J. Mol. Biol. (1973) **78**, 321–334

Messenger RNA in HeLa Cells: Kinetics of Formation and Decay

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(*Received 23 February 1973, and in revised form 4 May 1973*)

The polyadenylic acid-containing messenger RNA fraction of HeLa cells was measured by its affinity for oligodeoxythymidylate cellulose. Both the kinetics of initial labeling and the decay after a brief pulse of incorporation were examined.

The kinetics of decay are complex, but can be approximated by assuming two populations; a short-lived species with a half-life of seven hours and a long-lived component with a half-life of 24 hours. It is estimated that the short-lived material comprises 33% of total cellular mRNA, while the relatively stable species amounts to 67% of the steady-state mRNA content.

The two mRNA components with different decay times were observed simultaneously in the same cell population by measuring decay of 24-hour old mRNA labeled with ^{14}C and RNA briefly labeled with ^{3}H. The old mRNA had only a 24-hour decay component, while the new mRNA was biphasic. The decay of old and new mRNA was also observed after RNA synthesis was inhibited with actinomycin. Again, old mRNA decayed more slowly than recently labeled material. However, both decay times are significantly shorter in the presence of actinomycin and correspond to half-lives of approximately 4 and 12 hours.

There is a small but significant difference in sedimentation distribution of new and old mRNA, the old mRNA sedimenting more slowly than new material, suggesting that the more stable species has a lower average molecular weight.

The steady-state content of mRNA in HeLa cells amounts to 5·5% of the ribosomal RNA, or more than twice the amount of messenger RNA estimated to be on hemoglobin-synthesizing polyribosomes.

1. Introduction

A crucial parameter in any model of protein synthesis control is the lifetime of the messenger RNA. In prokaryotes, an mRNA molecule exists for only a few minutes and its availability regulates the synthesis of specific proteins. This report will show that in contrast to the prokaryotes, the mRNA in mammalian cells is comparatively stable. Thus, the control of transcription is probably not a major factor in the short-term regulation of protein synthesis, since in the mammalian cell the amount of mRNA would vary slowly. The regulation of protein synthesis in higher organisms may therefore involve mechanisms which control the utilization of a relatively stable mRNA population.

In *Bacillus subtilis* (as apparently in all prokaryotes), RNA transcription and messenger translation are closely coupled events. The mRNA complement turns over in

a few minutes (Levinthal *et al.*, 1962), or within 2 to 5% of a cell generation time. At the other extreme, with respect to messenger lifetime, are those eukaryotic cells which enter a state of terminal differentiation resulting in the production of only a few principal proteins. The mRNA in these special cells has a lifetime of several days. Particular examples of systems with stable mRNA are hemoglobin synthesis in reticulocytes (Rifkind *et al.*, 1964), the production of lens protein (Stewart & Papaconstantinou, 1967) and, most likely, the synthesis of silk fibroin (Suzuki & Brown, 1972).

Perhaps the most meaningful comparison between mRNAs in prokaryotic and metazoan eukaryotic systems can be made during exponential cell growth. Previous experiments, intended to determine the mRNA lifetime in cultured mammalian cells, measured the decay of protein synthesis after the inhibition of RNA synthesis with actinomycin. The lifetime of protein synthesis decay measured in these experiments was from three to four hours, compared with a cell generation time of 18 to 24 hours, indicating a minimum mRNA lifetime from 15 to 20% of a cellular generation time.

The implication that mRNA decay causes protein synthesis decay in mammalian cells in the presence of actinomycin assumes that mRNA is actually rate-limiting in protein synthesis. Recent experiments have indicated that this assumption is not valid. We have shown that there is a degradation of the translational system in the presence of actinomycin D that is not attributable to the loss of functional mRNA (Singer & Penman, 1972). On the contrary, in the presence of actinomycin the mRNA appears relatively stable, even while protein synthesis is decaying.

The direct measurement of the kinetics of mRNA formation and decay has been made possible by the discovery that a large percentage of mammalian cell mRNA contains a poly(A) segment (Edmonds *et al.*, 1971; Lee *et al.*, 1971; Darnell *et al.*, 1971). mRNA was isolated by virtue of its affinity for oligodeoxythymidylate immobilized on cellulose (Collaborative Research, Waltham, Mass.).

Two independent methods were used to measure mRNA lifetime in exponentially growing HeLa cells. The most direct method measures mRNA decay over several days after a brief labeling period. The second method compares the continuous labeling of mRNA and rRNA both initially and after the steady-state ratio of mRNA to rRNA has been reached. These two methods indicate that the kinetics of mRNA decay in human cells are not a simple exponential function, but rather, represent the decay of at least two classes of molecules that can be approximately represented as one with a half-life of about seven hours, and one with a half-life of 24 hours.

2. Materials and Methods

(a) *HeLa cell culture*

HeLa S3 cells were grown in suspension culture, as previously described (Eagle, 1959). Cell density varied between 2×10^5 and 4×10^5 cells/ml.

