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Poly(adenylic acid) Degradation by Two Distinct Processes in the Cytoplasmic RNA of *Physarum polycephalum*†

David S. Adams* and William R. Jeffery

ABSTRACT: The degradation of poly(adenylic acid) [poly(A)] and its relationship to translation were examined in the cytoplasmic RNA of *Physarum polycephalum*. Poly(A) sequences averaging about 80 and 65 nucleotides in length are present in *Physarum* nuclear and cytoplasmic RNA, respectively. These RNA classes also contain an oligo(A) sequence. Analysis of the electrophoretic mobility and total quantity of cytoplasmic RNase A and T_1-insensitive polynucleotides in a pulse-chase regime reveal that poly(A) degradation involves both a gradual shortening process, in which about 15 nucleotides are removed from this sequence, and a turnover process in which 95% of the radioactive poly(A) disappears. The shortening process, unlike the turnover process, is not responsible for the complete eradication of poly(A) since tracts of about 50 nucleotides in length appear in the steady-state cytoplasmic RNA and accumulate when the turnover process is suppressed. In contrast to the behavior of poly(A), the oligo(A) sequences did not appear to significantly decay during the chase. A 37 °C heat shock, which suppresses protein synthesis and disrupts polysome structure, does not interfere with either poly(A) degradation process, whereas cycloheximide, which hinders messenger RNA cycling through the polysome, substantially slows the turnover process but does not affect the shortening process. It is concluded that at least two processes, which exhibit differential linkage to the translation cycle, are involved in poly(A) degradation during the aging of cytoplasmic RNA in *Physarum*.

A gradual shortening of the poly(A)[1] sequence is initiated following the entry of messenger RNA into the cytoplasm of mammalian cells (Brawerman, 1973; Sheiness & Darnell, 1973). Although the physiological significance of this phenomenon is currently unresolved, its possible role in the regulation of mRNA turnover has frequently been postulated (Sheiness et al., 1975; Hieter et al., 1976; Marbaix et al., 1977). Most of the available information concerning the details of cytoplasmic poly(A) degradation has been derived from studies of cultured mammalian cells. However, the mRNA of lower eukaryotic organisms also contains poly(A) sequences which are subject to age-dependent shortening (Jaworski et al., 1976; Freer et al., 1977). Important information concerning the biochemical role of poly(A) degradation processes may be provided by these organisms since their poly(A) sequences are usually shorter (Firtel et al., 1972; McLaughlin et al., 1973; Jaworski, 1976; Lucas et al., 1977; Johnson et al., 1977) and their mRNA is sometimes of reduced longevity (Peterson et al., 1976; Vendov et al., 1977) in comparison to their mammalian counterparts. The present investigation is focused on the process of poly(A) degradation in the cytoplasmic RNA of the acellular slime mold *Physarum polycephalum*. We report the occurrence of two distinct varieties of poly(A) degradation in this organism and their differential relationship to the cycle of mRNA translation.

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

Experimental Procedure

Culture and Labeling. Physarum polycephalum (Carolina strain) microplasmodia were grown at 25 °C in axenic suspension cultures as described by Daniel & Baldwin (1964). The suspension cultures were labeled with 10 μCi/mL [2,8′-^{3}H]-adenosine (33 Ci/mmol; Schwarz/Mann, Orangeburg, N.Y.) or [5,6-^{3}H]uridine (41 Ci/mmol; Schwarz/Mann). In pulse-chase experiments exponentially growing cultures were concentrated in nutrient medium eightfold for labeling, labeled for the desired time period, then washed five times with 5 volumes of pH 4.6 balanced *Physarum salts* (Daniel & Baldwin, 1964) containing 1 mM adenosine or 1 mM uridine, and resuspended in the original volume of conditioned culture medium supplemented with 1 mM of the appropriate cold precursor.

Plasmodial Fractionation. Microplasmodia were harvested by centrifugation at 1000*g* for 0.5 min and washed three times in 50 mM Tris-HCl (pH 7.6), 10 mM NaCl, 1 mM $MgCl_2$. The final pellet was frozen in liquid N_2 and lyophilized (Melera & Rusch, 1973). The lyophilized material was resuspended in 3 volumes of ice-cold homogenization medium and disrupted by 20 strokes of a motor-driven Teflon pestle fitted in a Potter-Elvehjem glass homogenizer. The homogenization medium contained 0.25 M sucrose, 10 mM Tris-HCl (pH 7.6), 100 mM NaCl, 10 mM Na_2 EDTA, and 5 mM $CaCl_2$ to stabilize the nuclear membranes (Bradbury et al., 1973). The crude nuclear fraction was recovered from the homogenate by centrifugation at 1000*g* for 10 min at 4 °C and the mitochondrial pellet was deposited from the 1000*g* supernatant by a cycle of centrifugation at 9500*g* for 30 min at 4 °C. The 9500*g* supernatant was designated the cytoplasmic fraction.

