bind tightly to protein particles in the masticated food (Archer et al., 1973; Bhumiratana et al., 1977), thus suppressing the autolytic conversion of π- and δ-chymotrypsin to other active chymotrypsin species or the hydrolysis of chymotrypsinogen to neochymotrypsinogen.

References

Archer, M. C., Ragnarsson, J. O., Tannebaum, S. R., & Wang, D. I. C. (1973) *Biotech. Bioeng. 15*, 181.

Avery, M. J., & Hopkins, T. R. (1973) *Biochim. Biophys. Acta 310*, 142.

Bender, M. L., & Killheffer, J. V. (1973) *CRC Crit. Rev. Biochem. 1*, 149.

Bettelheim, F. R., & Neurath, H. (1955) *J. Biol. Chem. 212*, 241.

Bhumiratana, S., Hill, C. G., & Amundson, C. G. (1977) *J. Food Sci. 42*, 1016.

Corey, R. B., Battafay, O., Brueckner, D. A., & Mark, F. G. (1965) *Biochim. Biophys. Acta 94*, 535.

Davidson, R., Gertler, A., & Hofmann, T. (1975) *Biochem. J. 147*, 45.

Desnuelle, P. (1960) *Enzymes, 2nd Ed. 4*, 93.

Dixon, G. H., & Neurath, H. (1957) *J. Biol. Chem. 225*, 1049.

Guy, O., Rovery, M., & Desnuelle, P. (1966) *Biochim. Biophys. Acta 124*, 402.

Hopkins, T. R., & Spikes, J. D. (1967) *Biochem. Biophys. Res. Commun. 28*, 480.

Hopkins, T. R., & Spikes, J. D. (1968) *Biochem. Biophys. Res. Commun. 30*, 540.

Hummel, B. C. W. (1959) *Can. J. Biochem. Physiol. 37*, 1393.

Jacobsen, C. F. (1947) *C. R. Trav. Lab. Carlsberg, Ser. Chim. 25*, 325.

Miller, D. D., Horbett, T. A., & Teller, D. C. (1971) *Biochemistry 10*, 4641.

Rovery, M., & Bianchetta, J. (1972) *Biochim. Biophys. Acta 268*, 212.

Rovery, M., Poilroux, M., Curnier, A., & Desnuelle, P. (1955) *Biochim. Biophys. Acta 17*, 565.

Rovery, M., Poilroux, M., Yoshida, A., & Desnuelle, P. (1957) *Biochim. Biophys. Acta 23*, 608.

Schonbaum, G. R., Zerner, B., & Bender, M. L. (1961) *J. Biol. Chem. 236*, 2930.

Schwert, G. W., & Takenaka, Y. (1955) *Biochim. Biophys. Acta 16*, 570.

Sharma, S. K., & Hopkins, T. R. (1978a) *J. Biol. Chem. 253*, 3055.

Sharma, S. K., & Hopkins, T. R. (1978b) *Biochim. Biophys. Acta 527*, 465.

Valenzuela, P., & Bender, M. L. (1971) *J. Am. Chem. Soc. 93*, 3783.

Valenzuela, P., & Bender, M. L. (1973) *J. Biol. Chem. 248*, 4909.

Weber, K., Prigle, J. R., & Osborn, M. (1972) *Methods Enzymol. 26*, 3.

Wilcox, P. E., Kraut, J., Wade, R. D. & Neurath, H. (1957) *Biochim. Biophys. Acta 24*, 72.

Wright, H. T., Kraut, J., & Wilcox, P. E. (1968) *J. Mol. Biol. 37*, 363.

Zanetta, J. P., Vincedon, G., Mendel, P., & Gombas, G. (1970) *J. Chromatogr. 51*, 441.

Stability of Polysome-Associated Polyadenylated RNA from Soybean Suspension Culture Cells†

Carolyn D. Silflow* and Joe L. Key

ABSTRACT: The half-life of polysome-associated, poly(A)-RNA in exponentially growing soybean (*Glycine max*) suspension culture cells was determined with pulse-chase experiments. Based on a best fit from a computer analysis of the data, two decay components for poly(A)-RNA were found. One component had a half-life of approximately 0.6 h, while the other had a half-life of about 30 h, similar to the doubling time of the cultures. At the beginning of the chase period, the short-lived component represented approximately 90% of the total poly(A)-RNA in the polysomes. This percentage decreased with time so that, under steady-state conditions, the long-lived component probably represented the majority of poly(A)-RNA.

The relatively long half-life of messenger RNA in most higher eukaryotic cells allows for the temporal and spatial separation of mRNA and protein synthesis. Regulation of the stability of mRNA through differential turnover rates is a potential mechanism for post-transcriptional control of the steady-state abundance of various turnover species in the cytoplasm and, in turn, the rate of synthesis of various proteins (Kafatos, 1972). In several developmental systems, cell specialization is associated with unusual stability of the mRNA coding for the major protein synthesized by that cell type (Kafatos, 1972). Studies using various mammalian and insect cell cultures have indicated the existence of at least two major half-life components in the polyadenylated messenger RNA population (Singer & Penman, 1973; Pucket et al., 1975; Perry et al., 1975; Spradling et al., 1975).