(b) *RNA extraction*

Cells were harvested by centrifugation at 1000 revs/min (2 min) in an International refrigerated centrifuge (model PR2). After washing in isotonic saline, cells were suspended in 2 ml of reticulocyte standard buffer (10 mM-NaCl, 10 mM-Tris (pH 7·4), 1·5 mM-$MgCl_2$). NP40 (Shell Oil Co.) was added to the suspension to a concentration of 0·5% and cells were lysed by agitation on a Vortex mixer. Nuclei were removed by centrifugation at 1500 revs/min for 2 min. RNA was extracted by the method of Penman (1966). The cytoplasmic supernatant was made 0·1 M in NaCl, 0·01 M in EDTA, and 0·5%

in sodium dodecyl sulfate, shaken with a mixture (4 ml) of phenol/chloroform (1:1) with 1% isoamyl alcohol and, after centrifugation in a clinical centrifuge, the lower organic layer was removed. The mixture was then extracted with 3 successive additions of an isoamyl/chloroform mixture (2 ml), and then the aqueous phase (sample) was removed. The above procedure was repeated 3 more times, with the exception that phenol was added directly to the sample, which was then shaken and chloroform added before proceeding. Finally, the aqueous-phase layer was removed and precipitated in 2 vol. absolute alcohol.

(c) *Fractionation of mRNA using oligo-dT cellulose*

The use of oligodeoxythymidylate bound to cellulose to obtain mRNA was reported by Aviv & Leder (1972). The method was modified as follows: cellulose bound covalently with oligo-dT polymer (Collaborative Research, Waltham, Mass.) was centrifuged in "binding" solution (400 mM-NaCl, 10 mM-Tris (pH 7·4), 0·5% sodium dodecyl sulfate) to a pellet. The volume of the pellet of packed material occupied about 100 μl. The sample was added in 150 μl of binding solution, which contained 500 μg of yeast tRNA to reduce non-specific binding, and the poly(A)-containing RNA allowed to bind to the cellulose for 2 min. The cellulose was then washed by centrifugation in successive 1-ml quantities of binding solution. After the second 1-ml wash, less than 2% of the non-binding material is still associated with the cellulose, but washing was continued 3 more times. The cellulose pellet was then washed with successive 1-ml portions of elution solution (10 mM-Tris (pH 7·4), 0·05% in sodium dodecyl sulfate). After the second 1-ml portion, less than 1% of the binding material remains. The first 2 ml of the non-binding and that of the binding material (made 0·5% in sodium dodecyl sulfate and 0·1 M-NaCl) were pooled for analysis.

(d) *Analysis of mRNA*

The pooled samples from C were layered on 15% to 30% sodium dodecyl sulfate/sucrose gradients and centrifuged in the Spinco SW27 rotor for 17 h at 25,000 revs/min (24°C). Fractions were collected through a continuous-flow spectrophotometer and analyzed for radioactivity. The fractions from the gradient of material which had been eluted from oligo-dT cellulose were collected directly into scintillation vials, and scintillant added. The fractions from the gradient of material having no affinity to oligo-dT cellulose were precipitated with trichloroacetic acid on Millipore filters. The different counting efficiencies resulting from the different methods of fraction processing were measured and a correction applied to the data. The small amount of 28 S ribosomal material contaminating the material hybridizing to oligo-dT cellulose was determined by subtracting the estimated 28 S peak from the mRNA (see, for instance, Fig. 5). It should be noted that the non-specific RNA is composed principally of 28 S ribosomal and very little of the 18 S species binds.

3. Results

(a) *Inhibition of the labeling of RNA selected by oligodeoxythymidylate cellulose by Cordycepin*

Cordycepin (3′-deoxyadenosine, Sigma Chem. Co.) has been shown to be a specific inhibitor of the labeling of cytoplasmic mRNA (Penman *et al.*, 1970). The data shown in Figure 1 indicate that most of the labeling of material selected by affinity for oligo-dT cellulose is inhibited by Cordycepin. In addition, all of the poly(A)-containing cytoplasmic RNA is retained on the oligo-dT cellulose, as previously shown (Nakazato & Edmonds, 1972) and confirmed here.

(b) *Association of polydenylic acid-containing RNA with polyribosomes*

It has been shown previously that the poly(A)-containing RNA, found in the cytoplasm of HeLa cells, is primarily associated with polyribosomes and has the

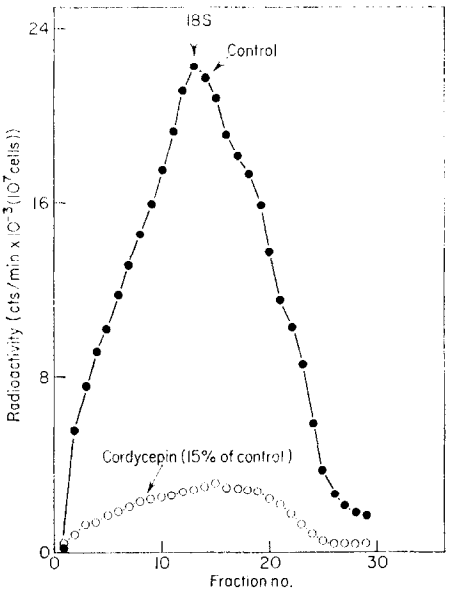

FIG. 1. Sensitivity to Cordycepin of oligo-dT cellulose-selected material.

