Differential Stability of Drosophila Embryonic mRNAs during Subsequent Larval Development

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ABSTRACT The relative stabilities of specific embryonic mRNAs that persist in Drosophila melanogaster larvae were determined using an approach that combined RNA density labeling with cell-free translation. Unlike the other methods commonly used to measure the decay of individual mRNAs, the density labeling approach does not depend on the use of transcriptional inhibitors or on the measurement of precursor pool specific activities. Using this approach, we have determined that different embryonic mRNA species persist for varying periods during subsequent development, with half-lives ranging from ~2 to ~30 h. The embryonic histone mRNAs are relatively unstable; they are no longer detectable by 9 h of larval development. By 41 h of larval development, 90% of the nonhistone mRNAs assayed have decayed considerably; computerized scanning densitometry of translation products indicates that these transcripts are not decaying as members of discrete half-life classes. The persisting mRNAs that remain are very long-lived; their in vitro translation products can still be detected after 91 h of larval development. We have tentatively identified the mRNAs that encode actin, tropomyosin, and tubulin as members of this stable mRNA population. Although embryonic mRNAs do fall into these three broad classes of stability, they appear to decay with a continuum of half-lives. Because the range of half-lives is so great, mRNA stability is probably an important factor controlling mRNA abundance during Drosophila development.

The cytoplasmic abundance of a particular mRNA can be controlled at both the transcriptional and posttranscriptional levels. One of the most poorly understood posttranscriptional controls is that which defines the stability of specific mRNA sequences. The importance of turnover rate in controlling mRNA abundance, and thus gene expression, is best illustrated by the observation that many of the mRNAs that encode the most highly abundant cell type–specific polypeptides are preferentially stabilized over other mRNAs present within the same cytoplasm (6, 21, 25, 29, 44).

The stability of specific eucaryotic mRNAs has most often been determined by assaying for the presence of particular mRNAs after treating cells with the transcriptional inhibitor actinomycin D (4, 13, 25, 34, 43, 46). However, this kind of analysis is subject to several possible artifacts. In addition to a secondary effect on cellular protein synthesis (12, 15, 39, 40) it has also been shown that actinomycin D treatment can both decrease the stability of total mRNA populations (41) and increase the half-life of specific mRNAs (9, 42). Also, if

the actinomycin D "chase" does not begin immediately or is not completely effective, then the measured half-life of a mRNA species will appear longer than it actually is.

The best experimental system to use for studying the differential stability of individual mRNAs would be one in which no transcriptional inhibition is necessary. For example, by measurement of the rate of radiolabel accumulation into specific mRNAs by using cDNA clone hybridization, the differential half-lives of nine mRNAs from Chinese hamster ovary cells (3.5–14 h; reference 22) and six mRNAs from sea urchin embryos (from 2.2 to > 11.5 h; reference 8) have been measured. Although useful, this approach to a steady state radiolabeling method for measuring mRNA half-lives requires corrections for various labeling inaccuracies (for example, the time for the precursor pool to reach constant specific activity) and also necessitates having cDNA probes for the mRNA species of interest.

Other methods, employing neither transcriptional inhibition nor conventional radiolabeling, have also been employed

to measure the decay of specific mRNA species, but these are not always generally applicable. For example, by translating in vitro the RNA present at various stages of rabbit reticulocyte maturation, Lodish and Small (29) have observed the preferential stability of globin mRNA. Since reticulocytes have no nuclei, the experiment does not require radiolabeling or actinomycin D treatment. Koch and Friesen (27) have detected a 20-fold variation (3.5–70 min) in mRNA half-lives in a temperature-sensitive mutant of *Saccharomyces* that cannot accumulate cytoplasmic RNA under restrictive conditions. In these experiments, the decay of specific mRNAs was determined by monitoring the pattern of protein synthesis at various times after the cells were transferred to the nonpermissive temperature.

Our method for measuring mRNA stability, which is of general use and also does not require transcriptional inhibition, relies on a pulse-chase density labeling strategy to follow the decay of pre-existent RNA (49). This strategy allows one to monitor chase effectiveness without measuring precursor pool specific activities and recover the pre-existent and newly synthesized RNA populations for further analysis. Bowman and Emerson (5) have used this methodology to demonstrate the uniform decay kinetics ($t_{1/2}$, 20 h) of quail muscle fiber mRNAs. We have used these techniques here, modified by a new gradient system to give better recoveries of translatable mRNA, for the measurement of mRNA stability during *Drosophila melanogaster* development.