Plants are known to contain polyadenylated, polysome-associated, heterodisperse RNA (Key & Silflow, 1975; Hammett & Katterman, 1975; Covey & Grierson, 1976), and this RNA has been shown to have mRNA activity in cell-free translation systems (Gray & Cashmore, 1976; Larkins et al., 1976). Direct investigations of the half-life of presumptive

† From the Department of Botany, University of Georgia, Athens, Georgia 30602. *Received August 1, 1978.* This work was supported by U.S. Public Health Service Research Grant CA 11624 from the National Cancer Institute and by Department of Energy (formerly ERDA) Contract Doe (38-1)643.

* Present address: Department of Biology, Yale University, New Haven, CT 06520.

0006-2960/79/0418-1013$01.00/0

mRNA in higher plant cells have not been reported. However, experiments with both intact (Lin & Key, 1968) and excised (Key et al., 1967) soybean tissue have indicated that RNA populations in soybean cells may have components with differing decay rates, and that some mRNA may have a relatively short half-life.

The experiments reported here were designed to measure the half-life of presumptive messenger RNA in soybean. Suspension culture cells were chosen for this work because they consist of a fairly homogeneous cell type, can be maintained in exponential growth for several days, and grow as single cells or small clumps, allowing each cell to be exposed to label or chase medium at approximately the same time. The pulse-chase method was used in these studies because it was considered to be more sensitive for the detection of multiple decay components than other techniques. An equation reported by Spradling (1975) which incorporated a correction for the slow decay of radioactivity in the RNA precursor pool during the chase was used in a computer analysis of the data. The results reported here indicate that the metabolism of mRNA in plant cells is similar in its kinetic aspects to many other higher eukaryotic cells.

Materials and Methods

Materials. Carrier-free [^{32}P]orthophosphate, [5-^{3}H]uridine (28.3 Ci/mmol), and [2-^{14}C]uridine (46.48 mCi/mmol) were purchased from New England Nuclear. Poly(U)[1] (potassium salt) was obtained from Calbiochem, and Nitex cloth (25-μm pore size) from Tetko Inc., Elmsford, NY.

Cell Cultures. Soybean suspension cultures were a gift from Dr. Ernest Jaworski (Monsanto Co., St. Louis, MO). The cultures, consisting of clumps of 10–100 cells, were maintained in darkness at 27 °C in flasks in Gamborg B5 medium (Gamborg et al., 1968). Aeration was provided by shaking the cultures at 150 rpm on a gyratory shaker. Every 4 days the cells were transferred to fresh medium at an initial density of approximately 40 μg of fresh weight mL^{-1}. The growth rate of the cultures was determined by pipetting aliquots of cell suspension onto Nitex filters, blotting the filters, and weighing the cells. This method of determining culture growth was rapid, and growth determinations could be made on the same cells from which polysomes or RNA were isolated.

Labeling Cells with [^{32}P]Orthophosphate. Exponentially growing cells (usually 72 h after transfer) were washed and transferred into phosphate-free Gamborg B5 medium. After 2–3 h, [^{32}P]orthophosphate, brought to pH 5.5 with NaOH, was added to the cultures (10–50 μCi mL^{-1}). After labeling, the cells were filtered through Nitex filters, washed with fresh medium, and frozen in liquid nitrogen.

Labeling Cells for Half-Life Experiments. Exponentially growing cultures were labeled for 18 h with [^{14}C]uridine (0.035–0.90 μCi mL^{-1}). [^{3}H]Uridine (5.0–10.0 μCi mL^{-1}) was then added to the cultures. After 1 h, cells were placed in fresh Gamborg medium containing 5–20 mM unlabeled uridine. At various times after the beginning of the chase, aliquots of cells were removed, and the cells were frozen in liquid nitrogen.

Polysome Isolation. For polysome isolation, frozen cells were quickly weighed and ground to a powder with an ice-cold mortar and pestle. The frozen powder was suspended with ten strokes of a Duall tissue homogenizer (Kontes) in 5 volumes of homogenization buffer containing 150 mM Tris-HCl, pH 8.5, 50 mM KCl, 20 mM $MgCl_2$, and 250 mM sucrose. Diethyl pyrocarbonate (0.01%) was added to the homogenization buffer immediately before use. The homogenate was filtered through Miracloth and centrifuged at 17300*g* for 15 min. The supernatant was loaded in Beckman Ti60 rotor tubes over a 6-mL cushion of 1.5 M sucrose, 50 mM Tris-HCl, pH 8.5, 50 mM KCl, 10 mM $MgCl_2$, and centrifuged at 260000*g* for 3 h. The polysome pellets were suspended in 50 mM Tris-HCl, pH 8.5, 25 mM KCl, 10 mM $MgCl_2$. The average yield in the polysome pellet was 2.5 A_{260} units per g of fresh weight of tissue. The yield was not increased when Triton X-100 was added to the homogenization buffer, suggesting that the cells contained few membrane-bound polysomes. Five A_{260} units of polysomes was loaded on each 12-mL isokinetic sucrose gradient (Noll, 1967) containing 10–34% (w/v) sucrose in suspension buffer. The parameters used to construct the gradients were V_m = 10 mL, C_t = 10% (w/v) sucrose, and C_r = 48% (w/v) sucrose. The gradients were centrifuged in a Beckman SW41 rotor at 60000*g* for 2 h, analyzed for absorbance at 260 nm by passage through a flow cell attached to a Gilford 2400 spectrophotometer, and collected in 0.5-mL fractions.