A culture of HeLa cells was concentrated to a volume of 50 ml (2×10^6 cells/ml), and to half the sample Cordycepin (3'-deoxyadenosine, Sigma Chem. Co.) was added to a concentration of 50 μg/ml; the other half received no drug. Both samples were incubated for 2 h with 10 μCi [^{3}H]uridine/ml. The cells were lysed and mRNA was isolated from each sample, as described in Materials and Methods. Fractions of a sucrose gradient of the oligo-dT-selected RNA were analyzed for radioactivity.

—●—●—, Cells incubated in the absence of drug; —○—○—, cells incubated in the presence of Cordycepin.

properties of functional mRNA, and that essentially all this messenger is poly(A)-containing (Adesnik & Darnell, 1972; Greenberg & Perry, 1972). However, these experiments dealt with newly labeled material. In the present experiments it was necessary to establish that the poly(A)-containing RNA, which has existed in the cell for long periods of time (comparable to, or greater than, a generation time), still retains the properties of functioning mRNA. The first experiment shows that even after labeling RNA and chasing for a period of time equivalent to a cell generation time, the poly(A)-containing RNA is found to sediment with polyribosomes and to be released by EDTA. Thus, with regard to this criterion at least, both newly and long-time labeled poly(A)-containing RNA behave as though they are still functioning mRNA.

The data shown in Figure 2 demonstrate that the majority of the material associated with polyribosomes (81% for new message, 85% for old message) is released by EDTA. The material remaining in EDTA probably represents heterogeneous cytoplasmic RNA that is of unknown significance (Penman *et al.*, 1968).

The data in Figure 3 show that the RNA selected by oligo-dT affinity is found co-sedimenting with polyribosomes. The RNA was labeled for 18 hours with [^{14}C]uridine and for 90 minutes with [^{3}H]uridine and extracted from various regions of a polyribosome distribution. Only three regions are shown in Figure 3. The ratio of old to new mRNA was identical throughout the polyribosomes. The mRNA from the

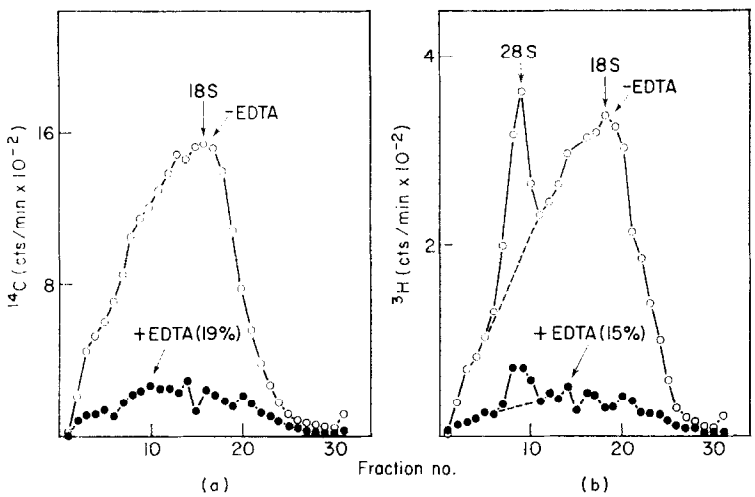

FIG. 2. Material retained on oligo-dT cellulose from polyribosomes prepared in the presence and absence of EDTA.

4×10^7 HeLa cells were labeled for 18 h with [^{14}C]uridine (0·1 μCi/ml, New England Nuclear, 40 μCi/mmol) and then pulse-labeled for 90 min with [^{3}H]uridine (New England Nuclear 20 Ci/mmol). Cytoplasm was extracted by cell lysis in NP40 (see Materials and Methods) and the nuclei removed by centrifugation. The extract was divided in half and one half made 10 mM in EDTA. The samples were centrifuged in 5-ml gradients of 15% to 30% sucrose in reticulocyte standard buffer. The EDTA was present in the gradient containing the sample treated with EDTA. Centrifugation was for 75 min at 50,000 revs/min in a Spinco SW65 rotor. The polysome fraction was isolated, RNA extracted from it and then mRNA was isolated by oligo-dT cellulose and analyzed on sucrose density gradients with sodium dodecyl sulfate buffer (see Materials and Methods). Fractions of these sucrose gradients were analyzed for radioactivity.

(a) —○—○—, ^{3}H-labeled sedimentation of mRNA isolated from normal polysomes; —●—●— ^{3}H-labeled mRNA from polysomes prepared in EDTA.