The crude nuclear fraction was washed 2× with 5 volumes of ice-cold 0.25 M sucrose, 1 mM $MgSO_4$, 1 mM KH_2PO_4 (pH 6.3), 5 mM $CaCl_2$, and 0.3% Triton N-101 (Berkowitz et al., 1969) and collected by centrifugation at 1000*g* for 10 min.

Extraction of Nuclear and Cytoplasmic RNA. The washed

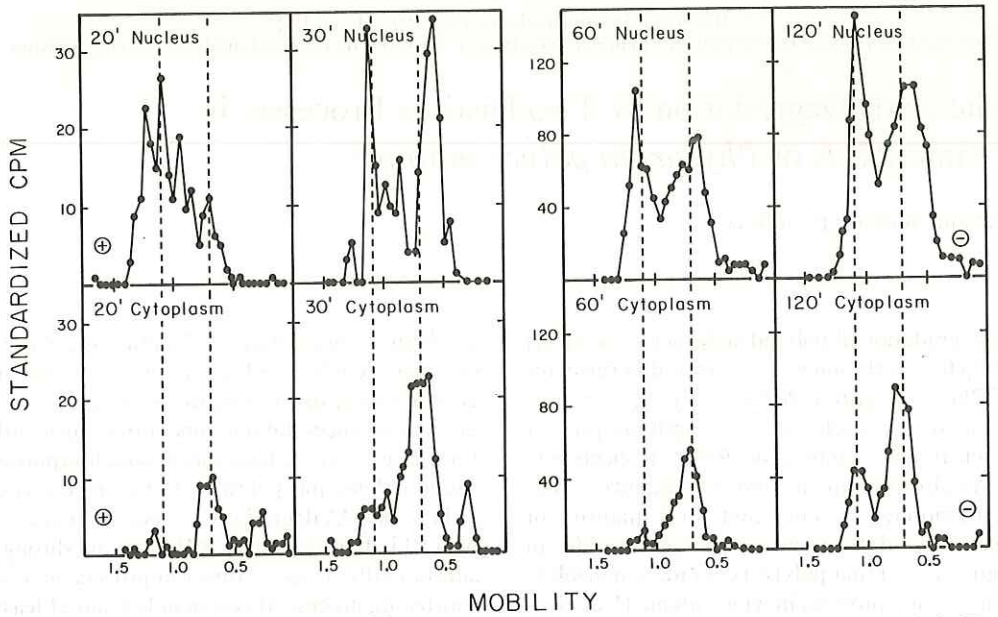

FIGURE 1: The electrophoretic mobility of *Physarum* nuclear and cytoplasmic poly(A) sequences prepared from RNA labeled for increasing periods of time with [^{3}H]adenosine. Poly(A) sequences were obtained from nuclear and cytoplasmic RNA by treatment with RNases T_1 and A and selection of the resistant fragments on oligo(dT)-cellulose. Dashed vertical lines represent the average mobility of 20-min-labeled cytoplasmic oligo(A) and poly(A). A mobility of 1.0 represents a migration distance of 4.8 ± 0.4 cm through the gel toward the anode.

nuclear pellet was resuspended in 10 mM Tris-HCl (pH 7.6) and the nuclear membrane was lysed by the addition of EGTA to a final concentration of 5 mM. The EGTA probably promotes nuclear lysis by chelation of Ca^{2+} required for nuclear membrane integrity. RNA was isolated from the lysed nuclei by a modified hot phenol extraction (Scherrer & Darnell, 1962). The lysed nuclear suspension was adjusted to 0.5% sodium dodecyl sulfate ($NaDodSO_4$) and thoroughly mixed with an equal volume of water-saturated phenol. After mixing at 55 °C for 5 min, the phases were separated by centrifugation and the aqueous phase was removed. An equal volume of 10 mM Tris-HCl (pH 7.6) was added to the phenol phase and the hot extraction was repeated. The combined aqueous phases were then reextracted three times with fresh phenol. RNA was precipitated from the final aqueous phase by adjusting the NaCl concentration to 0.1 M, adding 2.5 volumes of 95% ethanol, and cooling to −20 °C.