RNA Isolation from Polysomes. Pooled fractions of polysomes were made to 1% (w/v) sarkosyl and extracted once with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). RNA was precipitated from the aqueous phase by adding 0.06 volume of 2.5 M sodium acetate, pH 6.9, and 2.4 volumes of cold ethanol. The precipitated RNA was collected by centrifugation, resuspended in 0.01 M Tris-HCl, pH 7.6, containing 1% (w/v) sarkosyl, extracted with the phenol mixture, and precipitated as before.

RNA Binding to Poly(U) Filters. The methods of Sheldon et al. (1972) were modified slightly for poly(U) filter binding of poly(A)-RNA. Poly(U) solution (2 mg mL^{-1} in distilled water) was applied to Whatman GFA glass fiber filters. The binding buffer used was 200 mM NaCl, 10 mM Tris-HCl, pH 7.6, 0.01% (w/v) $NaDodSO_4$. Samples of 50–100 μg of RNA in 2 mL of binding buffer were applied to the filters and the eluate was collected and reapplied. The second eluate and an additional 4-mL wash of binding buffer were pooled and this fraction was designated as poly(A)$^-$-RNA. The filters were then washed with 40 mL of binding buffer followed by 20 mL of 120 mM NaCl, 10 mM Tris-HCl, pH 7.6. Dried filters were counted in a toluene-based scintillator using scintillation counter settings which minimized crossover of counts between ^{14}C and ^{3}H channels. The fraction of total counts in poly(A)-RNA was determined by subtracting the nonspecific binding to the control filters (containing no poly(U)) from the cpm bound to the poly(U) filters and dividing by the total acid precipitable cpm applied to the filters. The control filter cpm was usually 1–2% of poly(U) bound cpm.

Gel Electrophoresis. Polyacrylamide gel electrophoresis of RNA samples was carried out as described by Loening (1967). For analysis of poly(A)$^-$-RNA samples from the half-life experiments, cylindrical gels were made with a 3.5-cm 7.5% acrylamide gel on the bottom and a 4.5-cm 2.4% acrylamide gel on the top in order to provide good resolution of all size classes of RNA. The gels were scanned at 260 nm and sliced into 1-mm sections. Gel slices were digested in 0.2 mL of 15% hydrogen peroxide and 0.05 N HCl at 70 °C for 8 h and counted in an aqueous scintillation fluid. ^{3}H and ^{14}C ra-

[1] Abbreviations used: poly(U), poly(uridylic acid); hnRNA, heterogeneous nuclear RNA; poly(A)-RNA, RNA containing poly(adenylic acid); poly(A)$^-$-RNA, RNA not containing poly(adenylic acid); $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetate; AMP, adenosine monophosphate; Tris, tris(hydroxymethyl)aminomethane; A_{260} unit, quantity of material contained in 1 mL of a solution which has an absorbance of 1 at 260 nm when measured in a 1-cm pathlength cell; UV, ultraviolet; cpm, counts per minute; rpm, revolutions per minute.

dioactivities were determined for each RNA component on the gel including 25S, 18S, 5S, 4S, and heterodisperse RNA. The fraction of the total cpm on the gel which was in the 25S and 18S peaks and the ratio of ^3H to ^{14}C cpm in the ribosomal RNA were determined for each gel.

Data Analysis for Half-Life Experiments. The growth rate for each experiment was determined by measuring the fresh weight mL^{-1} for each aliquot of cells removed during the chase period.

The uridine pool decay rate for each experiment was determined from the ^3H to ^{14}C ratio of the ribosomal RNA from gel radioactivity profiles at each time point. The ratio was plotted vs. chase time and a plateau level determined. The difference between the $^3H/^{14}C$ ratio at each time point and the plateau ratio determined the percentage of [^{3}H]uridine yet to be incorporated at each time point prior to the plateau.

