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The Poly(adenylic acid)·Protein Complex Is Restricted to the Nonpolysomal Messenger Ribonucleoprotein of *Physarum polycephalum*†

David S. Adams,‡ Daniel Noonan, and William R. Jeffery*

ABSTRACT: The distribution of poly(adenylic acid) [poly(A)]·protein complexes in the polysomal and nonpolysomal messenger ribonucleoprotein (mRNP) fractions of *Physarum polycephalum* was examined in the present study. Poly(A)-containing components released from the nonpolysomal mRNP by ribonuclease (RNase) digestion were quantitatively adsorbed to nitrocellulose filters at low ionic strength, were highly resistant to micrococcal nuclease under conditions in which free poly(A) was completely degraded, and sedimented as a 10–15S particle which was disrupted by sodium dodecyl sulfate and protease treatment. These are characteristics of the poly(A)·protein complex. In contrast, poly(A)-containing molecules released from the polysomes by RNase were refractive to nitrocellulose, were completely sensitive to micrococcal nuclease, and sedimented at 2–4 S, identical with the sedimentation exhibited by protein-free poly(A). Examination of the poly(A) sequences present in polysomal and nonpolysomal mRNP by polyacylamide gel electrophoresis showed that the former contained only very short sequences, averaging ~15 nucleotides, while the latter exhibited only much longer segments, averaging ~65 nucleotides. It is concluded that poly(A)·protein complexes are restricted to the nonpolysomal mRNP of *Physarum* and that the limiting factor in complex formation may be the length of the available poly(A) binding site.

mRNA[1] is associated with proteins in the cytoplasm of eucaryotic cells forming mRNP particles (Greenberg, 1975; Preobrazhensky & Spirin, 1978). A significant protein binding site in mRNP is the 3′-poly(A) sequence of its mRNA moiety (Blobel, 1973). The resistance of poly(A) to RNase permits the isolation of the 3′-terminal domain of the mRNP as a poly(A)·protein complex (Kwan & Brawerman, 1972; Jeffery

† From the Department of Zoology, University of Texas at Austin, Austin, Texas 78712. *Received September 6, 1979.* Supported by grants from the National Science Foundation (PCM 77-24767) and the National Institutes of Health (GM-25119).

‡ Present address: The Rockefeller University, New York, NY 10021.

[1] Abbreviations used: poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); oligo(dT), oligo(deoxythymidylic acid); mRNA, messenger ribonucleic acid; mRNP, messenger ribonucleoprotein; RNP, ribonucleoprotein; tRNA, transfer ribonucleic acid; rRNA, ribosomal ribonucleic acid; Na_2EDTA, disodium ethylenediaminetetraacetate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; $NaDodSO_4$, sodium dodecyl sulfate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N′*-tetraacetic acid; Cl_3AcOH, trichloroacetic acid.

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& Brawerman, 1974). The poly(A)·protein complexes of a variety of mammalian cells have been examined and appear to contain one to four polypeptide species, usually with a 76 000-81 000 molecular weight protein as the predominant component (Blobel, 1973; Lindberg & Sundquist, 1974; Irwin et al., 1975; Barrieux et al., 1975; Kish & Pederson, 1976; Schwartz & Darnell, 1976; Jeffery, 1977; Jeffery & Peters, 1977). At present no information concerning the structure of poly(A)·protein complexes in lower eucaryotes is available. Information of this kind, however, could be important in relation to our understanding poly(A)–protein interactions since the poly(A) sequences of lower eucaryotes are much shorter than those of mammalian cells (Firtel et al., 1972; McLaughlin et al., 1973; Jaworski, 1976; Lucas et al., 1977; Adams & Jeffery, 1978). In the present investigation we have examined the organization and subcellular distribution of poly(A)·protein complexes in the cytoplasm of the plasmodial slime mold *Physarum polycephalum*. We report that proteins are exclusively associated with the long poly(A) sequences which are present in the nonpolysomal mRNP but not with the short poly(A) tracts in the polysomal mRNP of this organism. This attribute restricts the poly(A)·protein complex to the nonpolysomal mRNP.