(b) —○—○—, ^{14}C-labeled sedimentation of mRNA isolated from normal polysomes; —●—●—, ^{14}C-labeled mRNA from polysomes prepared in EDTA.

different regions was added. Eighty per cent of the oligo-dT-binding RNA sediments in structures heavier than ribosomal monomers, and 14 % cosediments approximately with monomers. Six per cent of the poly(A)-containing RNA sediments with the structures lighter than monomers, but this RNA has a very different sedimentation pattern in sodium dodecyl sulfate sucrose gradient (see Fig. 3) and may be of mitochondrial origin (Perlman *et al.*, 1973).

(c) *Kinetics of labeling with uridine*

The lifetime of an unstable species of RNA is reflected in the time necessary to reach saturation of radioactivity of that species during continuous labeling. Several experiments were done using low specific activity radioactive precursors such that incorporation was continuous. Portions of the culture were removed at two, four, six and eight hours and the radioactivity in mRNA (eluant) and rRNA (wash) (see Materials and Methods) was analyzed using sucrose density gradient centrifugation. Recovery was corrected using the integrated optical density in the 28 S rRNA. Radioactivity was then plotted as a function of time expressed as incorporation of uridine into RNA per ribosome in the culture. Figure 4 shows the graph obtained

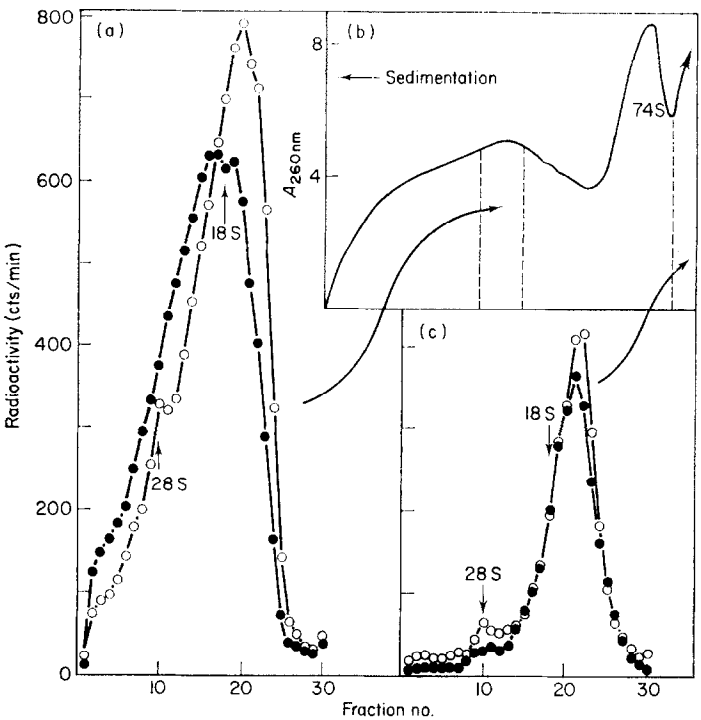

FIG. 3. Distribution and sedimentation of structures containing RNA with poly(A).

Polysomes were prepared by labeling and extracting cytoplasm as described in the legend to Fig. 2, except that no EDTA was used, and centrifugation was in a Spinco SW40 (12 ml vol.) at 40,000 revs/min for 55 min. Fractions were collected from the gradient with a Beckman-Gilford spectrophotometer continuously measuring absorbance. The gradient was divided into several pooled fractions, mRNA extracted and isolated (as described in Materials and Methods), analyzed on a sucrose gradient and measured for radioactivity.

(a) Representative gradient of ribosome-associated mRNA (94% of all mRNA) from section of polyribosomes in (c) denoted by arrows.

—●—●—, ^{3}H-labeled mRNA; —○—○—, ^{14}C-labeled mRNA.

(b) Absorbance of gradient of polyribosomes as a function of distance sedimented.

(c) The material with sedimentation values less than 70 S binding to the oligo-dT cellulose (6% of all mRNA) from section of polyribosomes in (c) denoted by arrows.

—●—●—, ^{3}H-labeled mRNA; —○—○—, ^{14}C-labeled mRNA.

from these data. The initial linearity of the points indicates that there was no apparent saturation of the mRNA species during the first six hours of the experiment, implying that a significant fraction of the mRNA has a half-life greater than six hours. It will be shown that there are at least two major components in the lifetime of HeLa cell mRNA. Therefore, the initial labeling kinetics are a composite, and thus difficult to interpret directly.

The data in Figure 4 allow us to determine the *rate* of mRNA incorporation compared to the rate for rRNA. The initial rate of labeling of mRNA compared to that of 18 S rRNA, which appears labeled in the cytoplasm approximately simultaneously with mRNA, gives a ratio of 0·74 (i.e. cts/min in mRNA = 0·74 cts/min 18 S rRNA).

