Eur. J. Biochem. 88, 395–402 (1978)

Stability of Polyadenylated RNA in Differentiating Myogenic Cells

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(Received January 26, 1977/March 22, 1978)

Three independent methods of measurement showed that cytoplasmic polyadenylated RNA from the differentiating myogenic cell line L8 consists of two main populations with regard to stability, one with a half-life of less than 4 h and the other with a half-life of 17–54 h. Similar results were obtained in the presence and absence of actinomycin D. During the fusion of mononucleated myoblasts into multinucleated fibers, there was an increase in both the steady-state pool of the more stable polyadenylated RNA and the proportion of stable polyadenylated RNA synthesized in pulse labelling.

In the last few years, techniques have become available for the isolation of messenger RNA by virtue of molecular hybridization of a 3′-terminal polyadenylate residue, poly(A), on the mRNA with a cellulose-bound residue of oligothymidylate, oligo(dT) [1]. Analysis of the kinetics of synthesis and decay of polyadenylated RNA in HeLa cells isolated in this way [2,22] showed that there existed two populations of RNA: a labile population with a half-life of 7 h, constituting 33% of the steady-state pool of polyadenylated RNA, and a more stable population with a half-life of 22 h (generation time of HeLa cells), constituting 67% of the steady-state pool. The stable RNA had a smaller average molecular size than the more labile population. The aim of the present work was to undertake a similar kind of analysis with differentiating cells, myoblasts, in order to ascertain whether such two populations of mRNA exist in these cells and whether they may be implicated in the process of cellular differentiation.

Although no systematic analysis has been undertaken of the stability of mRNA in differentiating cells, two lines of evidence indicate that proteins specific to differentiated cells may be coded for by long-lived mRNA. The first is a result of measurement of the accumulation of label into mRNAs in differentiated cells which produce large amounts of specific proteins such as ovalbumin [3], silk fibroin [4], and hemoglobin [5]. These measurements indicated that all these mRNAs have half-lives of the order of many hours. A second line of evidence has been obtained using actinomycin D and observing the continuation of protein synthesis. Research involving the use of actinomycin in differentiated cells has indicated stability of protein synthesis when RNA synthesis is suppressed [6–9].

There are several indirect indications for the involvement of stable mRNA in the differentiation of skeletal muscle cells. Multinucleated myofibers are relatively resistant to the effects of inhibition of RNA synthesis by actinomycin D. Fibers exposed to actinomycin D continue to contract and incorporate labelled amino acids for many hours [10–12]. Moreover, cell fusion, increase in the synthesis of myosin and in the activity of enzymes following cell fusion take place in spite of the presence of actinomycin D [13]. Buckingham et al. [14] have reported that a subpopulation of mRNA becomes more stable in muscle cells during differentiation.

In order to examine the stability of mRNA during the differentiation of muscle cells, a quantitative study was made of the kinetics of polyadenylated RNA decay in actinomycin-D-treated and untreated muscle cultures.

The cells used in most of the experiments were from a myogenic cell line, L8, of rat skeletal muscle origin [15,16]. This is a homogeneous, cloned population, and when it is plated at 2×10^5 cells/100-mm culture dish, it proliferates rapidly until confluent, then ceases growing. About 30 h later, the cells start to fuse and form multinucleated fibers. Fusion proceeds rapidly over the course of 2 days and is closely followed by the synthesis of muscle-specific proteins [12,13,16]. At the end of this period over 70% of the cells have fused into myotubes. The cultures subsist for several more days before showing signs of degeneration. The obvious morphological stages during the differentiation of these cultures make this system very convenient for a study of quantitative changes in metabolism of mRNA during cell differentiation.

MATERIALS AND METHODS

Cell Cultures

The myogenic cell line L8 was grown as described elsewhere [16, 17]. After removal by trypsin, cells were plated at an initial density of 2×10^5/100-mm plastic tissue-culture plate, in Waymouth's medium supplemented with 10% horse serum. Medium was changed every third day as well as 3 h prior to any experiment. Cultures taken for experiments prior to fusion contained a homogeneous population of mononucleated cells. In cultures at the postfusion stage, over 70% of the nuclei were in multinucleated fibers.

Chicken myoblasts were obtained from 12-day-old chicken embryo breast muscle, as described by Paterson et al. [18], and plated at a density of 3×10^6 cells/100-mm plate in Eagle's medium supplemented with 2% embryo extract and 10% horse serum. All culture plates were coated with a gelatin solution (0.1 mg/ml) before use.

Determination of the amounts of DNA in cultures was done following the procedure of Burton [19]. Determination of protein was performed according to Lowry et al. [20].