The ratio of poly(A)-RNA to ribosomal RNA was determined for ^{14}C and ^3H at each time point as follows: (1) cpm acid precipitable − cpm bound to poly(U) filter = cpm in poly(A)$^-$-RNA; (2) cpm in poly(A)$^-$-RNA × fraction of rRNA in total poly(A)$^-$-RNA = cpm in rRNA; (3) cpm bound to poly(U) filter/cpm in rRNA = cpm in poly(A)-RNA/cpm in rRNA. This ratio was plotted semilogarithmically vs. chase time. The resulting curve was analyzed using the BMDP3R nonlinear regression computer program (Health Sciences Computing Facility, UCLA) using an equation described by Spradling (1975). The equation for a curve containing one decay component is

$$F = \frac{P(1)(K_p - K_D)(K_D + P(2))[1 - e^{-(K_p - K_D - P(2)t}]e^{-P(2)t}}{K_D(K_p - K_D - P(2))[1 - e^{-(K_p - K_D)t}]}$$

F = cpm in poly(A)-RNA/cpm in rRNA; $P(1)$ = initial amount of poly(A)-RNA/initial amount of rRNA; $P(2)$ = decay rate for poly(A)-RNA; K_P = decay rate for precursor pool; K_D = growth rate; and t = time of chase. The data points for each experiment were fit with the program for one, two, and three poly(A)-RNA decay components. The combined data from several experiments were normalized to the 23-h point, plotted together, and analyzed in the same manner. In each case, the computer determined the best values of $P(1)$ and $P(2)$ by determining the lowest residual sum of squares (RSS) and residual mean square (RMS) values.

Results

Characterization of RNA. Preliminary experiments showed that the RNA to be used for the half-life determinations had characteristics of mRNA including EDTA-sensitive association with polysomes, polyadenylation, and a heterodisperse size distribution. Polysomes were isolated from exponentially growing cells which had been labeled for 1 h with [^{32}P]-orthophosphate (Figure 1a). The profile of acid-precipitable radioactivity across the sucrose gradient coincided with the absorbance profile suggesting that the labeled material was contained in the polysomes. In order to avoid contamination of polysomes with nonpolysome-associated ribonucleoprotein particles which might sediment near the top of the gradient (Spirin, 1969), all gradients were separated into a "heavy" and "light" region as shown in Figure 1a, and the pooled "heavy" region was used for subsequent experiments.

To learn whether newly synthesized RNA was polysome-associated, soybean polysomes were treated with EDTA. This treatment dissociates the ribosomes into subunits and releases polysome-associated RNA, but does not affect the sedimentation of nonpolysome associated RNA (Penman et al., 1968). When soybean polysomes were treated with EDTA, both the UV-absorbing material and the newly synthesized (labeled) material were released to the top of the gradient (Figure 1b). Table I shows the distribution of acid-precipitable radioactivity from three separate experiments in the "heavy" and "light" regions from polysomes run with and without EDTA. The percent of total cpm in the gradient which was in the "heavy" region ranged from 60% to 80% in the −EDTA gradients and from 10% to 16% in the +EDTA gradients. Thus, approximately 80% of the counts in the "heavy" region of the gradient was released by EDTA and was therefore presumably associated with polysomes. This value is a minimum estimate since a large proportion of the radioactivity in the "heavy" region of the +EDTA gradients was in the fractions adjacent to the arbitrary cut-off between "light" and "heavy" and probably represented material whose sedimentation was changed by EDTA treatment.

A similar analysis of the association of poly(A)-RNA (RNA which bound to poly(U) filters) with polysomes is shown in Table II. The percent of total poly(A)-RNA in the gradients which was found in the "heavy" region varied from 86% to 94% in the −EDTA gradients and from 12% to 19% in the +EDTA gradients. Therefore 80–86% of the poly(A)-RNA in the heavy region was EDTA-releasable and was presumably mRNA. EDTA treatment of polysomes released a comparable percentage of both total cpm (Table I) and poly(A)-RNA (Table II) suggesting that the release of polyadenylated and

Table I: Distribution of Labeled RNA in Polysome Gradients

expt		cpm in "heavy" region × 10^{-3} [a]	cpm in "light" region × 10^{-3}	% of total cpm in "heavy" region[b]	% of cpm in "heavy" region released by EDTA[c]
1	−EDTA	191	49	80	87
	+EDTA	35	322	10	
2	−EDTA	262	101	72	78
	+EDTA	74	398	16	
3	−EDTA	300	196	60	81
	+EDTA	123	939	11	

[a] cpm are total acid precipitable cpm. [b] (cpm in "heavy" region/cpm in "heavy" + "light" region) × 100 = % of total cpm in "heavy" region. [c] 1 − (% of total cpm in "heavy" region from +EDTA gradient/% of total cpm in "heavy" region from −EDTA gradient) × 100 = % of cpm in "heavy" region released by EDTA.

Table II: Distribution of Labeled Poly(A)-RNA in Polysome Gradients

expt		% of "heavy" cpm poly(U) bound	% of "light" cpm poly(U) bound	% of total poly(A)-RNA in "heavy" region[a]	% of poly(A)-RNA in "heavy" region released by EDTA[b]
1	−EDTA	51	14	94	80
	+EDTA		40	19	
2	−EDTA	32	11	89	86
	+EDTA	21		13	
3	−EDTA	27	7	86	
4	−EDTA	36	8	92	

[a] Calculations from numbers shown in Table I. (cpm in poly(A)-RNA in "heavy" region/cpm in poly(A)-RNA in "heavy" + "light" regions) × 100 = % of total poly(A)-RNA in "heavy" region. [b] 1 − (% of total poly(A)-RNA in "heavy" region from +EDTA gradient/% of total poly(A)-RNA in "heavy" region from −EDTA gradient) × 100 = % of poly(A)-RNA in "heavy" region released by EDTA.