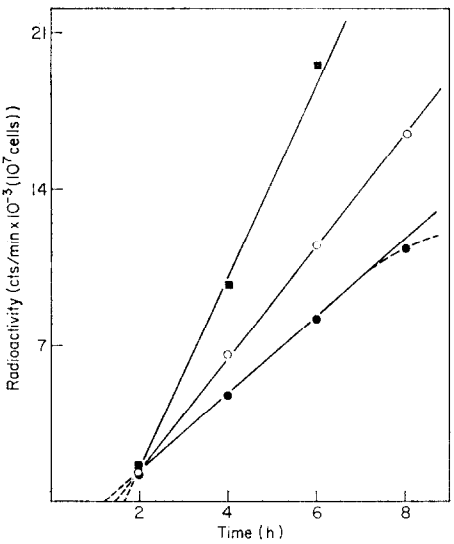

FIG. 4. Kinetics of synthesis of mRNA in uridine.
8×10^7 HeLa cells (at a density of 4×10^5 cells/ml) were labeled 5 μg uridine/ml plus 2 μCi/ml [^{3}H]uridine (20 Ci/mmol), as described in Materials and Methods. Portions were removed at specific times and cytoplasm prepared for extraction, isolation and analysis. Both the bound material and unbound material from the oligo-dT cellulose were analyzed by sucrose density gradients, and the cts/min in mRNA, 18 S rRNA and 28 S rRNA determined. The results were normalized to the 28 S ribosomal O.D. units in each sample. The increase in counts with time is shown for:
—●—●—, mRNA; —○—○—, 18 S ribosomal RNA; —■—■—, 28 S ribosomal RNA.

(d) *Steady-state ratio of messenger RNA to ribosomal RNA*

The cells were incubated in the presence of $^{32}PO_4$ for a time long enough to label all species of RNA to nearly uniform specific activity (6 days). This is the point at which nearly all RNA molecules in the cell are labeled. At this time, the "steady state" has been reached and the amount of mRNA has been measured to be 18% of the 18 S rRNA, or 5·2% (assuming that 28 S RNA is 2·5 times as large as 18 S) of the total rRNA (Fig. 5). Measurements made for different labeling times gave an approach to equilibrium in agreement with the mRNA lifetime described below.

(e) *Direct measurement of the decay of messenger RNA*

The following experiment allows a direct determination of mRNA half-life. Cells were labeled briefly with uridine and then measurements were made over a period of several days (see Materials and Methods). The ratio of mRNA to 18 S rRNA labeling is plotted in Figure 6(a) using a logarithmic scale. The data can be approximately fitted with two straight lines, one with a half-life of six to seven hours, the other having a slope which corresponds to a half-life of 24 hours. The results of this type of experiment were very reproducible, indicating the relatively constant behavior of mRNA under the culture conditions used here.

The presence of two components of mRNA decay is further examined in greater detail in Figure 6(b). mRNA was labeled first with [^{14}C]uridine and then with [^{3}H]-uridine 19 hours later (see Materials and Methods). Points were taken up to 13 hours after labeling with [^{3}H]uridine and the mRNA extracted. The isotopes represent two

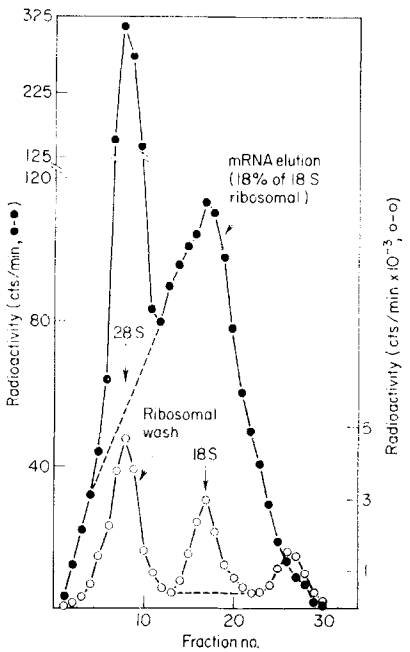

FIG. 5. Steady-state labeling of RNA.

10^5 HeLa cells were incubated for 3 days with 0·6 μCi $Na_2{}^{32}PO_4$/ml (New England Nuclear, 1 Ci/mmol), and their growth observed. At 3 days, they were diluted to 2×10^5 cells/ml with the same medium and allowed to continue growing for 3 more days. After a total of 6 days, cell growth was still continuing exponentially. The cells were harvested, cytoplasm prepared and the mRNA extracted and analyzed as described in Materials and Methods. In addition, the material not selected by oligo-dT affinity (rRNA) was analyzed by sucrose gradient sedimentation and the radioactivity in the 18 S rRNA determined. 28 S rRNA contamination was estimated and subtracted from the mRNA.

—●—●—, mRNA eluted from oligo-dT cellulose; —○—○—, material not bound to oligo-dT cellulose.

populations of mRNA, one old (^{14}C) and one new (^{3}H). The decay of both populations relative to their respective 18 S rRNA is plotted using a logarithmic scale. This method controls the data internally for variation in recovery or dilution due to cell growth. The slope of the new ^{3}H-labeled mRNA corresponds to a half-life of six hours, while that of the old ^{14}C-labeled mRNA *in the same cells* is 21 hours. Figure 6(c) illustrates that the chase becomes effective after five hours of labeling, at which time 85% of the total incorporation of [^{3}H]uridine, as measured by the 18 S rRNA, has occurred.