Labelling and Chasing

Cells were labelled with [^{3}H]uridine (Amersham, England) at a final concentration of 10 μCi/ml (20 Ci/mmol). For dilution of the cellular uridine pools, cells were exposed to final concentration of 5 mM uridine and 20 μM cytidine (Sigma), after washing with fresh medium. For procedures involving suppression of RNA synthesis, 200 μg/ml of actinomycin D (Calbiochem), made fresh in sterile phosphate-buffered saline, was added to the plates to a final concentration of 4 μg/ml.

Extraction of RNA

At the appropriate times, cells were washed thrice in cold buffer 1 (250 mM NaCl, 10 mM $MgCl_2$, 10 mM Tris pH 7.5 [21]) and removed from plates by scraping with a rubber policeman, in the presence of buffer 1 containing 1% of the detergent NP-40 (a gift from Paz Oil Company). The cells were gently pipetted to ensure complete disruption and nuclear and cytoplasmic fractions were separated at $2000 \times g$ for 3 min. The cytoplasmic supernatant was removed and made 1% in sodium dodecyl sulfate with a 20% stock solution and 10 mM in EDTA with a 0.2 M stock solution. The solution was deproteinized by the phenol/chloroform method, as described by Singer and Penman [2] and RNA was precipitated with two volumes of ethanol.

Isolation of Polyadenylated RNA

Polyadenylated RNA was isolated as described by Singer and Penman [2] on oligo(dT)-cellulose (type T-3, Collaborative Research, Waltham, Massachusetts). The oligo(dT)-cellulose was tested to accept 1.2 mg of polyadenylated RNA/g and rebind more than 85% of this isolated material. The oligo(dT)-cellulose was used in batches of 15–25 mg and the RNA from each sample in a volume of 100 μl was mixed with the wet oligo(dT)-cellulose in the presence of 250 μg of tRNA (Calbiochem) to reduce nonspecific binding of rRNA. Under these conditions, contaminating RNA species were found to comprise less than 10% of the total material binding to the oligo(dT)-cellulose (see also [22, 23]). The isolated RNA was bound to the oligo-(dT)-cellulose in 'binding' buffer (400 mM NaCl, 10 mM Tris pH 7.4, 0.5% sodium dodecyl sulfate) and then the slurry was washed and centrifuged in four small volumes of 'elution' buffer (10 mM Tris pH 7.4, 0.05% sodium dodecyl sulfate). The eluate, containing the isolated polyadenylated RNA, was subsequently made 100 mM NaCl and 1% in sodium dodecyl sulfate and precipitated with two volumes of ethanol. When necessary, 50 μg of tRNA were added for coprecipitation. Several batches of oligo(dT)-cellulose from Collaborative Research were tried until one was found which adequately bound polyadenylated RNA using this technique.

Formamide Gel Electrophoresis

Polyacrylamide gels were made with standard stock solutions but substituting formamide (Fluka Chemicals, F.R.G.) for water. The formamide was deionized by stirring with an ion-binding resin and then filtered through sintered glass. Gels were buffered at pH 9 (NaOH). The ethanol-precipitated RNA samples were centrifuged at $15000 \times g$, dried and resuspended in formamide for electrophoresis. Unlabelled rRNA (18 S and 28 S) was added to each gel as marker. The maximum acceptable amount per gel without overloading was 20 μg of RNA not including tRNA coprecipitated (up to 50 μg). Electrophoresis running buffer contained 20 mM diethyl barbituric acid, pH 9 (NaOH) and was recirculated because of the weak concentration of electrolytes. Gels were electrophoresed (125 V, 5 mA per gel) at room temperature for approximately 4 h. At this time, 18-S ribosomal RNA had moved 3.5 cm and 28-S rRNA 1.8 cm. The gels were stained with 0.1% pyronine in 0.1 M citric acid and 1% acetic acid, then equilibrated and destained in 15% glycerol for slicing. The stained bands of marker molecules were marked with Indian ink prior to slicing. Gels were frozen, sliced (1 mm) and hydrolyzed for 2 h at 50 °C in 0.2 ml NCS solubilizer (Amersham, Searle). The samples were counted

for radioactivity in a toluene-based scintillant. Efficiency for tritium was 36%.

Plotting of Decay Curves

Decay curves were evaluated by biexponential least-square fit, kindly programmed for us by Dr G. Yagil.

RESULTS

Mobility of Polyadenylated RNA on Gels

Without the use of formamide in the acrylamide gel electrophoresis system, consistent results were unobtainable due to the aggregation of the RNA. Part of the RNA did not enter the gel and a broad smear of high-molecular-weight material was evident. Moreover, the polyadenylated RNA which did enter the gel yielded spurious, nonreproducible peaks. Aggregation was also suspected in sodium dodecyl sulfate/sucrose gradients. In the presence of formamide, all the polyadenylated RNA entered the gel and the profile revealed a heterodisperse, reproducible population of molecules with a peak at 24–26 S (Fig. 1). Sharp, homogeneous peaks of rRNA, tRNA and 5-S RNA were obtained by this method. The heterodisperse distribution of polyadenylated RNA is similar to that of HeLa cells [2]. Little radioactivity was detected above background level between the 12-S and 4-S regions of the gel, indicating that there were no significant molecular breakdown products.

