in *Physarum* showing alterations which occur in the poly(A) region. The poly(A) sequence is represented by the zig-zag lines. The shaded ellipses represent poly(A) binding proteins. The poly(A) shortening process, which gradually removes the 15–20 adenylate residues from the poly(A) sequence of free mRNP, is not indicated in the diagram.

It appears from our results that poly(A) · protein complex dissociation may preceed the poly(A) turnover process (Fig. 5). The disengagement of proteins from the poly(A) sequence is probably an event which is necessary but insufficient in itself to cause poly(A) turnover. This is shown by our ability to recover long poly(A) sequences from cycloheximide-treated plasmodia which, on the basis of increased sensitivity to micrococcal nuclease digestion, are free of associated protein. Therefore, another event, which is currently unknown, must be required to initiate the turnover process. It is tempting to speculate that poly(A) turnover may be coupled to some aspect of the polypeptide elongation process since it is blocked by cycloheximide and appears to occur only in functional polysomes. This hypothesis, however, is not supported by all of our data since poly(A) turnover continued during a heat shock, a condition in which the polysomes are disrupted and release their messages as free mRNP.

Given the existence of the poly(A) processing events we have described in *Physarum* it is relevant to ask whether they represent a general phenomenon among the eukaryotes. There is some question, of course, whether they do since a number of laboratories have isolated long poly(A) in association with protein from the polysomal fraction of avian and mammalian cells (5). In fact, one laboratory reports that the poly(A) of globin mRNA is much shorter in

free mRNP than in the polysomes (21). Nevertheless there is considerable evidence that the situation we describe for *Physarum* may also exist in higher eukaryotes. Fertilized sea urchin eggs exhibit a rapid turnover of poly(A) tracts in their maternal mRNA which roughly corresponds to the time of increased translational utilization (22). Later during sea urchin development newly-synthesized poly(A) tracts are clearly shorter in the polysomes than the free mRNP (23). Bergmann and Brawerman (24) have recently described a situation in which the poly(A) · protein complex of mammalian cells is selectively removed from the remainder of the mRNP generating functional mRNA chains which lack detectable poly(A). They suggest that the 3′ non-coding region of mRNA is particularly sensitive to endonucleolytic cleavage, perhaps because of special configurational features, and that this attribute may be responsible for the biogenesis of the poly(A)-lacking mRNA class. Since these and other studies on poly(A)-lacking mRNA do not exclude the possibility that they contain very short poly(A) tracts at their 3′ termini we suggest that this class of message may be produced by processing events similar to those reported here for *Physarum*. Consistent with this idea, trout testes apparently contains both poly(A)$^+$ and poly(A)-lacking protamine mRNA. The poly(A) lacking molecules are restricted to the polysomes (25).

The poly(A) processing events described here can be employed to guide our thinking about the functional significance of the poly(A) sequence and its associated proteins. Since the poly(A)-associated proteins and most of the sequence itself is not found in *Physarum* polysomes they cannot be essential for translation once initiation has occurred. Instead their roles may be associated with translational initiation or the nature and structure of free mRNP particles. Since it has been shown that the presence of poly(A) confers a resistance to RNase digestion on the covalently-associated mRNA molecule (26) it is possible that configurations generated in free mRNP by the existence of a poly(A) · protein complex may protect non-polysomal mRNA. In this light it will be important to determine whether messages with short poly(A) tails undergo cytoplasmic polyadenylation and complex formation when prematurely released from the polysomes by temperature shock or amino acid deprivation.

Acknowledgements

This work was supported by grants from the National Institutes of Health (GM-24119) and the National Science Foundation (PCM-77-24767).

References

1. Shatkin, A. J., 1976. Cell 9: 645.
2. Baralle, F. E., 1977. Cell 12: 1085.
3. Proudfoot, N. J. & Brownlee, G. G., 1976. Nature 263: 211.
4. Brawerman, G., 1976. Prog. Nuc. Acid Res. Mol. Biol. 17: 7.
5. Preobrazhensky, A. & Spirin, A., 1978. Prog. Nuc. Acid Res. Mol. Biol. 21: 1.
6. Darnell, J. E., 1979. Prog. Nuc. Acid Res. Mol. Biol. 22: 327.
7. Brawerman, G., 1973. Mol. Biol. Rep. 1: 7.
8. Sheiness, D. & Darnell, J. E., 1973. Nature New Biol. 241: 265.
9. Merkel, C. G., Wood, G. T. & Lingrel, J. B., 1976. J. Biol. Chem. 251: 5512.
10. Adams, D. S. & Jeffery, W. R., 1978. Biochem. 17: 5419.
11. Jeffery, W. R. & Brawerman, G., 1974. Biochem. 13: 4633.
12. Adams, D. S., Noonan, D. & Jeffery, W. R., 1980. Biochem. 19: 1965.
13. Lindberg, U. & Persson, T., 1972. Eur. J. Biochem. 31: 246.
14. MacLeod, M., 1975. Biochem. 14: 4011.
15. Kwan, S.-W. & Brawerman, G., 1972. Proc. Nat. Acad. Sci. U.S.A. 69: 3247.
16. Sheiness, D., Puckett, L. & Darnell, J. E., 1975. Proc. Nat. Acad. Sci. U.S.A. 7: 1077.
17. Schiebel, W., Chayka, T. G., DeVries, A. & Rusch, H. P., 1969. Biochem. Biophys. Res. Commun. 35: 338.
18. Adams, D. S., Noonan, D. & Jeffery, W. R., 1981. Proc. Nat. Acad. Sci. U.S.A. 78: 83.
19. Jaworski, A. J., Wu, W. B. L. & Batema, O. M., 1976. Arch. Biochem. Biophys. 177: 516.
20. Freer, S. N., Mayama, M. & van Etten, J. L., 1977. Exp. Mycol. 1: 116.
21. Princen, H. M. G., van Eekelen, C. A. G., Asselbergs, F. A. M. & van Venrooij, W. J., 1979. Mol. Biol. Rep. 5: 59.
22. Dolecki, G. J., Duncan, R. F. & Humphreys, T., 1977. Cell 11: 339.
23. Dworkin, M. B., Rudensey, L. M. & Infante, A. A., 1977. Proc. Nat. Acad. Sci. U.S.A. 74: 2231.
24. Bergmann, I. G. & Brawerman, G., 1980. J. Mol. Biol. 139: 439.
25. Iatrou, K. & Dixon, G. H., 1977. Cell 10: 433.
26. Hieter, P. A. LeGendre, S. M. & Levy, C. C., 1976. J. Biol. Chem. 251: 3287.