of impurities in the glutathione used in our experiments. Nevertheless, experiments are presently in progress to ascertain whether or not the reduction of cytochrome *c* by glutathione as described in this paper is subject to the action of one or more catalytic species present in our reagents.

Acknowledgments

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Messenger Ribonucleic Acid Metabolism in Mammalian Mitochondria: Relationship between the Decay of Mitochondrial mRNA and Their Poly(A)†

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ABSTRACT: The mitochondrial poly(adenylic acid)-containing mRNAs in mouse ascites cells pulsed with radioactive precursors contain 35–55 nucleotide long poly(adenylic acid) sequences. These sequences are shortened with age in cells chased with cold medium. The possible relationship between the decay rates of mRNA and their poly(adenylic acid) sequences has been investigated by using mitochondrial RNA and protein synthesis inhibitors. The pattern of mRNA decay as determined by a new solid-phase-bound complementary DNA procedure indicates the presence of two classes of poly(adenylic acid) containing mRNA in mammalian mitochondria: one decaying with a $t_{1/2}$ of 45 min and the other class with a $t_{1/2}$ of 210 min. Inhibitors such as ethidium bromide and puromycin which accelerate the decay of mitochondrial mRNA also cause an enhanced decay of poly(adenylic acid) sequences. These results have been interpreted as evidence supporting the involvement of poly(adenylic acid) sequences in the mRNA stability.

Since the first observation by Lim & Canellakis (1970) on the occurrence of 3′-poly(A)[1] in globin mRNA, such sequences have been detected in a great many eukaryotic mRNAs. (For reviews, see Darnell et al., 1973; Greenberg, 1975; Lewin, 1975.) Despite intensive efforts, however, the precise functional and/or regulatory role of mRNA-associated poly(A) remains somewhat unclear. Recent experiments by Huez et al. (1974), Sheiness et al. (1975), and Nudel et al. (1976) appear to show a close relationship between the size of 3′-poly(A) tail and the turnover rate of mRNA molecule, im-

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[1] Abbreviations used: poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); oligo(dT), oligo(deoxythymidylic acid); cDNA, complementary DNA; EDTA, ethylenediaminetetraacetate; $NaDodSO_4$, sodium dodecyl sulfate.

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plicating a direct role for poly(A) in the mRNA stability. This view is further supported by experiments showing the inhibitory properties of poly(A) on RNase activity, suggesting that 3′-poly(A) in mRNA may be involved in modulating RNase activity, which is clearly an important factor in regulating the life of mRNA molecules (Levy et al., 1975; Hieter et al., 1976).

Poly(A) sequences considerably shorter than those found in the cytoplasmic mRNA have been detected in mitochondrial RNA of diverse cell types (for reviews, see Avadhani et al., 1975; Buetow & Wood, 1978). In two previous reports from this laboratory (Lewis et al., 1976a,b), it was shown that both poly(A)-containing and poly(A)-lacking mRNAs are associated with mitochondrial polysome structures capable of carrying out in vitro translation. These results provide direct evidence for the bona fide messenger function for at least part of the organelle poly(A)-containing RNA. The mRNA species dissociated from Ehrlich ascites mitochondrial polysomes contain 35–55 nucleotide long 3′-poly(A) sequences in contrast to a 150 nucleotide long poly(A) associated with the cytoplasmic mRNA (Avadhani et al., 1974). Because of these relatively short poly(A) sequences, the mitochondrial system appears to provide a natural test model to verify any direct relationship between the poly(A) size and life of mRNA.

In this paper, a new oligo(dT)-cellulose-bound cDNA method has been used to determine the turnover rate of mitochondrial mRNA. The mitochondrial mRNAs are turned over with a short $t_{1/2}$ ranging from 45 to 210 min. Further, there appears to be a rough correlation between the decay rates of poly(A) sequences and of mRNA since the experimental conditions which cause an accelerated decay of poly(A) also tend to accelerate the degradation of mRNA.