The extrapolation to the zero time of the two slopes in Figure 6(a) indicates the initial amounts of the two components. The intercepts at zero time give an initial ratio of radioactivity in the fast-decaying component to the slow-decaying component of 6:4, i.e. 60% of the briefly labeled message is the rapidly decaying species and 40% decays slowly.

(f) *Decay of messenger RNA in the presence of actinomycin*

A short-lived mRNA fraction would not have been seen in the previous experiments because of the long time period before a chase becomes effective. A rapidly decaying

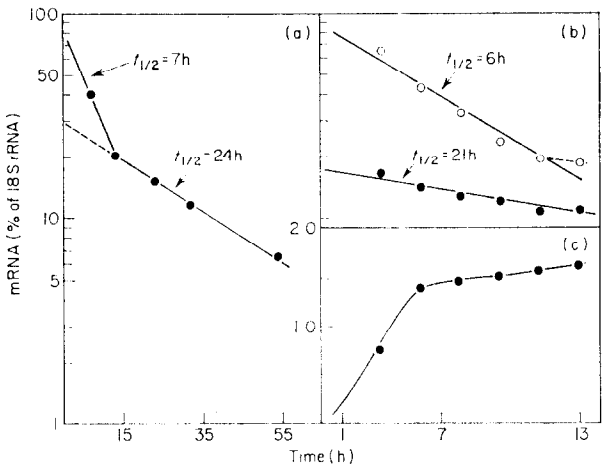

FIG. 6. Decay of mRNA after a pulse-label.

(a) HeLa cells (8×10^7) at a density of 4×10^5 cells/ml were labeled with [^{3}H]uridine (2 μCi/ml). After 3 h of incorporation, the cells were centrifuged and resuspended in fresh medium. Cell density was kept at less than 4×10^5/ml by dilution with medium. At intervals after the labeling, portions of the culture were removed for extraction of cytoplasm and isolation and analysis of mRNA (see Materials and Methods). The ratios of the amounts of mRNA to 18 S rRNA for each portion are plotted on a logarithmic scale as a function of time.

(b) HeLa cells (10^8) at a density of 2×10^5 cells/ml were exposed to [^{14}C]uridine (0·1 μCi/ml), for 18 h. At this time, 2 μCi [^{3}H]uridine/ml was added and the experiment continued for 13 h more with samples being removed at 2-h intervals after 3 h of labeling. Samples were processed for the isolation of mRNA and the amounts of ^{14}C-labeled "old" mRNA and ^{3}H-labeled "new" mRNA calculated. The new and old rRNA were also calculated from the material not bound to oligo-dT. The ratios of mRNA *versus* the 18 S rRNA of the respective isotopes were calculated and plotted on a logarithmic scale.

—●—●—, ^{14}C-labeled mRNA; —○—○—, ^{3}H-labeled mRNA.

(c) The increase of label in the ^{3}H and ^{14}C-labeled 18 S rRNA was determined for each sample in (b). For each point the ratio of ^{3}H/^{14}C-labeled 18 S rRNA is plotted on a linear scale.

component could be observed in the chase where actinomycin was used to inhibit mRNA formation.

Cells labeled 19 hours previously with [^{14}C]uridine (as above) were labeled with [^{3}H]uridine (4 μCi/ml), for one hour. Actinomycin (4 μg/ml) was added and portions were taken from the culture at intervals for six hours. The decay of [^{3}H]uridine-labeled mRNA relative to [^{14}C]uridine-labeled mRNA could then be observed. The results shown in Figure 7 indicate that the new mRNA decays more rapidly than the old mRNA, as predicted by the previous results.

The fast-decaying mRNA has a half-life of 4·5 hours, whereas the slow-decaying half-life is about 12 hours. In addition, ratios of the two decaying species when calculated on a logarithmic scale indicated a consistent decrease in the ratio of the ^{3}H *versus* ^{14}C-labeled mRNA. This result suggests that the mRNA may degrade more rapidly in the presence of actinomycin than in normal cells. However, the existence of a small, rapidly decaying component cannot be absolutely ruled out.

The data in Figure 8 show the sedimentations of the mRNA from two points four hours apart in the experiments measuring the decay in actinomycin. A comparison between the two figures indicates the relatively rapid decay in the ^{3}H-labeled mRNA relative to the ^{14}C-labeled mRNA.

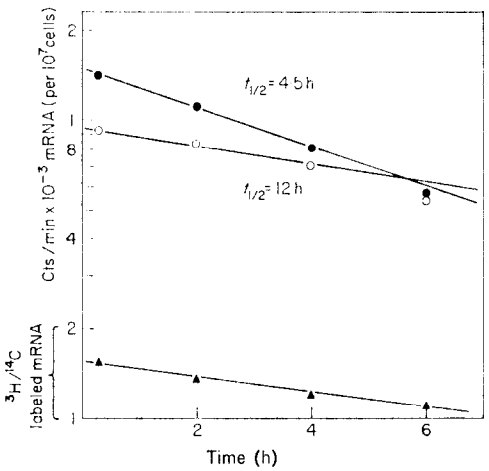

FIG. 7. Decay of mRNA during a chase in actinomycin.