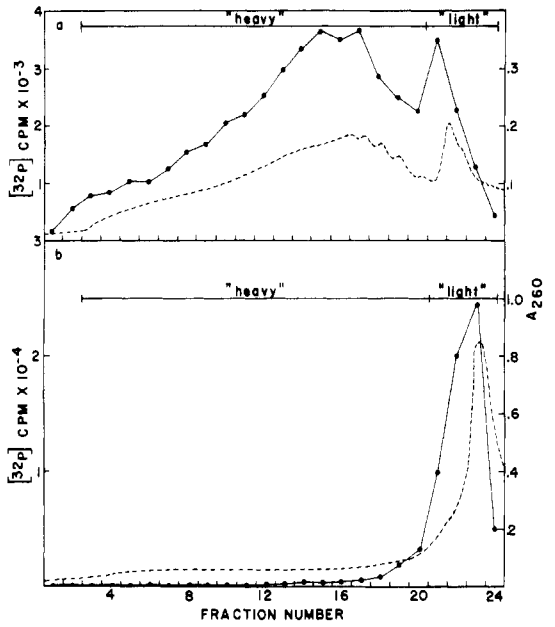

FIGURE 1: Sucrose gradient analysis of radioactivity associated with polysomes after labeling the cells for 1 h with [^{32}P]orthophosphate. Top of gradients to the right. Polysomes were prepared and loaded onto sucrose gradients (a) containing no EDTA, (b) containing 20 mM EDTA. Aliquots of 0.1 mL from gradient fractions were acid precipitated; the precipitate was collected on nitrocellulose filters and counted in a liquid scintillation counter. The "heavy" and "light" regions indicate fractions pooled for subsequent analysis. A_{260}, dotted line; radioactivity, solid line.

nonpolyadenylated RNA was similar.

Two lines of evidence indicated that the poly(A)-RNA isolated from polysomes was not degraded. Previous work on the soybean hypocotyl system (Key & Silflow, 1975) had shown that intact poly(A)-RNA labeled for 1–2 h with ^{32}P contained 40 mol % AMP, whether isolated from total RNA or from polysomes. If the RNA was partially degraded, the percent AMP in the polysome-associated RNA bound to poly(U) filters increased to 50% or more since only that portion of the molecule containing the poly(A) sequence bound to the filter. Base composition analysis of the tissue culture cell poly(U) filter-bound RNA from the "heavy" region of the −EDTA gradients indicated that the mole percent of AMP was consistently 42–43% and suggested that there was minimal degradation of the poly(A)-RNA. In addition, the size distribution on 2.4% polyacrylamide gels of the poly(A)-RNA isolated from tissue culture polysomes (Figure 2) is similar to total poly(A)-RNA of soybean hypocotyl or cultured cells and to poly(A)-RNA from other plant systems (Key & Silflow, 1975; Covey & Grierson, 1976). Although nonpolyadenylated heterodisperse RNA (up to 40% of total heterodisperse RNA) was not analyzed in these experiments, it was found associated with the polysomes of the tissue culture cells and may have messenger RNA activity as it does in other systems (Gray & Cashmore, 1976; Fromson & Verma, 1976).

Half-Life of Polysomal Poly(A)-RNA. In the experiments to be described, the stability of poly(A)-RNA isolated from polysomes in the "heavy" region of the polysome gradients was examined. These experiments followed the basic pulse-chase method used by Spradling et al. (1975). In this method, the decrease in specific activity in the unstable RNA component is determined using as a standard the specific activity of rRNA, which is assumed to be stable. The need to correct for dilution of radioactivity caused by cell growth during the chase is eliminated by comparing the counts in unstable RNA with counts in rRNA. Poly(A)-RNA and rRNA are assumed

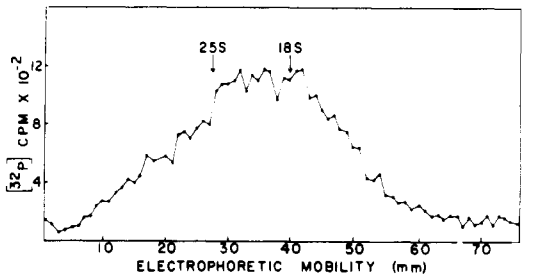

FIGURE 2: Distribution of poly(A)-RNA on 2.4% polyacrylamide gels. Cells were labeled for 2 h with [^{32}P]orthophosphate. Polysomes were prepared and RNA was extracted from the "heavy" region of the polysome sucrose gradients. The RNA was fractionated on oligo-(dT)-cellulose, and the RNA which bound to the column under high salt conditions was eluted and applied to the gel.