Cytoplasmic RNA was isolated from the 9500*g* supernatant fraction using a combination of alkaline phenol (Brawerman et al., 1972) and phenol–chloroform–isoamyl alcohol (Penman, 1966; Aviv & Leder, 1972) extraction methods. The cytoplasmic fraction was adjusted to 0.5% $NaDodSO_4$ and 0.1 M Tris-HCl (pH 9.0), an equal volume of cold phenol–chloroform–isoamyl alcohol (50:50:1) was added, the mixture was agitated, and the phases were separated by centrifugation. The organic phase was reextracted two times with 0.1 M Tris-HCl (pH 9.0), the aqueous phases were pooled, reextracted three times with equal volumes of the fresh phenol mixture, and RNA was recovered as described above.

Poly(A) Preparation. RNA preparations dissolved in 50 mM Tris-HCl (pH 7.6), 100 mM KCl, 1 mM $MgCl_2$ were incubated with a mixture of 5 μg/mL RNase A and 5 μg/mL RNase T_1 for 1 h at 37 °C. The digest was then adjusted to 0.3% $NaDodSO_4$ and 0.4 M NaCl and applied to a precequilibrated batch (0.3 mL) of oligo (dT)-cellulose (P-L Biochemicals, Milwaukee, Wis.) in a total volume of 5 mL (Jeffery & Brawerman, 1975). The mixture was agitated at room temperature for 1 h and the suspended oligo(dT)-cellulose was recovered by centrifugation. In order to maximize the yield of poly(A), the original oligo(dT)-cellulose unbound fraction was subjected to two additional cycles of oligo(dT)-cellulose binding and the three oligo(dT)-cellulose pellets were combined for washing and elution. After five successive washes with a total of 50 volumes of 50 mM Tris-HCl (pH 7.6), 500 mM NaCl, 1 mM $MgCl_2$, 0.3% $NaDodSO_4$, the bound polynucleotides were eluted by resuspension of the oligo(dT)-cellulose in 10 mM Tris-HCl (pH 7.6), 0.3% $NaDodSO_4$ and incubation for 15 min at 37 °C. The oligo(dT)-cellulose was then collected by centrifugation and the eluted material was recovered by decantation. The eluted polynucleotides were precipitated from the supernatant by the addition of ethanol in the presence of a 50 μg/mL *E. coli* tRNA carrier. This procedure yielded 84 ± 9% of the poly(U)-filter binding (Sheldon et al., 1972) radioactivity of the original digest.

Poly(acrylamide) Gel Electrophoresis. The size distribution of poly(A) segments was determined by electrophoresis on 7 cm, 6.5% polyacrylamide gels (Brawerman, 1973; Jeffery & Brawerman, 1975). The gels were cross-linked with 0.26% ethylene diacrylate or, when RNA was to be eluted from gel slices, with 0.23% bis(acrylamide). Poly(A) preparations in a total volume of 50–100 μL of 40 mM Tris-HCl (pH 7.6), 20 mM sodium acetate, 2 mM EDTA, 20% glycerol, and 0.03% bromphenol blue were layered on preelectrophoresed gels and electrophoresis was carried out at 1 mA per cm per gel until the tracking dye had traveled about 5 cm from the top of the gel. After electrophoresis was completed the gels were removed from the tubes and the positions of the bromphenol blue, 5S RNA, and tRNA markers were determined by spectrophotometric scanning of the gel. The gels were then frozen and sliced into 2-mm fractions. When labeled polynucleotides were analyzed, the gel slices were dissolved by incubation in 0.6 mL of 20% perchloric acid:20% hydrogen peroxide at 60 °C for 4 h and counted in 10 mL of Biofluor. When preparations of unlabeled polynucleotides were analyzed, gel slices were eluted in 0.5 mL of 50 mM Tris-HCl (pH 7.6), 50 mM KCl, 1 mM $MgCl_2$ for 12 h at 37 °C, and poly(A) was assayed by its ability to form paired complexes with [^{3}H]poly(U) as described by Jeffery & Brawerman (1974). Cl_3CCOOH insoluble radioactivity was then counted in a toluene-based scintillation fluid.