A culture of HeLa cells (4×10^7 cells) at a density of 2×10^5 cells/ml was labeled with [^{14}C]uridine (0·1 μCi/ml). 18 h later, the cells were labeled with 4 μCi [^{3}H]uridine/ml. 1 h later, actinomycin D (Merck, Sharpe and Dohme) was added to a concentration of 4 μg/ml. Portions were removed and mRNA isolated by oligo-dT cellulose affinity after the extraction of the cytoplasm. The amounts of ^{14}C and ^{3}H-labeled mRNA were measured, and their ratios calculated for each point during the 6-h decay. Samples were normalized by integration of the 28 S ribosomal optical density obtained from the non-binding material from each sample. Measurements of the mRNAs and their ratios are plotted on a logarithmic scale.

—●—●—, ^{3}H-labeled mRNA; —○—○—, ^{14}C-labeled mRNA; —▲—▲—, ratio of ^{3}H-labeled mRNA to ^{14}C-labeled mRNA.

(g) *Size difference of new and old messenger RNA*

The data in Figures 3 and 8 show a small but reproducible difference in the sedimentation of old and new mRNA. The most recently labeled material is somewhat larger on the average than the mRNA which is 24 hours old. At present it is not possible to determine whether this is due to a different size at the time of synthesis or to a limited reduction in size during the time spent in the cytoplasm. Similar results have been obtained with mouse cells.

4. Discussion

The results reported here represent the extension of previous observations which indicated that the lifetime of mRNA in mammalian cells was very long, using the cell generation time as a time scale (Singer & Penman, 1972; Greenberg, 1972). Greenberg described the kinetic behavior of the poly(A) segment in L cells, which are derived from mice. He found a ten-hour lifetime in cells whose generation time is approximately 14 hours. We have previously reported that the mRNA of HeLa cells decays much more slowly that the rate of protein synthesis after inhibition of RNA synthesis by actinomycin. This finding clearly indicated that the decay of protein synthesis in the presence of actinomycin is not a measure of mRNA metabolism.

Previous work was done using poly(U) immobilized on glass fiber filters for selecting poly(A)-containing RNA. In our experience, the glass fiber filter technique has

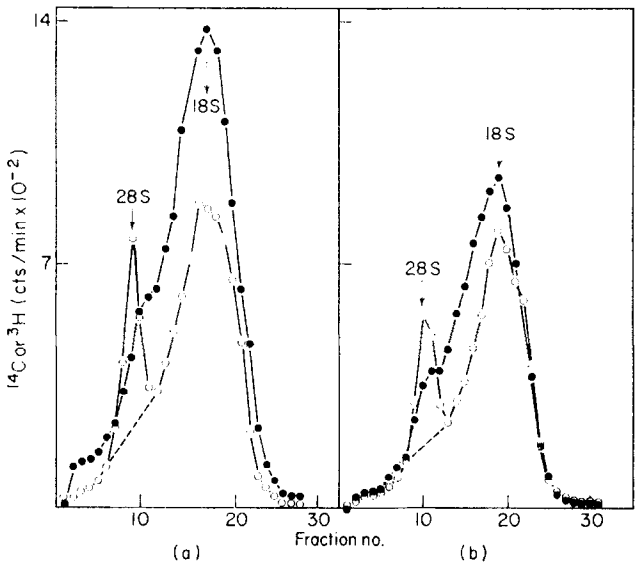

FIG. 8. Sedimentation profiles of mRNA decay in actinomycin.

The procedure was the same as described in Fig. 7. Two sedimentation profiles are included from 2 samples at 25 min and 4 h of actinomycin decay to illustrate the more rapid decay of the ^{3}H-labeled mRNA.

—●—●—, ^{3}H-labeled mRNA; —○—○—, ^{14}C-labeled mRNA.

(a) Sedimentation of mRNA after 25 min in actinomycin; (b) sedimentation of mRNA after 4 h in actinomycin.

not afforded the reproducibility necessary for the precision desired for the experiments described here. The availability of oligo-dT cellulose increased the reproducibility of the poly(A) affinity technique and made the experiments described here possible. One pitfall when using oligo-dT cellulose for measuring very small amounts of mRNA in the presence of vastly greater amounts of ribosomal RNA is the nonselective binding of ribosomal RNA to the cellulose. This was largely avoided by more extensive extraction with phenol and by the addition of a vast excess of yeast tRNA to the cellulose–sample mixture.

It is worth emphasizing the meaning of messenger half-life in these experiments. The results of the chase experiments are expresed as a ratio of mRNA to labeled rRNA. We have assumed that rRNA is stable in these cells for at least 54 hours. Previous reports have indicated that rRNA is indeed stable in growing cells (Emerson, 1971). In these experiments, it was found that the decrease in rRNA specific activity during a long chase could be accounted for completely by the increase in rRNA due to cell growth, indicating the probable stability of rRNA. An earlier report showed the absence of rRNA precursor labeling after an extensive chase period (Penman, 1966), indicating very little breakdown and re-utilization in exponentially growing HeLa cells.