Experimental Procedures

Culture and Labeling. Microplasmodia of *P. polycephalum* (Carolina strain) were grown at 25 °C on a rotary shaker using the axenic medium of Daniel & Baldwin (1964). The cultures were labeled by the addition of [2,8-$^3H_2$]adenosine (32 Ci/mM, ICN Pharmaceuticals, Irvine, CA) or [5,6-$^3H_2$]uridine (41 Ci/mM, ICN Pharmaceuticals) to a final concentration of 20 μCi/mL.

Plasmodial and RNP Fractionation. Microplasmodia were harvested in the mid-exponential growth phase by low-speed centrifugation and washed 3 times in 50 mM Tris-HCl (pH 7.6), 50 mM NaCl, and 1 mM $MgCl_2$. The washed plasmodial pellets were resuspended in 5 volumes of ice-cold 200 mM sucrose, 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, 100 mM $MgCl_2$, 50 mM Na_2EDTA, and 1 mM 2-mercaptoethanol (Jeffery, 1979), a homogenization medium slightly modified from that developed by Brewer (1972), and disrupted by 10 up and down strokes of a motor-driven Teflon pestle fitted into a Potter-Elvehjem glass homogenizer. A postmitochondrial supernatant fraction was prepared from the homogenate by centrifugation at 30000*g* for 10 min at 4 °C.

The postmitochondrial supernatant fraction was layered over 15 mL of 20% sucrose in 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 100 mM $MgCl_2$ and centrifuged at 27 000 rpm in a Beckman T30 rotor at 4 °C for 150 min in order to separate the polysomal RNP from the nonpolysomal RNP (free cytoplasmic RNP). This deposits the polysomal fraction in the pellet. The nonpolysomal RNP, which remains in the supernatant fraction, was recovered by another cycle of centrifugation at 27 000 rpm in the Beckman T30 rotor for 16 h at 4 °C. The polysomal and nonpolysomal RNP pellets were washed and then dissolved in 10 mM Tris-HCl (pH 7.6), 10 mM NaCl, and 1 mM $MgCl_2$.

Poly(A)·Protein Complex Preparation. For the preparation of poly(A)·protein complexes, the polysomal RNP and nonpolysomal RNP pellets were dissolved in 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, 1 mM $MgCl_2$, and 20 mM Na_2EDTA, a mixture of 5 μg/mL pancreatic RNase A and 1 μg/mL T_1 RNase was added, and the suspension was incubated on ice for 1 h. Ribosomes were removed from the digest by layering it over 5 mL of 20% sucrose in 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 1 mM $MgCl_2$ and centrifugation at 36 000 rpm for 2 h at 4 °C in a Beckman T40 rotor (Jeffery, 1977). The poly(A)·protein complex was examined by subjecting the supernatant fraction to centrifugation through sucrose density gradients. The supernatant fraction was layered over 12-mL, 10–30% linear sucrose gradients and centrifuged at 36 000 rpm for 16 h in a Beckman SW41 rotor at 4 °C. The gradients were spectrophotometrically scanned and fractionated, and the presence of radioactive poly(A) in the fractions was determined by the proteinase K–poly(U) filter assay as described below.

RNA Extraction and Poly(A) Preparation. RNA was prepared from the polysomes, the nonpolysomal RNP, or the total postmitochondrial supernatant by a combination of the alkaline phenol and phenol–chloroform–isoamyl alcohol methods as described previously (Adams & Jeffery, 1978).

For poly(A) preparation the extracted RNA samples were treated with 5 μg/mL pancreatic RNase A and 1 μg/mL T_1 RNase in the presence of 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 1 mM $MgCl_2$ for 1 h on ice. The digest was then adjusted to 500 mM NaCl and subjected to affinity chromatography on oligo(dT)–cellulose (Aviv & Leder, 1972). The poly(A) sequences eluted from oligo(dT)–cellulose were concentrated by nitrocellulose filter (0.45 μm, Millipore Corp., Bedford, MA) adsorbtion and elution (Lee et al., 1971) and analyzed by polyacrylamide gel electrophoresis as described by Adams & Jeffery (1978). The gel slices were eluted in 0.5 mL of 50 mM Tris-HCl (pH 7.6), 50 mM NaCl, and 1 mM $MgCl_2$ for 12 h at 37 °C, and the position of poly(A) was detected by its binding to poly(U)–glass fiber filters (Sheldon et al., 1972).