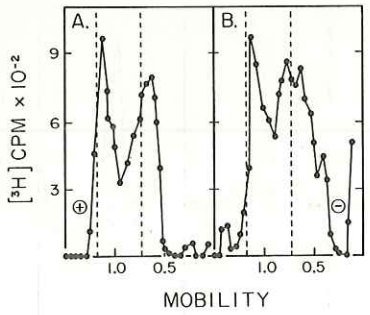

FIGURE 2: Ribonuclease sensitivity of *Physarum* nuclear poly(A)-containing RNA. Poly(A)-containing RNA prepared by selection of oligo(dT)-cellulose was digested with RNase T_1 (left frame) or a mixture of RNases T_1 and A (right frame), the resistant fragments were subjected to another cycle of selection on oligo(dT)-cellulose, and separated by polyacrylamide gel electrophoresis. Dashed vertical lines represent average mobilities of 2-h-labeled nuclear oligo(A) and poly(A). Mobility units are as indicated in Figure 1.

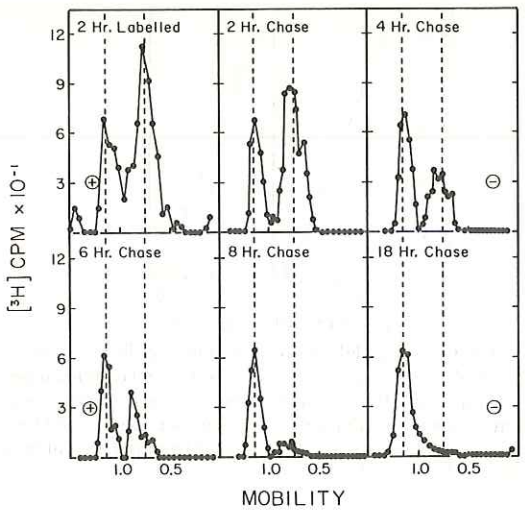

FIGURE 3: Age-dependent alterations in the electrophoretic mobility of poly(A) in *Physarum* cytoplasmic RNA. Cultures labeled with [^{3}H]-adenosine for 2 h were chased with cold adenosine-containing media. Equal aliquots were removed from the culture at various times during the chase; cytoplasmic RNA was prepared and digested with a mixture of RNases A and T_1. The RNase-resistant fragments of the digest were selected on oligo(dT)-cellulose and separated by polyacrylamide gel electrophoresis. Dashed vertical lines represent the average mobilities of 2 h-labeled cytoplasmic oligo(A) and poly(A). Mobility units are as indicated in Figure 1. A typical experiment is shown.

Results

Poly(A) in Nuclear and Cytoplasmic RNA. Figure 1 illustrates the electrophoretic mobility of the RNase A and T_1 resistant polynucleotides present in *Physarum* high molecular weight nuclear and cytoplasmic RNA (Fouquet et al., 1974). In most of these poly(acrylamide) gels, two distinct, although quite heterogeneous, nucleotide classes are evident. The electrophoretic mobility of the most rapidly migrating class resembles that of small adenylic acid rich [oligo(A)] sequences previously observed in the heterogeneous nuclear and cytoplasmic RNA of a number of types of eukaryotic cells (Nakazato et al., 1974; Mahoney & Brown, 1975; Kinniburgh & Martin, 1976; Cabada et al., 1977). These sequences may be transcribed from about 6500 (dA·dT) tracts of about 26 residues in length known to exist in the *Physarum* nuclear genome (Mol et al., 1976). The more slowly migrating polynucleotide class observed in Figure 1 falls within the mobility range expected of eukaryotic poly(A).

In order to gain insight into the kinetics of appearance of the two classes of RNase-resistant polynucleotides, these components were prepared from plasmodia which had been labeled with [^{3}H]adenosine for various time intervals. As shown in Figure 1 an extremely heterogeneous array of small polynucleotides, possibly representing nascent poly(A), was observed in the nuclear RNA early following radioisotope addition; but, as the labeling time increased, typical profiles of oligo(A) and poly(A) appeared in the gels. The average size of the nuclear poly(A) peak after 120 min of labeling, calculated by the comparison of its electrophoretic mobility with those of poly(A) standards, was about 80 nucleotides. This value was considerably larger than the 65 nucleotide average estimated for cytoplasmic poly(A) labeled for the same length of time. The data suggest that *Physarum* poly(A) sequences are substantially shorter than those of most mammalian poly(A) (Brawerman, 1974). Moreover, the poly(A) size decrease observed between nuclear and cytoplasmic RNA may be due to either shortening of this sequence during RNA transport or the selective transit of RNA with shorter than average poly(A) sequences.