The size distribution of polyadenylated RNA from muscle cells when analyzed by this method did not appear to exhibit significant qualitative changes during cell fusion. The polyadenylated RNA population, irrespective of cell fusion, showed a heterogeneous range in molecular weight from about 1.6×10^6 to about 0.4×10^6 and it was similar to polyadenylated RNA from mouse L cells which was extracted and analyzed with identical methods.

Aside from the main peak of the heterodisperse population, there was only one other prominent peak migrating at nearly 18 S (M_r 0.6×10^6). This peak increased in percentage of total mRNA during fusion. The nature of this fraction needs further investigation.

Effect of Actinomycin D on RNA Synthesis and Cell Viability

The concentration of actinomycin D used in all experiments was 4 μg/ml. This concentration inhibited within 30 min over 90% of total [^{3}H]uridine incorporation into the cells. No more than 6% of cytoplasmic RNA (polyadenylated and nonpolyadenylated) was synthesized in the cells during the first 2.5 h of exposure to the drug, as compared to untreated cells (Table 1). The same inhibitory effect was exerted in both unfused and fused cultures. L8 cultures survived in the presence of actinomycin D for over 30 h without any significant loss of cells. Likewise, no significant decrease in amount of DNA or of protein could be detected in cultures after long periods of exposure to actinomycin D (Fig. 2). The results were similar for fused and unfused cultures. Therefore, in the actinomycin-D-treated groups expression of the results per plate is equivalent to expressing them per weight of DNA.

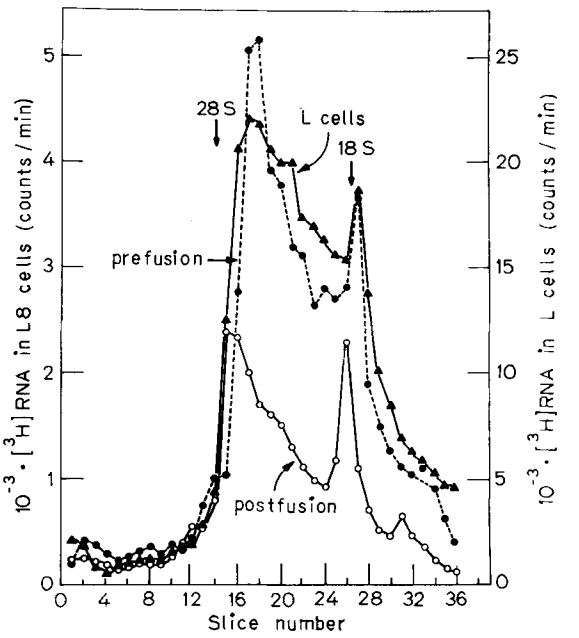

Fig. 1. *Formamide gel electrophoresis of polyadenylated RNA from myogenic cells and fibroblast L cell line.* One 100-mm plate of L8 cells 24 h prior to fusion, and one plate of L8 cells 24 h after onset of fusion were labelled with 10 μCi/ml of [^{3}H]uridine (50 μCi/plate) for 2 h. Three 60-mm plates of mouse L cell line (3×10^6 cells/plate), 2 h after growth was stimulated by fresh medium, were labelled with the same concentration of [^{3}H]uridine as the myogenic cells. All samples were extracted and polyadenylated RNA isolated and analyzed by formamide gel electrophoresis (Materials and Methods). The gel was sliced at 1-mm intervals and each slice was incubated in NCS solubilizer (Amersham, Searle) at 50 °C for 3 h and the radioactivity counted in a toluene-based scintillant. (●---●) L8 prefusion; (○——○) L8 postfusion; (▲——▲) L cells

Decay of Polyadenylated RNA in Cultures Treated with Actinomycin D

The rate of decay of polyadenylated RNA was measured in actinomycin-D-treated cultures. As can be seen from Fig. 3, a biphasic curve was obtained suggesting (by simplest approximation) the existence of two major populations of polyadenylated RNA.