Zone Sedimentation. Zone sedimentation analyses were carried out at 4 °C in the Beckman SW41 rotor through 12-mL linear sucrose density gradients made up in the appropriate buffered saline solution. The gradients were collected, monitored spectrophotometrically for absorbance at 254 nm, and fractionated. The fractions were assayed as described in the figure legends. Acid-insoluble radioactivity was determined by precipitation of part of each gradient fraction with cold 10% Cl_3AcOH using yeast rRNA as a carrier. The Cl_3AcOH precipitates were collected on glass fiber filters, and the filters were dried and counted in a toluene-based scintillation mixture (Lee et al., 1971).

Proteinase K–Poly(U) Filtration Assay for Labeled Poly(A). The position of labeled poly(A) and poly(A)-containing components in RNP fractionated on sucrose gradients and the poly(A) radioactivity in heterogeneous mixtures were determined by poly(U)–glass fiber filtration of proteinase K digested samples. The samples were brought to 500 μg/mL proteinase K (Beckman Chemicals, Palo Alto, CA) and, if necessary, 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 1 mM $MgCl_2$ and incubated at 4 °C for 12–16 h. After the incubation was completed, the digests were passed through poly(U)–glass fiber filters (Sheldon et al., 1972) and the filters were processed and counted.

[^3H]Poly(U) Assay for Unlabeled Poly(A). The poly(A) content of unlabeled RNA preparations was determined by a modified version of the [^{3}H]poly(U) binding assay (Jeffery & Brawerman, 1974). The RNA samples were brought to 10 mM Tris-HCl (pH 7.6), 200 mM NaCl, and 5 mM $MgCl_2$ in a total volume of 0.5 mL, and [^{3}H]poly(U) (sp act. 0.5 Ci/mmol of UMP; New England Nuclear, Boston, MA) was added to a final concentration of 0.1 μCi/mL. The mixture was annealed at 25 °C for 15 min, and then the volume was increased to 2.5 mL by the addition of more 10 mM Tris-HCl (pH 7.6), 200 mM NaCl, and 5 mM $MgCl_2$ and brought to 1 μg/mL RNase A and 1 μg/mL T_1 RNase. After incubation