In mRNA of the cellular slime mold *Dictyostelium discoideum* poly(A) and oligo(A) sequences are present in the same molecules separated by a short pyrimidine-containing region (Jacobson et al., 1974). In *Physarum* oligo(A) and poly(A) are either in different molecules or separated by a polynucleotide sequence including guanylic acid since distinct poly(A) and oligo(A) sequences were liberated by RNase T_1 digestion alone or by treatment with RNase A and T_1 in combination (Figure 2). The kinetic data of Figure 1, which indicate that radioactive poly(A) appears in the cytoplasm earlier than oligo(A), suggest that oligo(A) and poly(A) are not in the same molecules.

Poly(A) Degradation in Cytoplasmic RNA. The kinetics of poly(A) degradation in cytoplasmic RNA was examined using a [^{3}H]adenosine pulse-chase procedure. In this regime exponentially growing cultures were labeled with [^{3}H]adenosine for 2 h and then subjected to a cold adenosine chase. Effective chase conditions were rapidly attained since only about 16% more radioactivity was incorporated into the acid-insoluble fraction during the first 60 min after the plasmodia were washed into cold adenosine (data not shown).

In order to monitor the loss of labeled poly(A) from the cytoplasmic RNA and its shortening, RNase digests prepared from equal aliquots of culture were examined on poly(acrylamide) gels. As shown in Figure 3, both the average size and amount of labeled poly(A), but not oligo(A), were substantially decreased during an 8-h chase period. We calculate that approximately 15 nucleotides were always lost from the poly(A) during shortening and about 95% of the total poly(A) radioactivity disappeared from the cytoplasmic RNA during an 8-h chase (Figure 4A). Since entire poly(A) sequences were lost without the generation of heterogeneous polynucleotide intermediates (Figure 3), two separate processes appear to be responsible for the degradation of *Physarum* poly(A), an incomplete shortening process, involving the gradual size reduction of the sequence, and a turnover process, entailing its complete destruction.

In sea urchin zygotes poly(A) attached to maternal RNA is subject to complete removal and replacement with new poly(A) synthesized in the cytoplasm (Dolecki et al., 1977). In order to test the possibility that labeled poly(A) lost from *Physarum* cytoplasmic RNA is replaced by unlabeled poly(A), aliquots from [^{3}H]uridine-labeled cultures were assayed for oligo(dT)-cellulose binding at various intervals during the chase period. Labeled uridine, like labeled adenosine, could

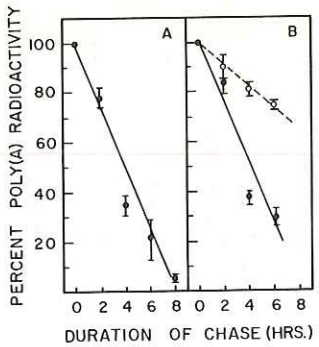

FIGURE 4: The kinetics of labeled poly(A) turnover in (A) normal and (B) cycloheximide-treated (open circles) or heat-shocked (closed circles) *Physarum* cytoplasmic RNA. Circles represent mean percent poly(A) remaining at various times during a cold adenosine chase ± SD (n = 3). Radioactivity remaining in poly(A) estimated from the area of poly(A) peak on polyacrylamide gels.

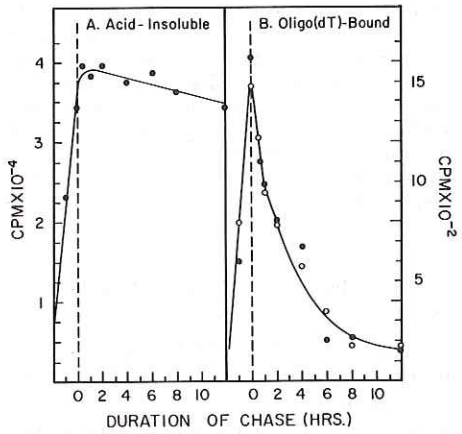

FIGURE 5: The kinetics of [^{3}H]uridine incorporation into *Physarum* cytoplasmic RNA during a pulse-chase regime. (A) Incorporation into Cl_3CCOOH-insoluble RNA. (B) Incorporation into oligo(dT)-cellulose bound RNA. Vertical dashed lines represent the time of the beginning of the chase. In B, filled and open circles represent data collected from two separate experiments.

be rapidly chased from the precursor pools (Figure 5A). As shown in Figure 5B, oligo(dT)-cellulose bound, [^{3}H]uridine-labeled RNA rapidly disappeared from the cytoplasmic fraction following the application of chase conditions implying that the molecules which lost poly(A) were not subject to extensive cytoplasmic polyadenylation. These results are also compatible with the complete turnover of the poly(A)-bearing molecules.