Experimental Procedures

Materials. All reagent grade chemicals were purchased from Sigma Chemical Co. Isotopes were purchased from New England Nuclear. Oligo(dT)-cellulose (T2 grade) and poly(U)-Sepharose were purchased from Collaborative Research Laboratories and Pharmacia Fine Chemicals, respectively. Poly(A) markers of varying size were from Miles Research Laboratories. AMV reverse transcriptase was a product of Boehringer Mannheim. Electrophoretically pure pancreatic RNase and T_1 RNase were purchased from Worthington Biochemicals. Inhibitors, such as actinomycin D, puromycin, chloramphenicol, and ethidium bromide, were purchased from Sigma Chemical Co.

Ehrlich ascites hypotetraploid cells grown for 7 days in the peritoneal cavity of Swiss colony mice were used in these experiments. Details of culture maintenance and cell growth were as described before (Avadhani et al., 1974).

Pulse and Chase Conditions. Freshly harvested cells were diluted with Locke–Ringer dextrose–salts medium to obtain a cell density of about 10^7 cells/mL and incubated at 37 °C for 30 min. After the cells had been acclimatized for 30 min, actinomycin D was added at a final concentration of 0.05 μg/mL. Fifteen minutes after the addition of actinomycin D, ^{3}H-labeled cytidine (30 Ci/mmol) and [^{3}H]uridine (50 Ci/mmol) or [^{3}H]adenosine (50 Ci/mmol) were added and incubation was continued for the required length of time. For chasing, cells were pelleted at 750*g* for 5 min at room temperature, washed once with Locke–Ringer medium, and resuspended in unlabeled medium (1 part of unlabeled ascites fluid and 3 parts of Locke–Ringer medium supplemented with 20 times excess of unlabeled nucleotides) to the original cell density. The media for washing and resuspension were preheated to 37 °C.

Cell Fractionation and Isolation of Mitochondrial Poly(A)-Containing RNA. Cells were washed with isotonic NaCl solution and a hypotonic medium as described before (Lewis et al., 1976a). Washed cells were homogenized in a medium containing 0.3 M sucrose, 2 mM EDTA, 50 mM KCl, and 10 mM Tris-HCl (pH 7.5) and crude mitochondria isolated by differential centrifugation. Details of mitochondrial isolation and purification by double digitonin treatment were described before (Avadhani et al., 1974). Such preparations have been shown to be essentially free of significant microsomal fragments and cytoplasmic rRNAs (Avadhani & Rutman, 1974; Lewis et al., 1976a). Mitochondrial specific polyribosomes were prepared by the high Mg^{2+} precipitation procedure (Lewis et al., 1976a). RNA was isolated from mitochondrial or submitochondrial preparations by the $NaDodSO_4$–phenol–chloroform method, also as described earlier (Avadhani et al., 1974).

Mitochondrial poly(A)-containing mRNA was purified by the poly(U)-Sepharose chromatography (slightly modified from Lindberg & Perrson, 1972). All the reagents used for poly(U)-Sepharose chromatography were pretreated with diethyl pyrocarbonate to reduce RNase activity. Up to 700 μg of total RNA or mitochondrial polyribosomal RNA dissolved in 5 mL of 10 mM Tris-HCl (pH 7.5), 0.6 M NaCl, 1 mM EDTA, and 10% formamide was applied to a poly(U)-Sepharose column (0.5 g of swollen poly(U)-Sepharose) and allowed to pass through at a rate of about 0.1 mL/min. The column was washed with 15 mL of the same buffer containing 20% formamide. Bound RNA was eluted by washing with 5 mL of a buffer containing 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA, and 60% formamide.

Electrophoretic Analysis of Poly(A). Poly(A)-containing RNA was purified either by binding to poly(U)-Sepharose as described above or by hybridization to a cDNA column by using mitochondrial polysomal RNA as the source (Lewis et al., 1976b). Purified RNA was digested with pancreatic RNase (Worthington Biochemicals Corp.) and RNase T_1 (Avadhani et al., 1974) and the RNase-resistant fragments were electrophoresed on 12% polyacrylamide gels (Bishop et al., 1967). Markers of 25, 45, and 100 nucleotide long poly(A) segments were used to determine the size of [^{3}H]poly(A).