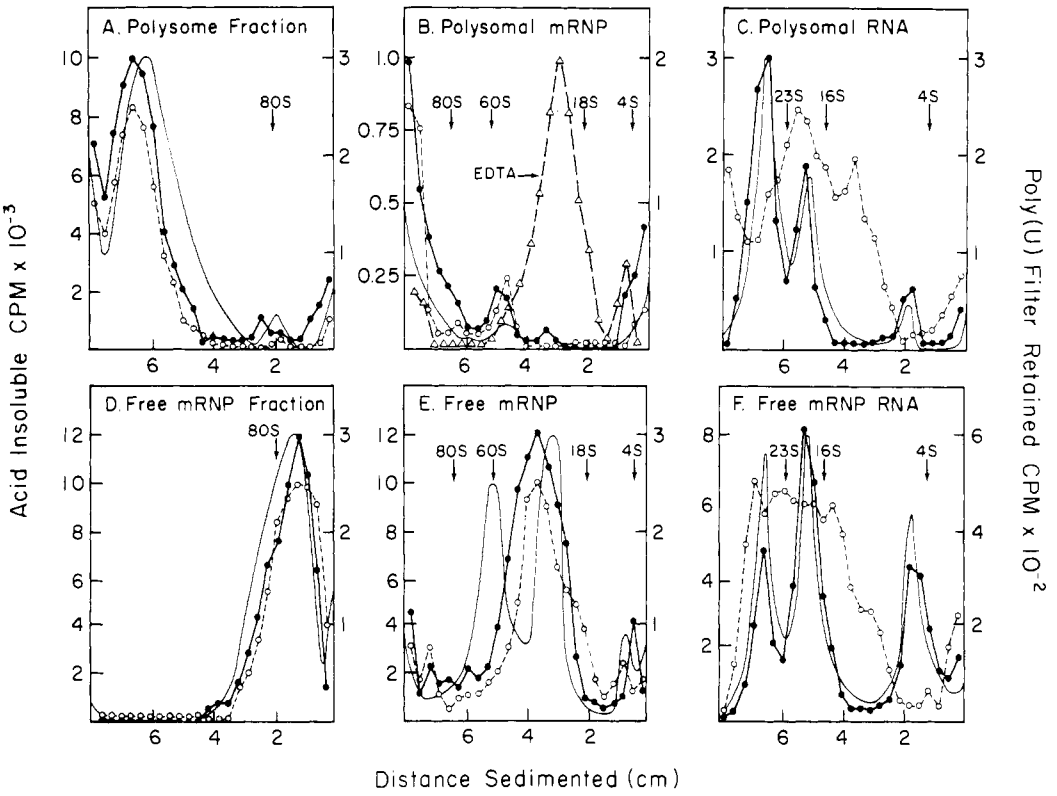

FIGURE 1: Separation and characterization of polysomal and nonpolysomal mRNPs and their derivatives in *P. polycephalum*. In the left-hand panels the polysomal (A) and nonpolysomal (D) RNPs, separated by differential centrifugation, were sedimented through 10–50% sucrose gradients containing 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 100 mM $MgCl_2$ for 90 min at 35000 rpm. In the center panels the polysomal (B) and nonpolysomal (E) RNPs were sedimented through 10–30% sucrose gradients containing 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 1 mM $MgCl_2$ at 35000 rpm for 5 h. In (B) the circles (○, ●) represent untreated RNP and the triangles (△) represent RNP treated with 30 mM Na_2EDTA for 10 min at 4 °C prior to centrifugation. In the right-hand panels phenol-extracted polysomal (C) or nonpolysomal (F) RNA was sedimented through 10–30% sucrose gradients in 10 mM Tris-HCl (pH 7.6), 10 mM NaCl, and 1 mM $MgCl_2$. Each RNP fraction was isolated from microplasmodia labeled for 2 h with [^{3}H]uridine. The solid line represents the absorbance at 254 nm. The closed circles (●) represent the total acid-insoluble radioactivity. The open circles (○) and open triangles (△) represent the radioactivity in poly(A)-containing molecules as determined by the proteinase K–poly(U) assay (A, B and D, E) or by poly(U) filtration (C, F). The markers used (arrows) were RNase-treated Ehrlich ascites cell polysomes (80 S), ribosomal subunits (60 and 40 S), and rRNA (18 S) and *Escherichia coli* rRNA (23 and 16 S) and tRNA (4 S) centrifuged on parallel gradients.

for 1 h at 25 °C, the digests were chilled and precipitated with 10% Cl_3AcOH in the presence of carrier rRNA. The Cl_3-AcOH precipitates were collected on glass fiber filters and counted.

Results

Characterization of Polysomal and Nonpolysomal mRNP. The sedimentation profiles of polysomal and nonpolysomal RNP prepared from the cytoplasmic fraction of *P. polycephalum* by differential centrifugation are shown in Figure 1. The polysomal fraction contained molecules which sedimented more rapidly than 100 S (Figure 1A) while the nonpolysomal RNP contained the more slowly sedimenting components (Figure 1D). As expected of the polysomal fraction, Na_2EDTA treatment released poly(A)-containing particles of a much lower sedimentation range than those present in the untreated polysomes (Figure 1B). Although the average sedimentation value for the Na_2EDTA-released particles was only 25 S, it was clear that they represented poly(A+) RNP since they exhibited quantitative binding to nitrocellulose filters at low ionic strength (data not shown) and yielded poly(A+) RNA molecules of lower sedimentation values following phenol extraction (Figure 1C). The nonpolysomal fraction, on the other hand, contained Na_2EDTA-resistant (data not shown) poly(A)-containing particles which sedimented more rapidly than the polysomal poly(A+) RNP (Figure 1E). Their average sedimentation value was 40 S, but, like their polysomal counterparts, they exhibited a relatively narrow sedimentation range. A very heterogeneous population of poly(A+) RNA molecules could be derived from the polysomal or nonpolysomal RNP by phenol extraction (parts C and F of Figure 1). Although *Physarum* mRNPs are smaller and less heterogeneous than those derived from mammalian cells (Henshaw, 1968), they resemble the mRNPs of other lower eucaryotes in their sedimentation behavior (Tønnesen, 1973; Mirkes, 1977).