When the half lives of the two components were calculated after subtracting one from the other at each point, the following values were obtained for the labile component: 3.7, 2.8, and 2 h in experiments made prior to, during, and after fusion of cultures, respec-

Table 1. *Inhibition of synthesis of cytoplasmic RNA by actinomycin D*
Four plates of unfused cultures and eight plates of fused cultures were taken for the experiment. At zero time, actinomycin D (drug) was added (4 μg/ml) to two plates of unfused cells and to four plates of fused cells; 30 min later [^{3}H]uridine was added (10 μCi/ml) for 2 h to two treated and two untreated plates of each group. The rest of the plates were labeled in the same way 26 h later. At the end of incubation, cytoplasmic RNA was isolated and separated into poly(A)-containing RNA and poly(A)-free RNA, by binding to oligo(dT)-cellulose. The radioactivity precipitable by trichloroacetic acid of each population of RNA was monitored in toluene scintillation fluid and the percentage incorporation into treated cultures was calculated in comparison with untreated cultures. n.d. = not determined

Time with drug	Fraction of cytoplasmic RNA	Incorporation of ^{3}H/plate of					
		prefusion stage			postfusion stage		
		− drug	+ drug	residual	− drug	+ drug	residual
h		counts/min		%	counts/min		%
2.5	− poly(A)	196250	4375	2.2	142950	2800	1.9
	+ poly(A)	42900	1240	2.9	35190	1990	5.6
28	− poly(A)	n.d.	n.d.		197320	180	0.09
	+ poly(A)	n.d.	n.d.		35000	1940	5.5

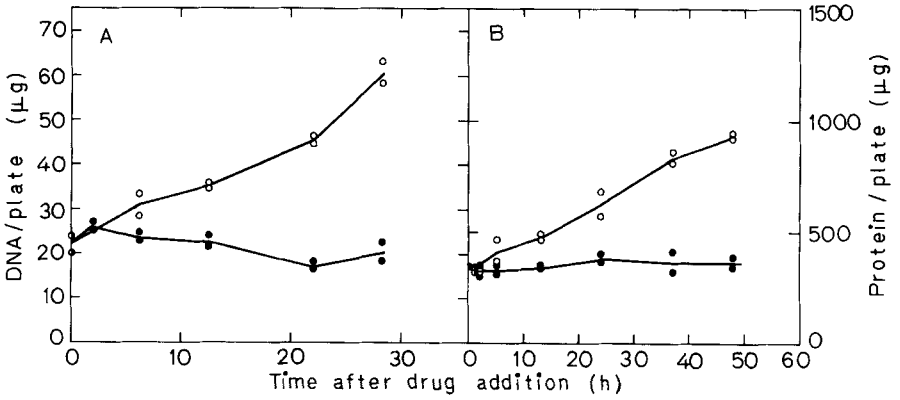

Fig. 2. *Effect of actinomycin D treatment on amount of (A) DNA and (B) protein in the cells.* Proliferating cultures of L8 cells were taken for the experiment. At zero time, actinomycin D (4 μg/ml) was added to half of the plates (●) and the rest were left untreated (○). At different times after addition of the drug, two plates were removed from each group, rinsed with cold phosphate-buffered saline and frozen at − 20 °C. When all the plates were collected the amounts of DNA (A) and protein (B) were determined

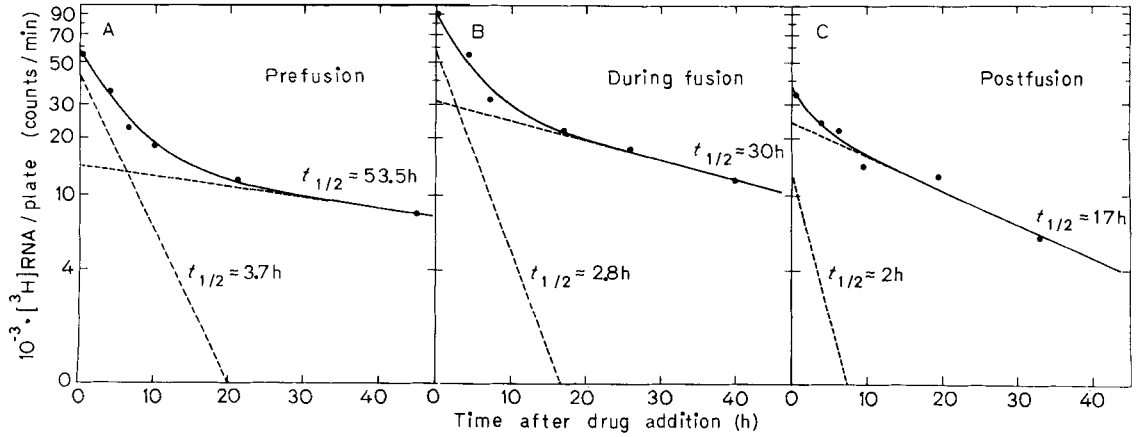

Fig. 3. *Decay of polyadenylated RNA in actinomycin-D-treated cultures.* 24 plates of L8 cells were divided into three groups. Six of each group were labelled at chosen times with 10 μCi/ml of [^{3}H]uridine (50 μCi/plate) for 2 h and then 4 μg/ml of actinomycin D was added. Two plates were left untreated for determination of the onset of cell fusion. Each of the three groups represents a different stage in the differentiation. In (A) the cells did not enter fusion until after the last decay point; in (B) fusion was first detected at 8 h after beginning the experiment and in (C) 24 h before beginning the experiment. Plates were removed at designated intervals after addition of actinomycin D, polyadenylated RNA was isolated and its radioactivity measured. The broken lines show the decay of each component of the curve after subtracting one from the other