Preparation of Solid-Phase-Bound cDNA. Oligo(dT)-cellulose bound cDNA was prepared by using AMV reverse transcriptase by the method modified from Venetiener & Leder (1974) and Efstratiadis et al. (1975). The reaction mixture contained 50 mM Tris-HCl (pH 8.3), 100 mM NaCl, 5 mM $Mg(CH_3COO)_2$, 5 mM dithiothreitol, 1 mM each dUTP, dATP, dGTP, and dTTP, 50 μg/mL actinomycin D, 15 μg/mL mitochondrial poly(A)-containing RNA, 30 μg/mL oligo(dT)-cellulose, and 200 units/mL of RNA-dependent DNA polymerase. The mixture was stirred at 37 °C for 120 min. Removal of the RNA and the recovery of the cDNA were as described by Venetiener & Leder (1974). The reaction conditions permit almost complete reverse transcription of template RNA (Efstratiadis et al., 1975). In the present system, about 20–30% of the input RNA is transcribed into oligo(dT)-cellulose-bound cDNA.

Hybridization reaction was carried out with 1 μg of solid-phase-bound cDNA (about 14–15 mg of cDNA-cellulose) in a final volume of 2 mL and the reaction mixture contained 50% formamide, 4 × SSC, and ^{3}H-labeled RNA as specified. The mixture was incubated at 45 °C for a specified duration. The hybridization mixture was quantitatively transferred to a water-jacketed column (0.3 × 5 cm) and the column was washed with 5 mL of 50% formamide in SSC at 40 °C to elute

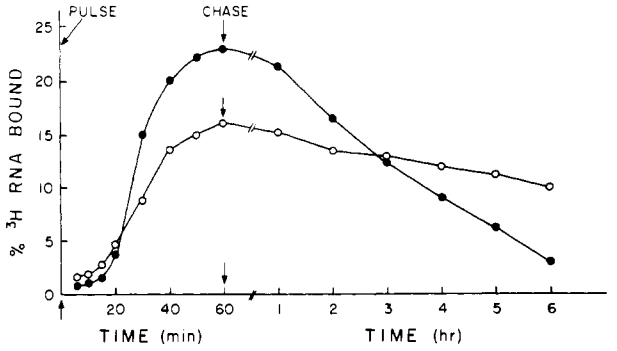

FIGURE 1: The kinetics of labeling of mitochondrial and cytoplasmic poly(A)-containing RNA. Cells were labeled with 5 μCi/mL each of [^{3}H]uridine and [^{3}H]cytidine and chased with unlabeled medium as described under Experimental Procedures. Samples (10 mL) containing about 10^8 cells each were withdrawn after intervals either during pulsing or chase and used for isolating total mitochondrial RNA (●—●) and cytoplasmic RNA (o—o). The % RNA binding to poly(U)-Sepharose was determined as described under Experimental Procedures.

unbound RNA. RNA specifically bound to the cDNA-cellulose was eluted with 1 mL of H_2O at 65 °C.

Analytical Procedures. RNA was estimated by extinction at 260 nm by using 1 mg of RNA = 25 OD. For radioactivity determinations, aqueous samples (0.5 or 1.0 mL) were counted with 10 mL of Cab-O-Sil scintillation mixture at about 30% efficiency. Polyacrylamide gel slices were swollen in NCS and counted in a toluene-based scintillation fluid as described before (Lewis et al., 1976a). All the counting was done in an Intertechnique SL-4000 counter.

Results

Because of their unique nucleotide pool characteristics, Ehrlich ascites carcinoma cells (hypotetraploid) provide a useful system for kinetic studies on nucleic acid synthesis and degradation. It has been shown that the nucleotide pools for uridine and thymidine exhibit typical self-chase characteristics (Rovera et al., 1970; Goldstein & Rutman, 1973, respectively). Unpublished results in our laboratory indicate that the cytidine pools in these cells behave very similarly to the uridine pools. However, the popular approach involving the use of transcription inhibitors to prevent reincorporation of label leads to several undefined complications in the mitochondrial system as described later in the Discussion. The free nucleotide pool was, therefore, chased with 20-fold excess of unlabeled nucleotides under conditions favorable to cell metabolism.