Presence of Poly(A)·Protein Complexes in Polysomal and Nonpolysomal mRNP. The existence of poly(A)·protein complexes in the polysomal and nonpolysomal RNP was determined by examining the sedimentation behavior of the poly(A)-containing material liberated from these fractions by RNase treatment (Kwan & Brawerman, 1972). The poly(A)-containing components derived in this fashion from the nonpolysomal RNP sedimented as 10–15S particles (Figure 2B), a sedimentation distribution typical of poly(A)·protein complexes (Kwan & Brawerman, 1972; Jeffery & Brawerman, 1974; Kish & Pederson, 1976; Schwartz & Darnell, 1976). The presence of proteins in these particles was confirmed by treatment of the RNase-digested RNP with $NaDodSO_4$ (Figure 2C) or protease (Figure 2D) prior to centrifugation which converted the 10–15S complex to 2–4S poly(A)-containing molecules. The 2–4S components were similar in sedimentation behavior to pure poly(A) prepared from *Physarum* cytoplasmic RNA. The poly(A)·protein complex could not be entirely accounted for by the adventitious association of basic proteins with poly(A) since a salt wash (Blobel, 1972)

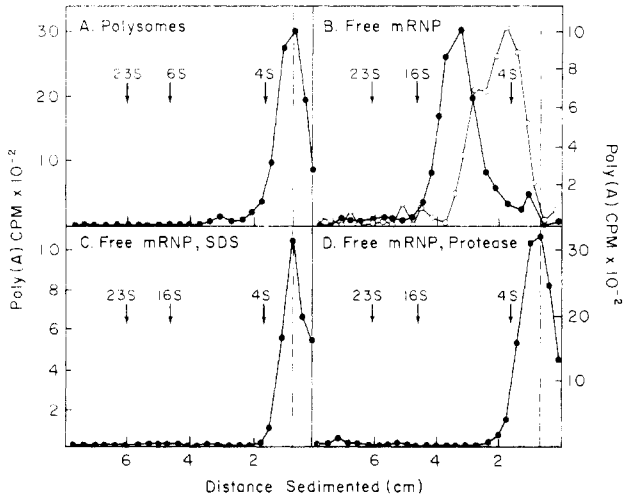

FIGURE 2: Zone sedimentation of poly(A)-containing components derived from the polysomal and nonpolysomal RNP of *P. polycephalum*. (A) RNase-treated polysomal RNP. (B) RNase-treated nonpolysomal RNP. (C) RNase-treated nonpolysomal RNP incubated with 1% $NaDodSO_4$ (SDS) for 2 min at 37 °C or (D) 500 μg/mL proteinase K for 60 min at 37 °C prior to sedimentation. In (B) the open circles (○) represent RNase-treated nonpolysomal RNP subjected to a salt wash for 60 min at 4 °C in 500 mM NaCl (Blobel, 1972) prior to centrifugation. The RNP fractions were isolated from microplasmodia labeled for 2 h with [^{3}H]adenosine. Zone sedimentation was carried out through 20–30% sucrose gradients containing 50 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 1 mM $MgCl_2$ for 16 h at 35 000 rpm. The radioactivity represented by poly(A)-containing molecules was determined by the proteinase K–poly(U) assay. The vertical dashed line represents the average sedimentation value of poly(A) derived from phenol-extracted *Physarum* cytoplasmic RNA. The markers used (arrows) were *E. coli* rRNA and tRNA centrifuged on parallel gradients.