The Relationship of Translation to Poly(A) Degradation. In order to further characterize the *Physarum* poly(A) degradation processes, their sensitivity to translational inhibition was examined. These studies employed cycloheximide (Cummins et al., 1965) and a 37 °C heat shock (Schiebel et al., 1969; Brewer, 1972) to inhibit protein synthesis. As shown in Figure 6, neither type of translational inhibition affects the extent of the poly(A) shortening process. The inability of translational inhibition to interfere with shortening was also established by examination of the electrophoretic mobility of the steady-state poly(A) sequences (Figure 7). In cycloheximide or heat-shocked plasmodia, as well as normal cultures, the average steady-state poly(A) chain (calculated from the data of Figure 7) was 45–50 nucleotide residues.

The poly(A) turnover process, on the other hand, appeared to be sensitive to cycloheximide but not heat-shock-induced translational inhibition (Figures 4B and 6). The cycloheximide

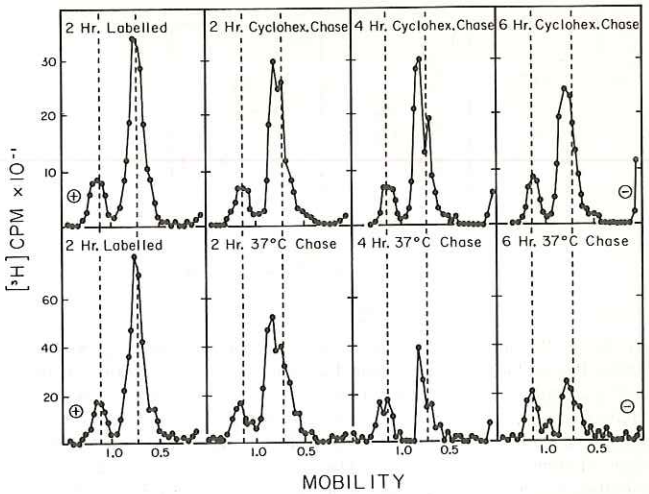

FIGURE 6: Age-dependent alterations in the electrophoretic mobility of poly(A) in *Physarum* cytoplasmic RNA during translational inhibition by cycloheximide or heat shock. Chase conducted in the presence of 20 μg/mL cycloheximide (upper frames) or at 37 °C (lower frames). Typical experiments are shown. Other details are similar to Figure 3.

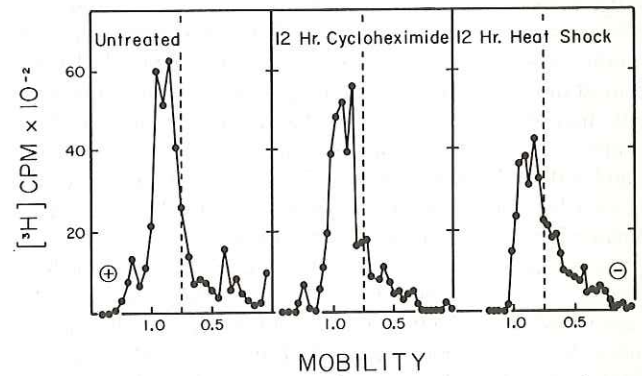

FIGURE 7: Electrophoretic mobility of poly(A) in *Physarum* steady-state cytoplasmic RNA. Cytoplasmic RNA was prepared from untreated (left frame), cycloheximide-treated (12 h, middle frame), or 37 °C incubated (12 h, right frame) cultures. Poly(A) was prepared from the RNA after RNase T_1 and RNase A treatment by oligo(dT)-cellulose selection of resistant fragments. Polyribonucleotides were separated by polyacrylamide gel electrophoresis and poly(A)-containing fragments were detected by complex formation with [^{3}H]poly(U). Dashed vertical lines represent the average mobility of 2-h-labeled cytoplasmic poly(A).

sensitivity was evidenced by the substantial decrease in the rate of labeled poly(A) disappearance in the presence of the drug (Figure 4B). The observed effect of cycloheximide on the poly(A) turnover process does not have a trivial basis, such as decreased viability of the drug-treated cultures, since the phenomenon is reversible (Figure 8). However, recovery from cycloheximide treatment may slow the turnover process since very heterogeneous polynucleotide fragments, some of which spanned the region of the gel between the characteristic positions of oligo(A) and the steady-state poly(A), appeared under these conditions.