Labeling of Mitochondrial and Cytoplasmic Poly(A)-Containing RNA. To determine the kinetics of appearance of radioactivity in the cytoplasmic and mitochondrial poly(A)-containing RNA fractions, cells were labeled with [^{3}H]cytidine and [^{3}H]uridine at 37 °C. At intervals, samples were withdrawn and mitochondrial components isolated as described under the Experimental Procedures. The total RNA isolated from these cell fractions was analyzed on poly(U)-Sepharose. Results of such an experiment during a 60-min pulse have been presented in Figure 1. In the case of the cytoplasmic fraction, percent [^{3}H]RNA binding to poly(U)-Sepharose increases to about 14.8% as against 22.7% for the mitochondrial RNA. When the pulse labeled cells are incubated in unlabeled medium, the cytoplasmic RNA binding to poly(U)-Sepharose steadily declines to about a 10% level by 6 h. In contrast, the % of poly(A)-containing RNA in the mitochondrial fraction declines rapidly to about a 2.5% level at the end of the 6-h chase. These results suggest a marked difference in the turnover rates of mitochondrial and cytoplasmic poly(A)-containing RNA and in principle agree with the reported results of Hirsch & Penman (1974) on the oligo(dT)-cellulose binding profiles of HeLa mitochondrial RNA.

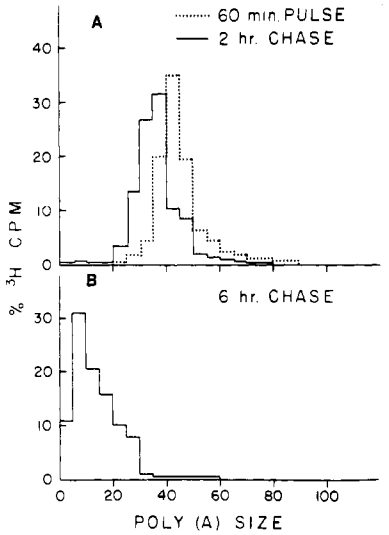

FIGURE 2: The size distribution of mitochondrial mRNA associated poly(A) during pulse–chase. Cells were labeled with 15 μCi/mL [^{3}H]adenosine for 60 min and chased for 2–6 h as described under Experimental Procedures. Mitochondrial-specific polysomes were isolated from about 5×10^8 cells, and RNA was isolated. Poly(A)-containing RNA was purified by using Poly(U)-Sepharose chromatography as described under Experimental Procedures. The RNase-resistant radioactive fragments were electrophoresed on 12% polyacrylamide gels. The electrophoretic pattern has been presented in the form of a histogram showing the size distribution of ^{3}H cpm.

Analysis of Mitochondrial mRNA-Associated Poly(A) Fragments. It is well known that the cytoplasmic poly(A) shortens progressively when pulse-labeled cells are chased either with actinomycin D or with unlabeled medium (Sheiness & Darnell, 1973; Sheiness et al., 1975; Merkel et al., 1976). Since there is no such information on the metabolic degradation of mitochondrial poly(A), it was decided to analyze the size distribution of mitochondrial poly(A) during a pulse–chase. As shown in Figure 2A, after 60 min pulsing, the RNase-resistant fraction of mitochondrial polysomal mRNA, representing the poly(A) fraction, resolves into heterogeneous size classes with about 76% radioactivity localized in the size range of 35–55 nucleotides. After a 2-h (Figure 2A) and 6-h (Figure 2B) chase, there are clearcut shifts in the size distributions, suggesting a progressive shortening of mitochondrial poly(A) with time. These results are in very good agreement with those reported for the degradation of cytoplasmic poly(A) (Sheiness & Darnell, 1973; Sheiness et al., 1975; Merkel et al., 1973).

It has been shown that ethidium bromide, a potent inhibitor of transcription, accelerates the degradation of mitochondrial RNA (Avadhani et al., 1973; Hirsch & Penman, 1974). Similarly, in HeLa cells inhibitors of protein synthesis such as emetine and cycloheximide tend to retard the decay of cytoplasmic poly(A) (Sheiness et al., 1975). In order to see if the inhibitors of mitochondrial transcription and translation have any effect on the turnover rates of mitochondrial poly(A), experiments were carried out to determine the size distribution of mitochondrial mRNA-associated poly(A) in the presence of ethidium bromide, puromycin, and chloramphenicol. The electrophoretic patterns (results not shown here) indicated that the mitochondrial poly(A) was shortened considerably faster in the presence of puromycin and ethidium bromide (see below) than in control cells without inhibitor. Because of the