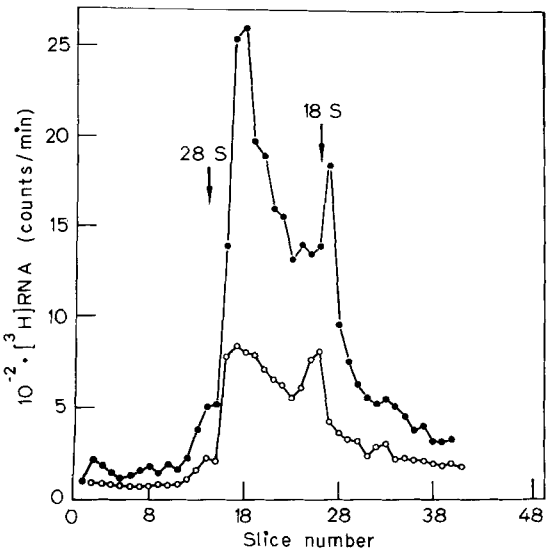

Fig. 4. *Formamide gel electrophoresis of polyadenylated RNA isolated from actinomycin-treated cells.* Two plates of prefusion L8 cells were labelled for 2 h with [^{3}H]uridine, 10 μCi/ml (50 μCi/plate), and to both plates were added 4 μg/ml actinomycin. One plate was extracted after 20 min in actinomycin (●——●) and one after 8 h (○——○). Polyadenylated RNA was isolated and analyzed by formamide gel electrophoresis. Gels were sliced at 1-mm intervals

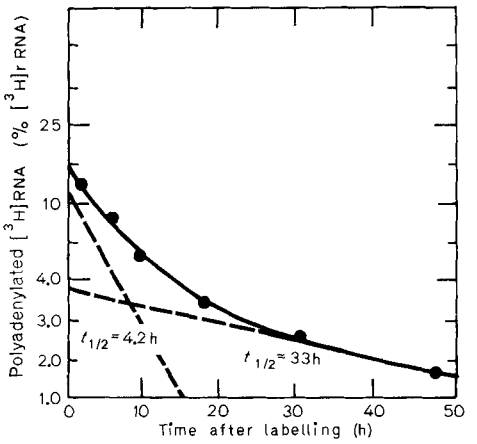

Fig. 5. *Decay of polyadenylated RNA in the presence of diluting concentrations of uridine.* Six plates of fused L8 cultures were labelled with [^{3}H]uridine (10 μCi/ml, 50 μCi/plate) for 2 h. Then 5 mM uridine and 20 μM cytidine were added. Plates were removed at varying intervals and polyadenylated RNA was isolated. The polyadenylated RNA radioactivity is expressed as a percentage of that of rRNA

tively. The values for the stable component were 53, 30, and 17 h respectively. The significance of the corresponding decrease in stability of both polyadenylated RNA populations during the course of differentiation is unknown.

The rate of synthesis was estimated for each population of polyadenylated RNA. The intercept point of each decay line with the zero time axis gives the proportion of incorporation into each component at the time actinomycin was added. The experiments indicate a change in the rate of incorporation into each polyadenylated RNA population during the course of differentiation. The labile component constitutes 74% of the newly synthesized polyadenylated RNA before fusion and only 34% after fusion.

Gel electrophoretic analysis of the polyadenylated RNA extracted from prefusion cultures after 8 h in actinomycin showed a decrease in the large-sized molecules of RNA compared to control cultures not exposed to actinomycin (Fig. 4). The resultant profile is reminiscent of the polyadenylated RNA extracted from cells after fusion (Fig. 1). Since most of the polyadenylated RNA which remains in prefusion cells after they are exposed to actinomycin D for 8 h is stable (Fig. 3A), then the profile of polyadenylated RNA extracted from cells postfusion may resemble the stable polyadenylated RNA present in prefusion cells.