The results of the translational inhibition experiments suggest that the poly(A) turnover process may be coupled to the protein synthetic cycle. This observation could also be explained by a nonspecific effect of the drug. In order to resolve this issue, a [^{3}H]adenosine-labeled culture was chased for 1 h at 37 °C in order to disrupt polysome structure, then cycloheximide was added and the 37 °C chase was continued for 5 h. If the effect of cycloheximide on the turnover process is unrelated to translational inhibition, very little poly(A) degradation would have been expected in this kind of experiment.

However, the results showed that less than half the original poly(A) radioactivity remained following a 6-h chase of this nature (Figure 8). Thus, the data suggest that the poly(A) turnover process, but not the poly(A) shortening process, is coupled to normal mRNA function in the polysome.

Discussion

Our results indicate that two distinct phenomena, which we have designated the shortening and the turnover processes, are responsible for poly(A) degradation during aging of *Physarum* cytoplasmic RNA. The two processes of poly(A) degradation can be readily distinguished in this organism because labeled adenosine is rapidly removed from the precursor pool during a chase. The data also imply that the poly(A) turnover process may be linked to translation in contrast to the behavior of the poly(A) shortening process.

The poly(A) shortening process which we have described in *Physarum* cytoplasmic RNA probably entails the gradual erosion of this sequence from its 3′ terminus in a similar fashion to that previously described in mammalian cells (Brawerman, 1973; Sheiness & Darnell, 1973; Merkel et al., 1976) and other lower eukaryotes (Jaworski et al., 1976; Freer et al., 1977). Furthermore, in *Physarum,* like in mammalian cells (Sheiness & Darnell, 1973; Jeffery & Brawerman, 1974; Brawerman & Diez, 1975), the shortening process does not completely destroy the poly(A) sequence since tracts consisting of about 50 nucleotide residues remain in the steady-state RNA and accumulate when the poly(A) turnover process is suppressed by cycloheximide. It is probably significant that the steady-state length of *Physarum* and mammalian poly(A) (about 60 nucleotides in mouse sarcoma 180 cells and 40 nucleotides in rabbit reticulocytes; Brawerman, 1976) is quite similar, even though *Physarum* cytoplasmic poly(A) exhibits an initial size which is considerably shorter than that found in most mammalian cells. Thus, our present findings call attention to the possible significance of the 50 nucleotides present in the 5′ sector of the poly(A) sequence. The inability of the shortening process to proceed through this region in such divergent groups as slime molds and mammals may be related to its interaction with specific proteins (Kwan & Brawerman, 1972; Blobel, 1973; Schwartz & Darnell, 1976; Jeffery, 1977) or its association with other polynucleotide sequences (Jeffery & Brawerman, 1975). It is also possible that poly(A) shortening may reach equilibrium with an opposed cytoplasmic poly(A) extension process (Slater et al., 1972; Brawerman & Diez, 1975).

The other mode of poly(A) degradation observed in cytoplasmic RNA, the turnover process, is responsible for the degradation of entire poly(A) sequences. The rapid nature of the eradication of a poly(A) sequence by this process is evidenced by the disappearance of large poly(A) without the gradual appearance of shorter fragments. Although we have no direct evidence that oligo(A) tracts are not produced as a result of poly(A) degradation, these sequences are unlikely candidates for intermediates in the turnover process since they do not appear to fluctuate quantitatively in accordance with changes in the rate of poly(A) decay and are probably contained within non-poly(A)-bearing RNA. Although intermediates of the turnover process could not be detected in cytoplasmic RNA under normal conditions, they may appear in cultures which gradually recover from the effects of cycloheximide.