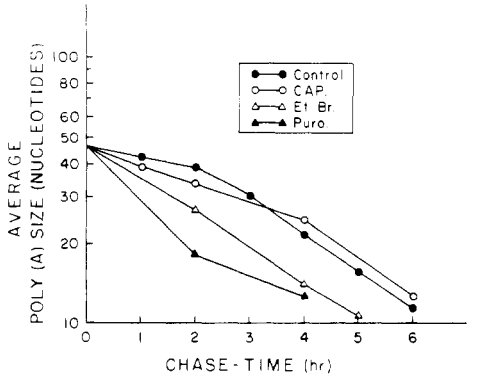

FIGURE 3: Decay of mitochondrial poly(A) in presence of inhibitors. Cells were labeled with [^{3}H]adenosine for 60 min as described in Figure 2 and chased with unlabeled medium either with or without added inhibitors. Chloroamphenicol (CAP) and puromycin (Puro.) when added were at a concentration of 100 μg/mL each. Ethidium bromide (Et Br.) was added at a concentration of 5 μg/mL. Details of isolation of mitochondrial poly(A)-containing mRNA and electrophoretic analysis of RNase-resistant poly(A) runs were described in Figure 2. The "average" poly(A) size in each sample was calculated as the summation of % ^{3}H cpm × poly(A) size class/100.

heterogeneous size distribution of poly(A) segments in these samples, a clear presentation and interpretation of results is difficult. In almost all cases, however, a considerable amount of radioactivity (60–78%) was present as a major peak cutting across roughly 3–4 size classes shown in Figure 2. It was, therefore, thought that a rough approximation of poly(A) size can be estimated by using Figure 2 data determining the "average size", i.e., (summation of % ^{3}H cpm × the size class)/100. By applying this formula to the size distribution results (as in Figure 2), the "average size" of mt poly(A) at various stages of chase either in the presence or absence of inhibitors was calculated. Results presented in Figure 3 show that chloramphenicol, an inhibitor of mitochondrial translation, has no significant effect on the poly(A) decay. Ethidium bromide, a potent inhibitor of mitochondrial transcription (Penman et al., 1970) which is also known to inhibit mitochondrial translation (Avadhani & Rutman, 1975), as well as puromycin, which is known to inhibit mitochondrial protein synthesis by dissociating the polysome structures, accelerates the rate of mitochondrial poly(A) decay.

Determination of mRNA Half-Life Using Oligo(dT)–Cellulose-Bound cDNA. Since a relatively small number of mRNAs are expected to be involved in mitochondrial protein synthesis (Mahler, 1973; Avadhani et al., 1975; Buetow & Wood, 1978; Lewis et al., 1976b), it was thought that solid-phase-bound cDNA prepared against mitochondrial poly(A)-containing mRNA might provide a direct probe to determine the life of mRNA species. Conditions which permit the full length reverse transcription of template mRNA were used to synthesize the cDNA (Efstratiadis et al., 1975). The cDNA used in these experiments contained heterogeneous strands in the range of about 2.7×10^5 to 6.9×10^5 molecular weight (results not shown).

The effect of input RNA concentration on the rate as well as the extent of hybridization to solid-phase-bound cDNA was investigated by using varied amounts of mitochondrial polysomal RNA pulse labeled for 60 min with [^{3}H]cytidine and [^{3}H]uridine. Kinetic data presented in Figure 4 indicate a saturation of hybridization in the range of 15 μg of RNA. For all the subsequent experiments, therefore, 10–12 μg of mitochondrial polysomal RNA was used to avoid any overloading of the cDNA and also to ensure a near optimal level of hybridization. The cDNA–cellulose is highly specific for mi-