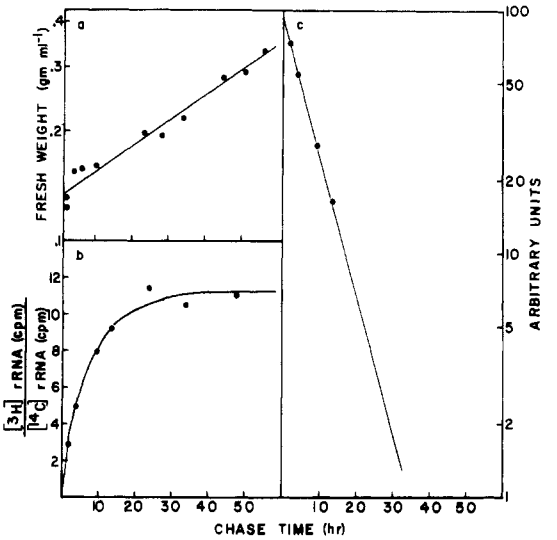

FIGURE 3: Cell growth and determination of decay of radioactivity in uridine nucleotide pool under chase conditions. (a) Growth of cultures as determined by fresh weight increase. (b) Ratio of ^{3}H to ^{14}C in the polysomal rRNA during the chase. (c) Percent of remaining incorporation of [^{3}H]uridine during the chase. Cells were labeled for 18 h with [^{14}C]uridine, then for 1 h with [^{3}H]uridine. Unlabeled uridine was added to effect the chase, cell aliquots were withdrawn, the media was filtered away, and the cells were blotted and weighed (a). Polysomes were isolated, RNA was extracted from the "heavy" region of polysome sucrose gradients, and the RNA was fractionated on poly(U) filters. The RNA which did not bind to the filters was electrophoresed on polyacrylamide gels and the ratio of ^{3}H cpm to ^{14}C cpm in the total rRNA was determined from the gel radioactivity profiles (b). The percent difference between each point in b and the plateau level was calculated and plotted to show the percent of remaining incorporation of [^{3}H]uridine at various times after the beginning of the chase (c).

to be labeled from the same precursor pool. The assumption that plant rRNA is stable (Trewavas, 1970; Sutton & Kemp, 1976) was found to be reasonable for the cells in the present study by labeling to approximate steady state and measuring the decrease in specific activity of total cell rRNA during a chase period; the half-time for decay of specific activity was approximately equal to the doubling time of the cells. The decay data were analyzed using an equation described by Spradling (1975). This analysis requires the determination of two parameters: (1) the cell growth rate; and (2) the rate of decay of radioactivity in the uridine nucleotide precursor pool during the chase.

The cell growth rate during the half-life experiments was determined from growth curves such as that shown in Figure 3a. The increase in fresh weight shown by these curves reflected cell division rather than cell expansion, because previous experiments in which culture growth was determined

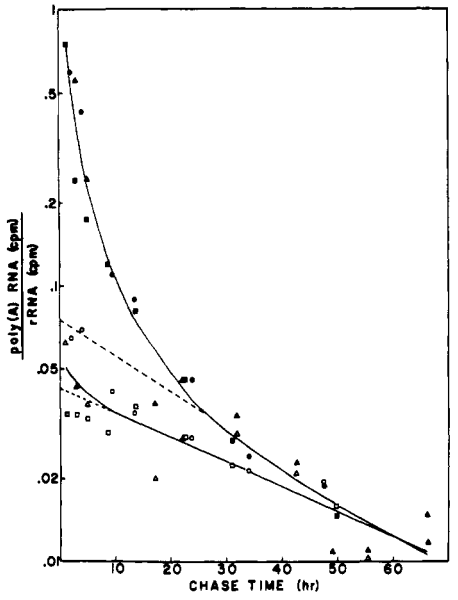

FIGURE 4: Kinetics of decay of polysome-associated poly(A)-RNA. Labeling conditions and experimental procedure were the same as in Figure 3. The ratio of cpm bound to poly(U) filters to cpm in rRNA was determined for both ^{14}C- and ^{3}H-labeled RNA at each time point. Different symbol shapes indicate separate experiments. ^{14}C, open symbols; ^{3}H, closed symbols. All experiments were normalized around the 23-h time point. The data from three experiments were combined in order to increase the number of data points analyzed by the computer and thus increase the statistical accuracy of the computer solution.

by cell counts had shown that the cultures grew exponentially from about 36 h after transfer until at least 100 h. The growth rate of the cultures was not affected by the addition of uridine to the medium.

Measuring the precursor pool decay rate involved chasing labeled uridine from the cell with unlabeled uridine. A rapid chase of uridine is difficult to achieve with plant cells (Cox et al., 1973), presumably because of the large size of the intracellular pool of uridine derivatives and/or because of complex compartmentalization of the pool. Continued incorporation of radioactivity into the mRNA during the chase would result in an apparent half-life value higher than the true value, and this effect would be larger for RNA components with shorter half-lives. A correction for the decay of counts in the precursor pool during the chase period was made by prelabeling the cells with [^{14}C]uridine for 18 h (to approximate steady-state labeling) before beginning the 1-h pulse label with [^{3}H]uridine. The ratio of ^{3}H to ^{14}C in polysomal rRNA during the chase with cold uridine reached a constant level when ^{3}H labeling of the rRNA had terminated; that is, when the radioactive precursors were diluted from the pool (Figure 3b). For each time point, the percent of incorporation of label needed to reach the plateau level was calculated as described in Materials and Methods and plotted semilogarithmically vs. chase time (Figure 3c). The data were consistent with a single-component, first-order decay rate. The half-life of precursor pool decay ranged from 3 to 7 h in various experiments.