Uridine Chase Experiments

It was of importance to check whether the application of actinomycin D affects in some way the stability of polyadenylated RNA [24]. For this purpose, we measured the decay of pulse-labelled RNA in cultures not treated with actinomycin D after diluting the cellular uridine pool with high amounts of unlabelled uridine [25]. The radioactivity in polyadenylated RNA was calculated and plotted as a percentage of that of rRNA which is very stable and provides an internal calibration for polyadenylated RNA (a mathematical justification for this method is discussed by Spradling et al. [25]). These experiments also show biphasic kinetics for the decay of polyadenylated RNA, similar to the results obtained with actinomycin D treatment (Fig. 5). Some variability in the half lives of the more labile components may be related to a delay in the manifestation of effect of the uridine chase versus a more rapid effect of actinomycin. The results indicated that actinomycin had no significant effect on the decay kinetics of polyadenylated RNA in these cultures.

A chase with excess uridine was also applied to study the decay of polyadenylated RNA in cultured muscle cells from chicken breast. Since these cells are very sensitive to the toxic effect of actinomycin, we could not use it in experiments with these cells. As shown in Fig. 6, polyadenylated RNA from chicken breast muscle cultures has the same biphasic kinetics as RNA from rat myogenic lines. The results in Fig. 6B and 6C demonstrate the effectiveness of using a uridine dilution to stop all incorporation into total RNA within 4–5 h after exposure. By contrast, cells not exposed to 5 mM uridine incorporated label exponentially. Fig. 6B also confirms that ribosomal RNA was stable in this system as it is in the others. These

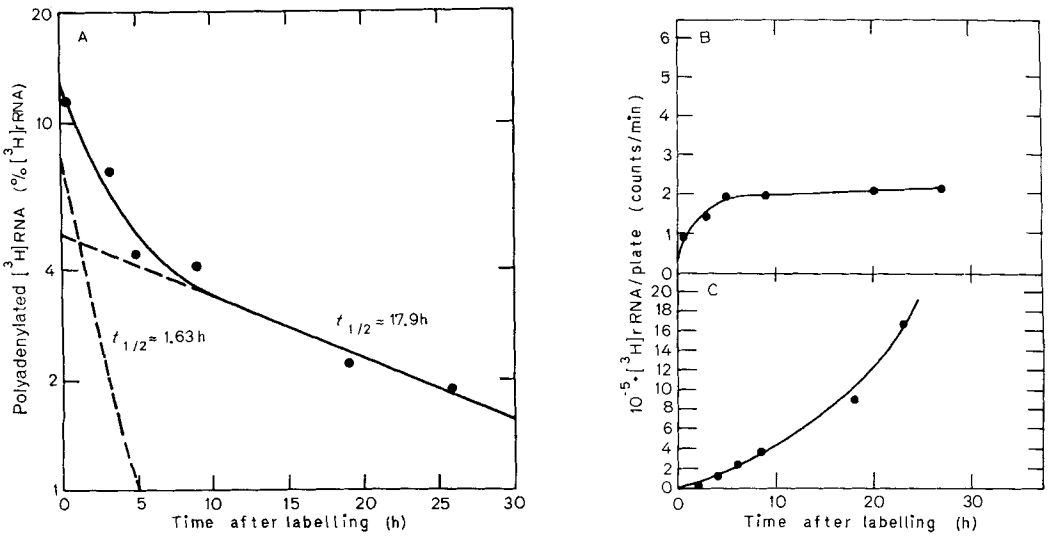

Fig. 6. *Decay of polyadenylated RNA in primary chicken muscle cell cultures.* 12 plates (100 mm) of chicken breast muscle cells (3×10^6/plate) at the onset of fusion were labelled for 2 h with [^{3}H]uridine (10 μCi/ml, 50 μCi/plate). After this time, uridine (5 mM) and cytidine (20 μM) were added to six plates and the remaining six were kept untreated for a control. From each group of plates cytoplasmic RNA was extracted at various times and polyadenylated RNA was isolated from the plates chased by uridine dilution. (A) Labelled polyadenylated RNA, and (B) labelled rRNA from plates taken at various intervals after addition of unlabelled uridine. (C) The labelling of ribosomal RNA in cultures which did not receive unlabelled uridine. The broken line shows the decay of each component after subtracting one from the other