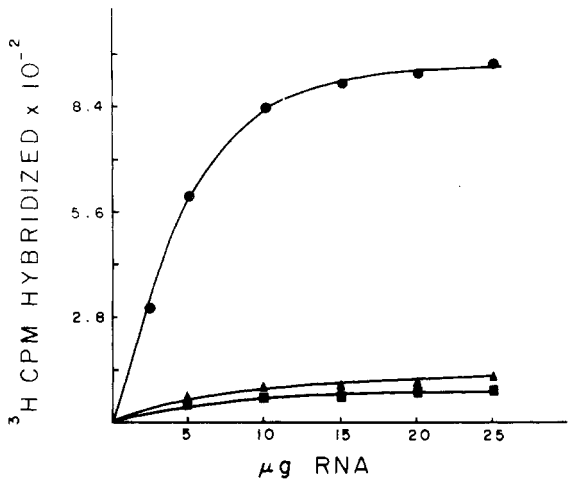

FIGURE 4: Kinetics of hybridization to oligo(dT)-cellulose bound cDNA at varied RNA concentrations of mitochondrial polysomal RNA (●—●), *E. coli* RNA (■—■), and cytoplasmic 28S rRNA (▲—▲). Mitochondrial polysomal RNA was prepared from cells labeled for 60 min with 20 μCi/mL each of [^{3}H]cytidine and [^{3}H]uridine and hybridized with 1 μg of oligo(dT)-cellulose bound cDNA for 12 h as described under Experimental Procedures. The specific activity of the RNA was about 5100 cpm/μg. The specificity of cDNA binding was verified by using ^{3}H-labeled *E. coli* RNA (8500 cpm/μg) and Ehrlich ascites cytoplasmic 28S rRNAs (6800 cpm/μg).

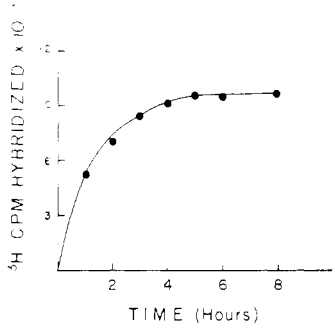

FIGURE 5: Rate of hybridization to oligo(dT)-cellulose bound cDNA as a function of time. Hybridization was carried out by using 12 μg of ^{3}H-labeled mitochondrial polysomal RNA (5060 cpm/μg) and 1 μg of oligo(dT)-cellulose-bound cDNA for varied lengths of time. The details of labeling with [^{3}H]cytidine and [^{3}H]uridine, isolation of mitochondrial polysomal RNA, and hybridization conditions were as described in Figure 4 and under Experimenal Procedures.

tochondrial mRNA since ^{3}H-labeled *E. coli* RNA or cytoplasmic 28S RNA does not hybridize significantly (Figure 4). The time course of hybridization to solid-phase-bound cDNA has been presented in Figure 5. Results, by using 12 μg of ^{3}H-labeled polysomal RNA, indicate that a saturating level of hybridization is reached in about 6 h. These optimal conditions of input RNA and time of hybridization were used to determine the turnover rates of mitochondrial poly(A)-containing mRNA.

The control decay curve for mitochondrial mRNA (Figure 6) shows a distinct break in the range of a 1–3-h chase period. These results strongly suggest the presence of at least two distinct classes of mRNA molecules in Ehrlich ascites mitochondria. One class turns over very rapidly with a $t_{1/2}$ of about 45 min and the other class turns over with a $t_{1/2}$ of 210 min. It is also seen that the rapidly turning over species may be composed of as much as 50% of the total poly(A)-containing mRNA species. Both ethidium bromide and puromycin cause rapid degradation of mitochondrial mRNA as indicated by sharp decay curves (Figure 6). In contrast, chloramphenicol, which did not have any detectable effect on the poly(A) decay