Very little is known about the contribution of mRNA stability in regulating gene expression in *Drosophila*; only the half-life of embryonic histone mRNA has been reported (2). We have found that individual embryonic mRNAs persist for very different periods during larval development. The great range of mRNA half-lives implies that mRNA stability probably plays an important role in determining mRNA abundance during *Drosophila* development.

MATERIALS AND METHODS

Light Pulse–Dense Chase Method for Determining Stability of Embryonic mRNA:
D. *melanogaster* females (Ore-R, 2–3 d posteclosion) were anesthetized, and ~400 flies were placed into a labeling chamber. The construction of these labeling chambers and procedures for feeding have been described (47). In brief, the flies are kept in polypropylene tubes with a nylon mesh covering each end; one end of the tube is then placed on a base, consisting of 2% (wt/vol) agar–1% (wt/vol) dextrose, which provides a surface for feeding and egg laying. For the light pulse, flies were fed a yeast paste containing [³H]uridine for 60 h. The yeast paste was prepared by mixing 140 mg yeast with 72 μl distilled H₂O, 8 μl 50× phosphate stock (0.53 M KH₂PO₄, 2.1 M K₂HPO₄), 40 μl 10× MgSO₄/Na₂CO₃ stock (0.02 M MgSO₄, 0.24 M Na₂CO₃), 80 μl 5× vitamin stock, and 200 μl of 2.5 mCi/ml [5,6-³H]uridine (30–42 Ci/mmol, Schwarz-Mann, Orangeburg, NY). The 5× vitamin stock contained 0.03 mM thiamine-HCl, 0.49 mM nicotinic acid, 0.13 mM riboflavin-5-PO₄, 0.34 mM Ca⁺-pantothenate, 0.06 mM pyridoxine-HCl, 0.006 mM biotin, 0.11 mM folic acid, and 2.97 mM choline chloride. To prevent radiolabel diffusion into the agar base, the yeast paste was placed on a small plastic disk embedded in the center of the base. Flies were fed every 3–4 h and the agar bases were changed after 24 and 48 h of labeling. The labeling was done at 25°C in a humid plastic chamber.

Embryos deposited during the last 12 h of this 60-h adult radioactive labeling were collected, and an aliquot was washed with distilled H₂O, dechorionated with 3% (vol/vol) sodium hypochlorite, and frozen at −70°C. The remaining embryos were surface-sterilized by a 2-min treatment in 2% (wt/vol) cetyltrimethylammonium bromide and then transferred into a sterile medium that contained 20 mg ¹³C-¹⁵N-labeled *Chlorella*, 20 mg ²H-¹³C-¹⁵N-labeled *Chlorella*, 8 mg agarose, 20 μl 50× phosphate stock, 100 μl 10× MgSO₄/Na₂CO₃ stock, 200 μl 5× vitamin stock, 580 μl distilled H₂O, and 100 μl of 10 μCi/ml [2-¹⁴C]-uridine (58 mCi/mmol, Schwarz-Mann). The embryos were then placed at 25°C for subsequent development.

RNA Extractions:
Embryonic and larval RNA were prepared as described (48) except that it was not necessary to remove high molecular weight RNA from DNA by 2 M LiCl precipitation since DNA and RNA are well resolved in KI/NaI gradients.

Equilibrium Gradient Centrifugation of RNA Preparations in KI/NaI:
RNA samples in sterile distilled H₂O were added to 0.305 ml NaI stock (density, 1.896 gm/ml [MCB Manufacturing Chemists, Inc., Cincinnati, OH], prepared in 0.8 mM Na₂SO₃ to prevent oxidation), 0.984 ml KI stock (density = 1.718 gm/ml [Mallinckrodt Inc., St. Louis, MO], also prepared in 0.8 mM Na₂SO₃), 0.05 ml deionized formamide, and enough additional sterile distilled H₂O to give a final volume of 1.5 ml. Gradients were overlaid with mineral oil and centrifuged in the Beckman SW60 Ti swinging bucket rotor (48,000 rpm for 48 h at 20°C; Beckman Instruments Inc., Palo Alto, CA). Four-drop fractions (~50 μl each) were then collected from the tube bottom and the density of selected fractions was determined by measuring the weight of fraction aliquots. The density distribution of radioactive RNA was determined by liquid scintillation counting of an aliquot of each fraction after treatment to prevent chemiluminescence by free iodine. This was accomplished by mixing ~25% of each gradient fraction with 0.5 ml of 1% (vol/vol) beta-mercaptoethanol (Sigma Chemical Co., St. Louis, MO). This mixture was then counted in Beckman Ready-Solv MP.