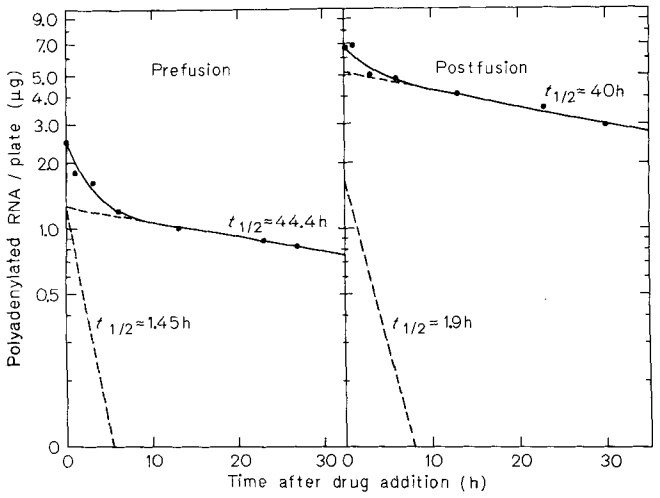

Fig. 7. *Decay of polyadenylated RNA measured by ultraviolet absorbance.* L8 culture plates were divided into two groups, one taken before cell fusion and one after. To each group actinomycin D was added (4 μg/ml). At designated intervals after actinomycin addition, plates were removed (12 plates/point at prefusion and 7 plates/point at postfusion) and polyadenylated RNA was isolated and measured by absorbance at 260 nm (1 A_{260} unit = 40 μg RNA/ml). The broken lines show the decay of each component after subtracting one from the other

results show the biphasic decay kinetics to be characteristic also of primary chicken skeletal muscle cultures. We have also found similar kinetics in primary rat thigh muscle cultures.

Decay of Polyadenylated RNA as Measured by Absorbance at 260 nm

A third method for measuring polyadenylated RNA decay involved isolating RNA from a large number of cultures exposed to actinomycin for varying times and then detecting, by ultraviolet absorbance, the remaining amount of polyadenylated RNA. This method monitors changes in the amount of steady-state RNA. Thus, analysis was not restricted to a small percentage of polyadenylated RNA which had recently been labelled, nor was analysis complicated by the possibility that a small population of cells might have been utilizing precursors at a more rapid rate.

Similar to the experiments utilizing labelled precursors, this measurement also indicates the existence in the cells of two classes of polyadenylated RNA each with a different stability (Fig. 7). Prior to fusion, the more stable species of polyadenylated RNA constitutes 50–60% of the total polyadenylated RNA population. After fusion, the long-lived polyadenylated RNA reaches 90% of the total. As will be discussed later, these observations are consistent with the data based on the follow-up of labelled RNA populations.

DISCUSSION

The results indicate that differentiating primary cultures of skeletal muscle cells and an established myogenic cell line have two main populations of polyadenylated RNA with regard to stability. Our ob-

servations are similar to those obtained for HeLa cells, for a mosquito cell line and for red blood cells [22,25,26]. In the myogenic cell line the ratios of the two RNA populations changed with differentiation: the stabler population increased during cell fusion and became the major polyadenylated RNA component. No significant decay of rRNA was noticed during the duration of the experiments (data not shown).

The significance of the biphasic kinetics of decay is not clear as yet. Although the interference of actinomycin D in RNA metabolism may affect the stability of particular RNA molecules [24], it is not likely that the biphasic decay is an artefact created by actinomycin D, since biphasic kinetics have also been obtained when actinomycin D was not used (see also [22, 25,26]). It is also unlikely that the two populations of RNA represent two different populations of cells, since the experiments with the cell line L8 were performed in cloned populations which show very high uniformity in differentiation capacity [16,27]. In this cell system, the cell population becomes heterogeneous only during differentiation, when about 70% of the cells fuse into multinucleated fibers. However, throughout the proliferation stage, the cells can be considered genetically and developmentally very homogeneous. Since all these studies involved a step of isolation of polyadenylated RNA, the possibility that the biphasic decay reflects differences in stability of the poly(A) segment rather than decay of the coding part of the mRNA molecule cannot be ruled out.

Experiments in which the decay of a short-time-labelled polyadenylated RNA was followed illustrated the decay kinetics of polyadenylated RNA which had been recently synthesized. Whereas experiments in which the amount of isolated polyadenylated RNA was measured by ultraviolet absorbance provided information on the net changes in the total population of this RNA in the cells. A study of the kinetics of newly synthesized polyadenylated RNA yielded an opportunity to estimate its contribution to the steady-state pool of this RNA and to calculate the expected steady-state ratio of each population of polyadenylated RNA. These calculations and the mathematical basis for them are presented in the Appendix. Before fusion, the rate of synthesis of the stable population was 25% of the total polyadenylated RNA being synthesized. Applying this to the equation in the Appendix yields the result that after a steady state is reached, the stable RNA will be 83% of the total steady-state polyadenylated RNA. When the instantaneous amount of polyadenylated RNA was measured in confluent unfused cultures, it was found to be 50% (Fig. 7), indicating that a steady state had not yet been reached. The stable population of polyadenylated RNA synthesized after fusion accounted for 65% of the newly synthesized polyadenylated RNA and substituting this into the equation in the Appendix, we expect the stable population to constitute 94% of the steady-state polyadenylated RNA. In our experiments, values between 75% and 90% were obtained. The increased ratio of the long-lived RNA population following cell fusion is consistent with earlier observations, which showed that the muscle fibers are more resistant to inhibition of RNA synthesis by actinomycin D [10,11]. Observations suggesting stabilization of mRNA during the differentiation of other cell types have been reported [8].