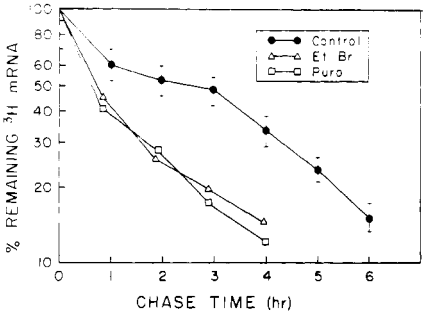

FIGURE 6: Rates of decay of mitochondrial poly(A)-containing mRNA. Cells were labeled for 60 min with [^{3}H]cytidine and [^{3}H]uridine as described in Figure 4 and chased with unlabeled medium either in the presence or absence of ethidium bromide (Et Br., 5 μg/mL) or puromycin (Puro., 100 μg/mL). Mitochondrial polysomal RNA (12 μg) was hybridized with 1 μg of oligo(dT)-cellulose-bound cDNA for 6 h, and the amount of bound [^{3}H]RNA was determined as described under Experimental Procedures. The amount of [^{3}H]RNA bound to the cDNA–cellulose at the beginning of chase in each case was in the range of 960 to 1190 ^{3}H cpm and was considered to be 100% [^{3}H]mRNA. The control values have been derived from three separate hybridization experiments, and the values for ethidium bromide and puromycin are the mean of two separate estimates.

(Figure 3), also showed no change in the mRNA decay pattern (results not shown). These results on the effects of puromycin, ethidium bromide, and chloramphenicol on the decay patterns of mitochondrial mRNA and their associated poly(A) together show that conditions which stimulate the rapid decay of the mRNA strand also cause a rapid decay of the poly(A). It is therefore possible that these two events are closely related and are regulated by a common mechanism.

Discussion

A large fraction of cytoplasmic mRNAs (Darnell et al., 1973; Greenberg, 1975; Lewin, 1975) and a great majority of monospecific, mRNAs purified from the cytoplasm contain poly(A) tracts. Such observations have led to a generally accepted notion that 3′-polyadenylation is the characteristic of cytoplasmic mRNA. This criterion was directly applied to mitochondrial poly(A)-containing RNA from varied cell types (see Avadhani et al., 1975; Buetow & Wood, 1978) to assign it a messenger role. Consequently, a previously reported study aimed at determining the turnover rate of mitochondrial mRNA was carried out with total mitochondrial RNA selected for poly(A) by using oligo(dT)-cellulose chromatography (Hirsch & Penman, 1974). The presence of poly(A)-containing RNA as large as the full complement of mt genome in HeLa mitochondria (Grohmann et al., 1978) and the occurrence of 20S poly(A) containing RNA having the properties of 17S mitochondrial rRNA precursors in BHK mitochondria (Cleaver et al., 1976) strongly suggest a precursor role for at least part of mitochondrial poly(A)-containing RNA (for discussion, see Buetow & Wood, 1978). In this paper, I have investigated the turnover rates of poly(A)-containing mRNA associated with the mitochondrial translation complex.

A majority of the published work on the decay rates of mRNA species from varied cell types has been carried out by using the oligo(dT)-cellulose binding method to determine amounts of mRNA (Greenberg, 1972; Singer & Penman, 1973; Perry & Kelley, 1973; Murphy & Attardi, 1973). This method has a serious limitation since oligo(dT) might not be recognized by some mRNAs containing short poly(A) tails. This method described in this paper is based on the hybridization of mRNA species to a solid phase bound cDNA. It has been shown in a globin mRNA system that, in the presence of excess cDNA, kinetics of hybridization is dependent on the cDNA concentration and not on RNA concentration (Levy & Aviv, 1976). In our experience, approximately 1–1.2% steady-state mitochondrial polysomal RNA binds to poly-(U)-Sepharose. The conditions of hybridization used in the present experiments, therefore, allow an mRNA to cDNA ratio of 1:10. Furthermore, overall conditions permitted maximal hybridization. The amount of radioactive RNA hybridized to the solid-phase-bound cDNA during the chase, therefore, directly reflects the decay rates. This method offers at least two distinct advantages: (1) the amount of remaining mRNA is determined on the basis of specific sequence complementarity rather than the size of poly(A); and (2) the cDNA can be used for several experiments without any detectable loss of binding ability.