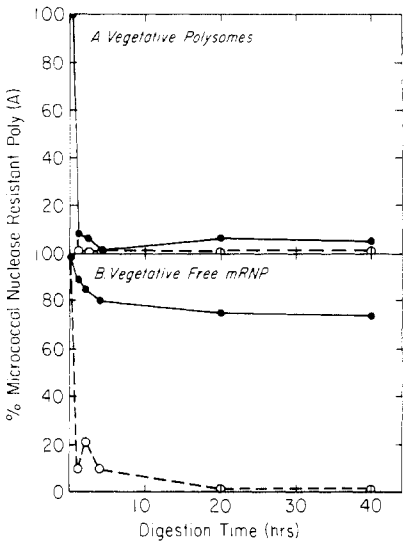

FIGURE 3: Micrococcal nuclease sensitivity of poly(A)-containing components derived from the polysomal and nonpolysomal RNP of *P. polycephalum*. The polysomal and nonpolysomal RNP was isolated from microplasmodia labeled with [^{3}H]adenosine for 2 h. The poly(A)-containing components derived from this material by RNase treatment (see Experimental Procedures) 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) assay. The decrease in radioactivity in the fractions which lacked micrococcal nuclease was used to correct the nuclease digestion curves. Poly(A)-containing components were derived from the polysomal (A) and nonpolysomal (B) mRNP. Closed circles (●) represent radioactivity present in the poly(A)-containing material released from RNP by RNase digestion. The open circles (○) represent radioactivity present in the poly-(A)-containing components of their corresponding phenol-extracted samples.

did not reduce its sedimentation to that typical of free poly(A) (Figure 2B). The ability of $NaDodSO_4$ or protease to disrupt the salt-washed complex and its quantitative adsorption to nitrocellulose filters (these data not shown) suggest that it represents protein tenaciously bound to poly(A).

In contrast to the results obtained for nonpolysomal RNP, RNase treatment of the polysomal fraction did not yield a poly(A)·protein complex (Figure 2A). Instead, a component which cosedimented with pure poly(A) was obtained, suggesting that a poly(A)·protein complex may be absent from the polysomes.

Further evidence for the restriction of poly(A)·protein complexes in the nonpolysomal mRNP was obtained by testing the nitrocellulose binding ability and the micrococcal nuclease sensitivity of the RNase-released materials. Poly(A) tracts associated with proteins bind to nitrocellulose filters at low ionic strength (Peters & Jeffery, 1978) and should be at least partially resistant to micrococcal nuclease (Reddi, 1959). It was observed that 90–95% of the poly(A)-containing material released from the nonpolysomal RNP was adsorbed to nitrocellulose filters while only 10–15% of that derived from the polysomes showed this feature. Moreover, as indicated in Figure 3, the poly(A)-containing molecules released from the polysomes, like pure poly(A), were completely digested by micrococcal nuclease while those obtained from the nonpolysomal fraction were ~80% resistant to this enzyme. The resistance of the nonpolysomal poly(A)-containing molecules to micrococcal nuclease was not due to special properties of their poly(A) sequences since poly(A) extracted from this fraction was completely sensitive to digestion (Figure 3B). Thus, three independent lines of evidence suggest that poly-(A)·protein complexes are released from nonpolysomal mRNP but not from the polysomal mRNP of *Physarum*.

It is possible that poly(A)·protein complexes cannot be released from the polysomes by RNase treatment due to conformational differences between polysomal and nonpolysomal mRNPs. The polysomal and nonpolysomal RNP fractions were directly treated with micrococcal nuclease and the proportion of resistant poly(A) was measured by the proteinase K–poly(U) filtration assay in order to test this possibility. Only 4–8% of the polysomal poly(A) survived nuclease treatment while 75–80% of the nonpolysomal poly(A) proved resistant. These results suggest that polysomal mRNP is severely deficient in poly(A)·protein complexes.

Poly(A) Sequences in Polysomal and Nonpolysomal mRNP. It is possible that poly(A)·protein complexes do not exist in the polysomes due to modification of the poly(A) binding sites. RNA was extracted from the polysomal and nonpolysomal fractions and its poly(A) sequences were sized by polyacrylamide gel electrophoresis in order to test this possibility. As shown previously (Adams & Jeffery, 1978) and in Figure 4, poly(A) prepared from 2-h-labeled total cytoplasmic RNA consists of two distinct very heterogeneous sequence populations, one averaging about 15 nucleotide residues and the other 65 residues. Kinetic studies suggest that these poly(A) sequence classes are not present in the same molecules (Adams & Jeffery, 1978). Poly(A) prepared from the polysomal fraction comigrated exclusively with the short poly(A) sequences whereas the nonpolysomal RNP derived poly(A) showed the same mobility as the longer segments (Figure 4). The presence of only very short poly(A) in the polysomal RNA suggests that poly(A)·protein complex formation in this