The ratio of poly(A)-RNA to ribosomal RNA for both ^{3}H and ^{14}C was determined at various points during the chase. The composite results of three separate half-life experiments are shown in Figure 4, where all points have been normalized to the 23-h time point. The number of components and the decay rate of each component were obtained by fitting the data with a computer program based on an equation derived by Spradling (1975). The computer program, unlike a graphical solution, incorporates the previously determined correction factors for cell growth rate and precursor pool decay rate. The fit for two components (RMS of 0.063) was approximately sixfold better than for one (RMS of 0.404) and is indicated by the upper solid line in Figure 4. The best estimates for the half-lives of the two components were 0.6 and 27 h. Extrapolation back to zero time of the fraction of the curve which corresponds to the 27-h half-life component (dashed line, upper curve) allows a comparison of the relative initial amounts of each component. At the beginning of the chase, the long-lived component represents approximately 10% of the total labeled poly(A)-RNA. Attempts to solve the data for more than two components were unsuccessful, possibly due to an inadequate number of data points. Thus, while a one-component curve can be ruled out, it is possible that more than two components exist. It is also possible that the half-life values of the two components represent average values, and that poly(A)-RNA species with a range of decay rates exist within each component.

The results from steady-state labeling with ^{14}C for the same three experiments were also normalized to the 23-h point and analyzed as above (Figure 4, open symbols). The computer fit for these data for one or two components was essentially the same. For one component, the RMS was 0.069 and the half-life was 26.6 h. For two components (Figure 4, lower solid line), the RMS was 0.068 and the half-life values were 32 h for the more stable component and 1.2 h for the unstable component, which represented less than 10% of the total at the beginning of the chase. The ^{14}C curve, therefore, reflected mostly the long half-life component. The ^{3}H and ^{14}C data suggest that, after short labeling periods, the short-lived component makes up the large majority of the total labeled poly(A)-RNA. However, as labeling times increase, the proportion of long-lived RNA increases until it becomes the majority under steady-state conditions. The half-life estimates for the long-lived component are similar within experimental error for the ^{3}H and ^{14}C curves. The half-life value for the unstable component in the two-component solution of the ^{14}C curve is larger than the value obtained from the ^{3}H curve; however, the ^{3}H value is probably more accurate because the short-lived component makes up a larger proportion of the ^{3}H curve. Because of scatter in the data points, possible errors in the determination of pool decay rate, and the low amount of radioactivity in poly(A)-RNA relative to rRNA, it is estimated that the value for the half-life of the unstable component is accurate to within ±50%, while for the stable component the accuracy is probably ±20%.

Discussion

An attempt has been made to assess the half-life of polysome-associated presumptive mRNA in a higher plant system. The polysomal poly(A)-RNA in these cells consists of at least two stability components. After 1 h of labeling, approximately 90% of the total labeled polysomal poly(A)-RNA is composed of an unstable population with an average half-life of 0.6 h. The stable component has a half-life of approximately 30 h and represents approximately 10% of the poly(A)-RNA after 1 h of labeling. However, under steady-state conditions it represents the majority of the polysomal poly(A)-RNA.

These half-life measurements depend upon the poly(A) sequence to separate poly(A)-RNA molecules from other RNAs. A metabolic process which removed the poly(A) fragment would destroy the ability of the poly(A)-RNA to bind poly(U) filters, making the lifetime of the poly(A)-RNA appear shorter. Poly(A) removal was not detectable during the half-life experiments. The amount of heterodisperse

poly(A)$^-$-RNA appearing on gel profiles of the material not bound to poly(U) filters during the early time points did not increase throughout the experiment, but decreased rapidly and was not detectable by 8 h after the beginning of the chase. Because newly synthesized poly(A) in soybean poly(A)-RNA is 100 to 140 nucleotides long (Key & Silflow, 1975), a considerable amount of shortening of poly(A) sequences (Sheiness & Darnell, 1973) could occur before the poly(A)-RNA would not be retained on poly(U) filters.