To our knowledge the poly(A) turnover process we observe in *Physarum* has not been reported in mammalian cells, although such a phenomenon may well occur and have been obscured due to the large size of the precursor pools (see

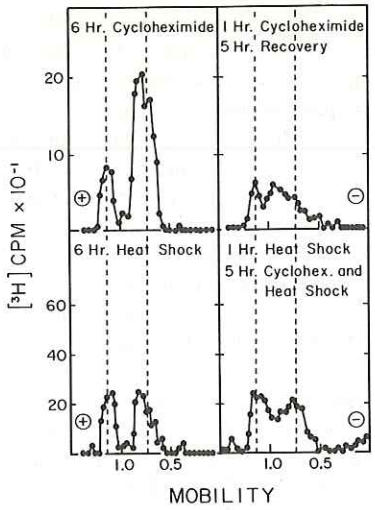

FIGURE 8: Age-dependent alterations in electrophoretic mobility of poly(A) in *Physarum* cytoplasmic RNA under various experimental conditions. Cultures were labeled for 2 h with [^{3}H]adenosine prior to the beginning of the chase in the presence of 20 μg/mL cycloheximide for 6 h (upper left frame), in the presence of 20 μg/mL cycloheximide for 1 h, then continuation of the chase for 5 h in cycloheximide-free medium (upper right frame); at 37 °C for 6 h (lower left frame), and at 37 °C for 1 h, then continuation of the heat shock for 5 h in the presence of 20 μg/mL cycloheximide (lower right frame). Poly(A) prepared from cytoplasmic RNA was electrophoresed as described in the preceding figures. Dashed vertical lines represent the average mobilities of 2-h-labeled cytoplasmic poly(A) and oligo(A). Other details are similar to Figure 3.

Sheiness et al., 1975; Figure 5). The *Physarum* poly(A) turnover process may be related to the mode of poly(A) degradation recently discovered in fertilized sea urchin eggs in which sequences associated with maternal RNA are subject to complete and rapid destruction prior to their replacement with newly synthesized poly(A) (Dolecki et al., 1977). If this is so, interference with a turnover process similar to the one we have described in *Physarum* could explain the surprising stimulation in poly(A) titer seen when sea urchin zygotes are treated with emetine (Slater et al., 1974), a drug which resembles cycloheximide in its effects on eukaryotic protein synthesis (Grollman, 1966). However, it should be noted that the *Physarum* poly(A) turnover process is distinct from that recently reported in sea urchin zygotes in that the covalently associated RNA does not serve as a primer for subsequent polyadenylation in the cytoplasm.

There are two consequences of our studies concerning the effect of translational inhibition on the poly(A) degradation processes which deserve further comment. First, we have demonstrated that poly(A) sequences which are protected from eradication during suppression of the turnover process by cycloheximide uniformly undergo the usual age-dependent shortening. Hence, the poly(A) shortening process is not restricted to a particular class of poly(A)-containing RNA which is refractive to complete turnover. Second, poly(A) turnover, but not shortening, was interrupted by cycloheximide, a drug which blocks polypeptide elongation trapping the mRNA in the polysome (Siegel & Sisler, 1964), while neither process was suppressed by a heat shock which interferes with protein synthesis by causing polysome disruption and premature mRNA liberation (Schiebel et al., 1969). Thus, it appears that poly(A) turnover is linked with the translation cycle. The cycloheximide-induced stabilization of poly(A) is clearly not a result of interference with the synthesis of a nuclease which normally catalyzes degradation since this phenomenon is not seen when protein synthesis is blocked by heat shock. In contrast to the turnover process, the *Physarum* poly(A) shortening process,

like that observed in other eukaryotic cells (Brawerman, 1973; Merkel et al., 1976; Dworkin et al., 1977) does not appear to be linked to translation since it can be carried out when poly(A) + RNA is incorporated into polysomes or free cytoplasmic ribonucleoprotein. These findings are consistent with the possibility that poly(A) shortening is caused by a component integrated within the mRNP structure whereas the factor responsible for the turnover process might be transiently associated with the mRNA involved in translation.

At present our findings concerning the two modes of poly(A) degradation cannot be evaluated in relation to the turnover of the entire mRNA molecule since the kinetics of poly(A) + RNA decay in the cytoplasm of *Physarum* plasmodia are unknown. Elucidation of the stability of these molecules will require the utilization of methods which do not rely upon poly(A)-containing RNA detection since poly(A) is rapidly lost from *Physarum* cytoplasmic RNA. In the present study we have excluded the possibility that poly(A) turnover is followed by the large-scale replacement of this sequence through cytoplasmic polyadenylation, but it is still possible that the poly(A) turnover process we describe may reflect either total mRNA degradation or selective removal of poly(A) tails from mRNA.

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