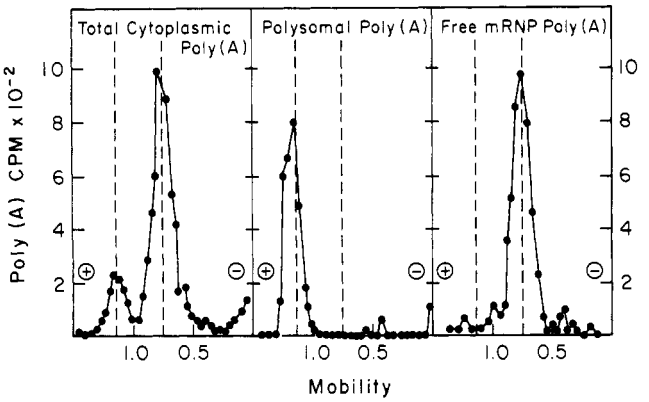

FIGURE 4: Electrophoretic mobility of poly(A) sequences extracted from the polysomal and nonpolysomal RNP in *P. polycephalum*. Left-hand panel: total cytoplasmic poly(A). Center panel: polysomal RNP poly(A). Right-hand panel: nonpolysomal RNP poly(A). Poly(A) was prepared from RNP fractions derived from microplasmodia labeled for 2 h with [^{3}H]adenosine. Poly(A) radioactivity was determined by poly(U) filtration of the RNA eluted from the gel slices. In each panel 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 as represented in the total cytoplasmic poly(A). The average poly(A) class sizes were determined by comparison with the mobility of poly(A) markers run on parallel gels. A mobility of 1.0 represents a migration distance of 4.8 ± 0.4 cm through the gel toward the anode.

fraction may be limited by poly(A) length.

The presence of poly(A) tracts with a mass average size of 65 nucleotides in the free mRNP fraction suggests that this material is not derived from nuclear leakage during the fractionation procedure because the nuclear RNA of *Physarum* contains poly(A) sequences exhibiting a mass average length of ~80 nucleotides (Adams & Jeffery, 1978). Since some 80-nucleotide poly(A) sequences do occur in the gels shown in Figure 4, we cannot entirely exclude the possibility that a portion of the nonpolysomal RNP is of nuclear origin, but certainly most of it is not.

For the purpose of determining whether the polysome fraction contains a significant titer of RNA molecules containing short poly(A) sequences, its poly(A) content was measured by the [^{3}H]poly(U) binding assay (Jeffery & Brawerman, 1974). This procedure allows the proportion of poly(A+) RNA sequences in a particular RNA fraction to be estimated if the poly(A) tract length is known and it is assumed that only one poly(A) segment exists per molecule. By application of this analysis to the *Physarum* polysomal and nonpolysomal RNP fractions, it can be shown that ~68% of the cytoplasmic poly(A+) RNA complement was present in the polysomes (Table I). The distribution of poly(A+) RNA molecules between the polysomes and free cytoplasmic mRNP in *Physarum* is similar to that previously reported for mammalian cells (Lindberg & Persson, 1972; Jelinek et al., 1973; MacLeod, 1975).

Table I: Estimation of the Distribution of Poly(A+) RNA Molecules between the Polysomal and Nonpolysomal RNP Fraction of *P. polycephalum*

RNP fraction	cpm of [^{3}H]-poly(U) per mL	av poly(A) size	cpm of [^{3}H] poly(U) rel to poly(A) size	poly(A+) RNA distribution (%)
polysomal	55220	15	3675	67.6
nonpolysomal	114560	65	1763	32.4

Discussion

In this report we demonstrate that poly(A)·protein complexes are restricted to the nonpolysomal mRNP in *P. polycephalum*. Three independent lines of evidence support this conclusion. The poly(A)-containing components released from the nonpolysomal RNP fraction sediment as 10–15S particles which can be disrupted by $NaDodSO_4$ or protease treatment. They are also quantitatively adsorbed to nitrocellulose filters, a characteristic of poly(A)·protein (Kwan & Brawerman, 1972; Peters & Jeffery, 1978). Finally, the nonpolysomal particles are highly resistant to micrococcal nuclease digestion under conditions in which free poly(A) is completely degraded. None of these features are shared with poly(A) sequences released from the polysomes. They proved to be nitrocellulose refractive and micrococcal nuclease sensitive, and they sedimented in the 2–4S range. These characteristics are typical of free poly(A) tracts.