Because of the possibility of nuclear RNA contamination of the polysomes, estimates were made of the contribution of such contamination to the poly(A)-RNA decay results. The half-life determination of the unstable component would be most affected by the presence of nuclear RNA since the turnover rates of nuclear poly(A)-RNA in animal systems have been reported to be similar to that of the short-lived component in the soybean cells (Levis & Penman, 1977). RNA gel profiles of total and polysomal RNA isolated after pulse labeling were compared. Using rRNA precursor as a marker for nuclear RNA, it was estimated that a maximum of 30% of the nuclear poly(A)-RNA could contaminate the polysomes. Cell fractionation experiments showed that, for labeling times of 10 min to 1 h, only 10–20% of the total labeled poly(A)-RNA was found in the nuclear fraction. Taking into account nuclear contamination of the cytoplasm, it was estimated that 15–30% of the total labeled poly(A)-RNA in the cell is in the nucleus after labeling times of 1 h or less. The short-lived component represents 90% of the total labeled poly(A)-RNA at the beginning of the chase. It is unlikely that the 5–15% of the total labeled poly(A)-RNA in the cell which could be nuclear contamination of the polysomes would contribute 90% of the polysome-associated poly(A)-RNA, and therefore unlikely that the short-lived component is simply nuclear contamination. Another line of evidence which indicates that the unstable component is not nuclear contamination is the fact that at least 80% of the poly(A)-RNA used in these experiments was released to the top of the sucrose gradient by EDTA treatment (Table II) and was therefore polysome-associated RNA.

The results obtained in the half-life experiments were not due to differences in rates of processing of rRNA and poly(A)-RNA from the nucleus into the cytoplasm. Half-life experiments in which total cell poly(A)-RNA was analyzed gave results similar to those obtained in the polysome experiments in both the number of components and the half-life of each component.

The possibility that mitochondrial poly(A)-RNA may have contributed a decay component is unlikely. Gel profiles of polysomal poly(A)$^-$-RNA did not contain any radioactivity peaks in the areas expected for plant mitochondrial ribosomal RNA (24 and 18.5 S, Leaver & Pope, 1976).

An obvious question about the results obtained in these experiments is whether they would apply to plant cells in general or only to cells in culture. A final answer will be found only through direct studies on the stability of mRNA in an intact plant. Indirect studies with both excised (Key et al., 1967) and intact (Lin & Key, 1968) plant tissue have indicated that short- and long-lived mRNAs occur in plant cells, and that the mRNA for some proteins has a very short half-life.

Acknowledgments

We wish to thank Dr. James Rawson for help in preparing the computer program.

References

Covey, S. N., & Grierson, D. (1976) *Eur. J. Biochem. 63*, 599–606.

Cox, B. J., Turnock, G., & Street, H. E. (1973) *J. Exp. Bot. 24*, 159–174.

Fromson, D., & Verma, D. P. S. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 148–151.

Gamborg, O. L., Miller, R. A., & Ojima, K. (1968) *Exp. Cell Res. 50*, 151–158.

Gray, R. E., & Cashmore, A. R. (1976) *J. Mol. Biol. 108*, 595–608.

Hammett, J. R., & Katterman, F. R. (1975) *Biochemistry 14*, 4375–4379.

Kafatos, F. C. (1972) *Gene Transcription Reprod. Tissue, Trans. Karolinska Symp. Res. Methods Reprod. Endocrinol., 5th*, 319–345.

Key, J. L., & Silflow, C. D. (1975) *Plant Physiol. 56*, 364–369.

Key, J. L., Barnett, N. M., & Lin, C. Y. (1967) *Ann. N.Y. Acad. Sci. 144*, 49–62.

Larkins, B. A., Jones, R. A., & Tsai, C. Y. (1976) *Biochemistry 15*, 5506–5511.

Leaver, C. J., & Pope, P. K. (1976) in *Nucleic Acids & Protein Synthesis in Plants* (Bogorad, L., & Weil, J. H., Eds.) pp 213–237, Plenum Press, New York.

Levis, R., & Penman, S. (1977) *Cell 11*, 105–113.

Lin, C. Y., & Key, J. L. (1968) *Plant Cell Physiol. 9*, 553–560.

Loening, U. E. (1967) *Biochem. J. 102*, 251–257.

Noll, H. (1967) *Nature (London) 215*, 360–363.

Penman, S., Vesco, C., & Penman, M. (1968) *J. Mol. Biol. 34*, 49–69.

Perry, R. P., Bard, E., Hames, B. D., & Kelley, D. E. (1975) in *Biochemistry of the Cell Nucleus Mechanism & Regulation of Gene Expression* (Hidvegi, E. J., Sumegi, J., & Venetianer, P., Eds.) Vol. 33, pp 17–34, Akademiai Kiado, Budapest.

Puckett, L., Chambers, S., & Darnell, J. E. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 389–393.

Sheiness, D., & Darnell, J. E. (1973) *Nature (London), New Biol. 241*, 265–268.

Sheldon, R., Jurale, C., & Kates, J. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 417–421.

Singer, R. H., & Penman, S. (1973) *J. Mol. Biol. 78*, 321–334.

Spirin, A. S. (1969) *Eur. J. Biochem. 10*, 20–35.

Spradling, A. (1975) *Cell 4*, 139–140.

Spradling, A., Hui, H., & Penman, S. (1975) *Cell 4*, 131–137.

Sutton, D. W., & Kemp, J. D. (1976) *Biochemistry 15*, 3153–3157.

Trewavas, A. (1970) *Plant Physiol. 45*, 742–751.