Some investigators have used high levels of actinomycin D to prevent further synthesis or reincorporation of label (Sheiness et al., 1975; Singer & Penman, 1973), although pulsing with unlabeled medium in the absence of inhibitors has proved useful in the reticulocyte system (Merkel et al., 1976). It has been shown that a transcription inhibitor such as ethidium bromide causes an excessive degradation of mitochondrial RNA in several mammalian cells (Zylber et al., 1969; Hirsch & Penman, 1974; Avadhani et al., 1973). To overcome these problems, cells have been chased with large excesses of unlabeled nucleotides in a nonradioactive medium. The cytidine and uridine pools (Rovera et al., 1970; E. Dowden and R. Rutman, unpublished results) in Ehrlich cells are extremely small and self-chasing in nature. The amounts of labeled nucleotides left after pulsing and subsequent washing of the cells, or the contribution of nucleotides resulting from the decay of RNA, are relatively very small. Although the adenosine pool is considerably larger, results presented in Figure 2 indicate that the amount of radioactivity in 45–55 nucleotide long poly(A) is only about 20% after a 2-h chase and less than 3% after the 6-h chase. In view of results showing that initially synthesized poly(A) is invariably in the largest size class (Merkel et al., 1976), these results indicate that negligible [^{3}H]adenine nucleotides are reused for the synthesis of new poly(A) chains during chasing.

As reported for several cytoplasmic systems (Mendecki et al., 1972; Sheiness & Darnell, 1973; Sheiness et al., 1975; Merkel et al., 1973), in this paper I have shown that mitochondrial poly(A) is progressively shortened with age (see Figures 2 and 3). Since published work in various mitochondrial systems has indicated the degradative effects of ethidium bromide on poly(A)-containing and poly(A)-lacking mitochondrial RNA (Hirsch & Penman, 1974) in HeLa cells, it was decided to verify the effects of both RNA and protein synthesis inhibitors on the turnover rates of mitochondrial mRNA and compare them with the effects on poly(A) decay. It is seen that both ethidium bromide and puromycin caused a relatively fast decay of mRNAs (Figure 6) as well as of their associated poly(A) (Figure 3). Chloramphenicol, on the other hand, has no significant effect on both the rates of mRNA turnover and poly(A) decay. Although both chloramphenicol and puromycin inhibit mitochondrial protein synthesis (Kroon & Devries, 1971), their mode of action is entirely different. It is possible that dissociation of ribosome particles and release of mRNA by puromycin might render the molecules more open to nuclease attack.

Many of the transcription and translation inhibitors have undefined influence on mRNA synthesis and turnover. It should be recognized that the relationship between the turnover rates of poly(A) (Figure 3) and of mRNA (Figure 6) might

indeed be more complex than the results would indicate. Furthermore, the kinetic analysis method used in the poly(A) decay experiments (Figure 3) is expected to show only approximate but not absolute decay rates.

It has been shown that the majority of cytoplasmic mRNAs in varied mammalian cells turnover with a $t_{1/2}$ of 9–22 h (Darnell et al., 1973; Greenberg, 1975; Lewin, 1975). The mitochondrial mRNAs, on the other hand, show a considerably shorter life span ranging from $t_{1/2}$ of 45–210 min. This marked difference in the decay rates between mitochondrial and cytoplasmic mRNAs may be due to the size differences in their 3′-poly(A). Alternatively, it might also be due to specific regulatory features characteristic for these two cellular compartments. Nevertheless, results with transcription and translation inhibitors do indicate an approximate correlation between the rates of poly(A) decay and mRNA decay.

Extensive kinetic analysis of labeled mRNA indicated a close correlation between decay rates of poly(A) and mRNA, suggesting a nonrandom decay of the 3′-poly(A) (Sheiness et al., 1975). Although deadenylated mRNA can be translated faithfully in both in vivo and in vitro systems, the work of Huez et al. (1974) and Nudel et al. (1976) has shown that deadenylation leads to a rapid decay of mRNA. There are also reports showing that 3′-poly(A) may render protection to the mRNA by inhibiting exonucleolitic activity (Levy et al., 1975; Hieter et al., 1976). These observations support the idea that 3′-poly(A) may be a factor in regulating the life of mRNA. Results showing nearly comparable decay rates for naturally occurring poly(A)-lacking and polyadenylated mRNAs appear to argue against the role of poly(A) in mRNA turnover (Greenberg, 1972; Singer & Penman, 1973). The poly(A)-containing and poly(A)-lacking mRNAs are now known to represent distinct species (Fromson & Verma, 1976), and they may be regulated by independent mechanisms. Present results on mitochondrial poly(A)-containing mRNA decay support the hypothesis that 3′-poly(A) may be involved in the stability of mRNA.

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