The restriction of the poly(A)·protein complex to the nonpolysomal mRNP does not appear to be a universal feature of eucaryotic cells. A number of laboratories have reported the occurrence of poly(A)-containing particles, usually consisting of one to four polypeptides, in mammalian polysomes (Kwan & Brawerman, 1972; Blobel, 1973; Jeffery & Brawerman, 1974; Barrieux et al., 1975; Irwin et al., 1975; Kish & Pederson, 1976; Jeffery, 1977; Jeffery & Peters, 1977). In these studies, however, the polysomes were probably not completely separated from more slowly sedimenting free mRNP particles. Even in the investigation of Schwartz & Darnell (1976), in which small HeLa cell polysomes were carefully separated from the postpolysomal particles prior to the RNase-mediated release of the poly(A)·protein complex, some contamination by the larger nonpolysomal mRNP could have been present. In *Physarum* the relatively narrow sedimentation range of the nonpolysomal mRNP and the deficiency in mRNA–monosome complexes (Jeffery, 1979) may facilitate a distinct separation between these particles and the polysomes. Clearly, the present results and the uncertainties cited above call for a more detailed investigation of the subcellular distribution of the poly(A)·protein complex in eucaryotic cells.

If the absence of poly(A)·protein complexes in polysomal mRNP proves to be a unique characteristic of *Physarum* or other lower eucaryotes, it may be a consequence of the presence of very short poly(A) sequences. The short poly(A) segments present in *Physarum* polysomal RNA are probably relatively inefficient protein binding sites. In this regard, it was previously reported that many mRNA molecules in unfertilized sea urchin eggs, which also contain relatively short poly(A) sequences, lack poly(A)·protein complexes. Poly(A)·protein complex formation on these molcules occurs after fertilization when their poly(A) tails are extended by cytoplasmic polyadenylation (Peters & Jeffery, 1978). In *Physarum* the opposite situation may occur; that is, poly(A)–protein interactions may be destabilized as a result of the poly(A) shortening and turnover events which accompany mRNA aging (Adams & Jeffery, 1978). At present the length of the poly(A) segment needed to provide a stable binding site for protein is unknown. The micrococcal nuclease sensitivity data, however, suggest that 80% or ~50 of the AMP residues are protected from degradation in the poly(A)·protein complex if it is assumed that these particles show a uniform response to the enzyme.

Since *Physarum* polysomal mRNP lacks a poly(A)·protein complex and contains very short poly(A) sequences, it is proposed that the incorporation of mRNA into the polysomes in this organism is accompanied by poly(A)·protein complex

disruption and extensive poly(A) turnover. By making this suggestion, we also imply that the free cytoplasmic mRNP must at least in part be the precursor to the polysomal mRNA. We are confident that this is so because it has recently been possible to recover labeled free mRNP in polysomes after its microinjection into unlabeled macroplasmodia of *Physarum* (Noonan et al., 1979).

It is now possible to explain our previous results on poly(A) metabolism in *Physarum* which showed that poly(A) degradation occurred by two processes, a shortening process in which only ~15 nucleotides were removed and a turnover process in which entire poly(A) tracts disappeared leaving only short oligo(A) sequences in the cytoplasmic RNA (Adams & Jeffery, 1978). The poly(A) turnover process probably occurs when mRNA from the free cytoplasmic mRNP enters the polysomes. This explains the presence of oligo(A) in the polysomal RNA. The poly(A) turnover process could either be the cause or the result of the dissociation of the poly(A)·protein complex. Preliminary data from this laboratory, in which cycloheximide was used to block translocation, provide indirect evidence for the former possibility since under these conditions the proportion of nonpolysomal poly(A) protected from micrococcal nuclease digestion is gradually reduced. Thus, we feel that this phenomenon results from the translation process itself rather than being a preliminary to the initiation of protein synthesis. Perhaps poly(A) shortening, which continues in cycloheximide-treated *Physarum* (Adams & Jeffery, 1978), eventually triggers the disruption of the poly(A)·protein complex and subsequent poly(A) turnover.

A general conclusion that can be drawn from the data reported in the present investigation is that the poly(A)·protein complex is not essential for mRNA function once protein synthesis has been initiated. This implies that its physiological role may be related to earlier events in the life cycle of mRNA such as transport from nucleus to cytoplasm, metabolic stability, and storage in the form of a free cytoplasmic mRNP or the initiation steps of translation.

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