The comparison of radioactivity in mRNA to that in a stable RNA species yields a lifetime that is independent of cell growth. Thus the lifetime of a molecule is measured, rather than the content per cell. The dilution of the radioactive species by

normal cell growth is removed from the results and the kinetic behavior is more easily understood.

Decomposition of the data presented in Figure 6 leads to two decay curves which can be fitted by the assumption of two populations, with a half-life of seven hours for the short-lived component and one with an approximately 24-hour half-life. However, the data do not exclude the existence of a range of lifetimes. It is interesting to note that the cell generation time in these cultures is approximately 24 hours.

The observation of two lifetimes implies that old messenger RNA and newly labeled material behave differently. That this is indeed the case is emphasized by the data shown in Figures 6 and 7, where the decay of mRNA which is about one day old is compared to the simultaneous decay of newly labeled material. In Figure 7, the decay of mRNA populations is compared in the presence of actinomyin. In this case the decay times are somewhat shorter than in a chase experiment, but the very different behavior of old and new mRNA is readily apparent.

The relative amounts of rapidly decaying and slowly decaying mRNA in a pulse-label is estimated by extrapolating the decay curves of Figure 6 back to zero time. This is only approximate, since an error arises from the finite length of labeling time. However, the error introduced is probably smaller than the precision of the experiment. It is estimated that newly formed mRNA is composed of 60% rapidly decaying component and 40% stable component. Of course, the steady-state content of mRNA in the cells is quite different; it is estimated that 33% of total cellular mRNA is comprised of the rapidly decaying component and 67% of the relatively stable species.

The observation of two distinct lifetime components appears to be at variance with the report of Greenberg (1972) in which mRNA kinetics were measured in L cells. The difference may be the result of the different cell type. Also, the approach-to-equilibrium method used by Greenberg may not have been sensitive for a long-lived component, especially if such a species were smaller in amount than that in HeLa cells. There is an earlier report by Cheevers & Sheinin (1970) which describes two components of mRNA in mouse cells, one of a two-hour half-life and the other greater than six. This work antedated the poly(A) affinity technique and possibly the mRNA fraction was contaminated.

The steady-state ratio of mRNA to rRNA appears to be rather large. The value of 5·2% is considerably larger than the value of 2·2% reported for the ratio of mRNA to the ribosomes synthesizing hemoglobin (Lodish, 1971). This apparent discrepancy could be accounted for by either a considerable amount of mRNA which is not on polyribosomes or by a significantly lesser packing of ribosomes on mRNA in HeLa cells. The first possibility seems unlikely in view of the data presented here, which indicate that a large majority of poly(A)-containing mRNA (94%) is associated with polyribosomes. The second possibility is more difficult to rule out.

It should be noted that the half-life of the short-lived mRNA has not been corrected for the admixture of the long-lived component. Such a correction would yield a slightly larger value for the half-life. The accuracy of the data is probably not adequate to make the correction meaningful.

The oligo-dT cellulose technique described in this work quantitatively isolated all poly(A)-containing molecules (Darnell *et al.*, 1971; Edmonds *et al.*, 1971; Lee *et al.*, 1971) and an examination of the non-binding material showed that it contained no poly(A) material. This does not exclude the possibility that mRNA comprises some

fraction of the non-binding material, because of the absence of poly(A) on these messengers. The technique of measuring mRNA decay used in this work does not exclude the possibility that mRNA is eventually deadenylated.

The lifetime measurements described here emphasize that in mammalian cells, at least, the supply of mRNA is probably not the rate-determining factor on protein synthesis. Relatively slow variations of mRNA content cannot account for relatively dramatic changes in rates of protein synthesis. It is therefore very likely that a translational level control exists, very probably at the level of initiation of translation (McCormick & Penman, 1969).

Some protein products, specific to cell differentiation, appear to be synthesized on long-lived mRNAs (McAuslan, 1963; Stewart & Papaconstantinou, 1967; Marks *et al.*, 1962; Kafatos, 1972). Estimates of these half-lives vary from 24 hours to 100 hours, depending on the system. It appears that these lifetimes are quantitatively but not qualitatively different from the long-lived component of HeLa mRNA. There are models of differentiation which depend on mRNA differential stability (see, for instance, Kafatos, 1972). These models would be equally well served by a differential stability of a translation control mechanism. It may be noted, however, that the presence of at least two populations of mRNA with different lifetimes within a single cell type indicates that a mechanism for selective stability of mRNA exists.

This work was supported by grants from the National Institutes of Health (CA-08416-06 and CA-12174) and the National Science Foundation (GB-27684). One of us (R. S.) expresses appreciation to the Damon Runyon Fund for Cancer Research for their generous support and to the American Cancer Society for their assistance in continuing this research to its conclusion.

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