We found no significant decay of rRNA in L8 cultures either before or after cell fusion. Our method of isolation of polyadenylated RNA did not allow more than 10% contamination by rRNA. Thus, any undetected turnover of rRNA would not affect our measurements when data are expressed in absolute values. However, Bowman and Emerson have shown [34] that in quail myoblasts rRNA is not stable at the post-fusion stage, determining a single half-life value of about 45 h. Had this been the case for L8 cells it might have affected data expressed relatively to rRNA. A recalculation of the data in Fig. 5 using their given value ($t_{1/2}$ of rRNA = 45 h) changes the half life of the more stable species of polyadenylated RNA from 33 to 19 h and of the less stable species from 4.2 to 2.1 h. These new figures correspond exactly to the values determined in the presence of actinomycin D (Fig. 3). Therefore even if there existed a limited degradation of rRNA, which we could not detect, our basic findings regarding polyadenylated RNA (namely, the biphasic kinetics of its decay and the increased proportion of more stable molecules in differentiated cells) would not be altered.

It is now apparent that mRNAs in a great variety of animal cells show stability in the order of many hours. Therefore, the fine control of the time and rate of synthesis of many proteins may be based, to a certain extent, on post-transcriptional mechanisms. This is especially true for cells which undergo rapid differentiation and great changes in the pattern of protein synthesis. Fusion of myoblasts is associated with great increase in myosin synthesis and change in its subunits [13,16,28], great increase in activity and isozymic changes in creatine kinase and other enzymes [29–31], and a decrease in DNA polymerase activity [32]. Our data suggest that a considerable proportion of the polyadenylated RNA population which is present in the cells several hours after fusion was formed prior to cell fusion.

In this study, as well as in many other studies, the stability of mRNA was inferred from the decay of polyadenylated RNA. In order to obtain more direct information on the functional stability of mRNA and the nature of biphasic decay, it is of importance to test the capacity of the polyadenylated RNA isolated from actinomycin-D-treated cultures to direct protein synthesis. This is described in the following paper [33].

APPENDIX

Calculation of Contribution of the Synthesis of mRNA Populations to the Steady-State Pool

Robert H. SINGER

The change in the amount (A) of mRNA in a cell at any given time is given by the equation:

$$\frac{dA}{dt} = S - kA$$

where S = rate of synthesis of mRNA and k is the first-order rate constant of mRNA degradation related to half-life by the empirical formula:

$$k = \frac{\ln 2}{t_{1/2}}$$

where $t_{1/2}$ is the half-life. Since we have observed that there are two components of mRNA with different rates of decay, we can approach their relationship mathematically by writing an equation for each population:

For component 1:

$$\frac{dA_1}{dt} = S_1 - kA_1 = S_1 - \frac{\ln 2}{t1_{1/2}} A_1.$$

For component 2:

$$\frac{dA_2}{dt} = S_2 - kA_2 = S_2 - \frac{\ln 2}{t2_{1/2}} A_2.$$

When the steady state is reached $dA/dt = 0$ and the two populations can be reduced to the ratio:

$$S_1/S_2 = \frac{A_1 t2_{1/2}}{A_2 t1_{1/2}} \quad \text{or} \quad \frac{S_1 t1_{1/2}}{S_2 t2_{1/2}} = \frac{A_1}{A_2}.$$

Since we have observed that at prefusion (Fig. 1) $t1_{1/2}$ (short half-life) = 3.7 h and $t2_{1/2}$ (long half-life) = 53.5 h, then $t1_{1/2}/t2_{1/2} \approx 1/15$.

Extrapolating the slopes of the two populations to zero time, the ratios of rate of synthesis $S_1/S_2 = 3$.

Thus,

$$\frac{S_1 t1_{1/2}}{S_2 t2_{1/2}} = \frac{1}{5} = \frac{A_1}{A_2}.$$

Since $A_1 + A_2 = 100\%$, the ratios of each mRNA component in cells pre-fusion extrapolated to the steady state are 83% for the long-lived component and 17% for the short-lived component.

We wish to thank Dr David Yaffe for the invaluable advice and encouragement throughout this work. R.H.S. would like to express his appreciation to the Muscular Dystrophy Association of America for their past and continuing support, and to the grant support of the National Institutes of Health (NS 11329-01). This research has also been supported by grants to Dr David Yaffe from the Muscular Dystrophy Association of America, the U.S.-Israel Binational Foundation, and the National Institutes of Health (GM 22767-01). The authors express their appreciation to Dr Bruce Paterson and Dr S. Penman for their very helpful suggestions. The skilful technical assistance of Mrs Sara Neuman is gratefully acknowledged.

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