Recovery of RNA from KI/NaI Gradients:
Since stable embryonic [³H]RNA served as an internal marker for the position of light RNA in larval RNA gradients, heavy and light RNA fractions could be easily identified. To minimize cross-contamination between heavy and light RNA species, we always pooled fractions in only the extreme heavy side or the extreme light side of the heavy and light RNA peaks, respectively. These RNA fractions were precipitated at −20°C after the addition of 2 vol of 0.2 M KOacetate, phenol-extracted calf liver tRNA (Boehringer-Mannheim Biochemicals, Indianapolis, IN) to 25 μg/ml, and 6 vol cold ethanol. The RNA was then pelleted, resuspended in 0.2 M KOacetate, and reprecipitated. At least 90% of the radioactive RNA initially present in the pooled gradient fractions was recovered by this procedure.

Quantitation of RNA Mass Recovered from Gradients:
The amount of RNA in individual light (embryonic) or dense (larval) RNA samples was estimated as follows. From the specific activity of the 48–60-h pulse [³H]RNA sample, the mass of light embryonic [³H]RNA present in larval RNA samples could be approximated. To determine the amount of dense larval [¹⁴C]RNA, the number of larvae used in the RNA centrifugation was multiplied by the amount of RNA/larva at various times during larval development (as estimated by Church and Robertson [10]). The age of the growing larvae was estimated after correction for the fact that larvae fed an algae medium develop at half the rate as those fed a yeast medium.

Cell-free Translation and Analysis of Products by One- and Two-Dimensional PAGE:
Total RNA was added to a rabbit reticulocyte cell-free translation system; translation products were fractionated using gel electrophoresis and visualized using fluorography as previously described (48). Unless otherwise noted, all gel lanes in one-dimensional gels were loaded with an equal amount of trichloracetic acid–precipitable [³⁵S]methionine radioactivity, and all two-dimensional gels within a particular experiment were exposed to film for equivalent (³⁵S-disintegrations per minute loaded) × (days exposure) values.

Scanning Densitometry of Two-Dimensional Gels:
The optical density of specific polypeptides on the fluorograms was quantitated by computerized densitometry. Flurograms were scanned with a Photoscan P-1000 densitometer (Optronics, Chelmsford, MA) using a 100-μm path length. The data from the scan was processed using the computer program of Garrison and Johnson (18).

RESULTS

Density Labeling/Cell-free Translation Strategy for Measuring Drosophila mRNA Stability

We have developed a light pulse–dense chase labeling strategy to measure embryonic mRNA persistence during subsequent larval development. In these experiments, embryos containing radioactive RNA were collected from adult females that had been fed a yeast paste containing [³H]uridine for 2 d. These embryos were then transferred into a medium containing 50% ¹³C-¹⁵N-*Chlorella* and 50% ²H-¹³C-¹⁵N-*Chlorella*, as well as [¹⁴C]uridine, for subsequent development.

When feeding commences at the end of embryonic development, the newly hatched larvae contain [^{3}H]RNA with a normal buoyant density. As larval development progresses and the isotopically substituted *Chlorella* is ingested, newly synthesized RNA becomes density labeled and labeled with [^{14}C]uridine as well. As embryonic [^{3}H]RNA decays, it would be expected that there would be reuse of some ^{3}H-labeled precursors, which would then be incorporated at the heavy buoyant density. RNA was prepared from larvae collected at various times during this dense chase period. Stable embryonic [^{3}H]RNA (light buoyant density) was separated from the newly synthesized larval [^{14}C]RNA (heavy buoyant density) in KI/NaI equilibrium gradients. One or both classes of RNA were recovered and added to a rabbit reticulocyte cell-free translation system.

Several technical points need to be addressed with regard to the basic strategy outlined above. First, the effectiveness of the pulse-chase can be easily determined in this type of experiment. If the chase is effective, there should not be any ^{14}C radiolabel in the light region of the larval RNA equilibrium gradients. Second, there are no discernable isotope effects during the larval chase period. Although larval development in the dense algae mixture occurs at half the rate of larvae raised on a yeast medium, this is also true when larvae are grown in unlabeled algae. There may be an isotope effect during pupation, however, since larvae raised in this dense algae mixture mentioned above rarely eclose. Third, although it is possible to separate dense and light RNAs on cesium formate equilibrium gradients (5, 20, 49), RNA from these gradients has a greatly diminished capacity to stimulate in vitro translation systems. Therefore, we developed a gradient system using KI and NaI that can adequately resolve dense and light RNAs and does not subsequently affect mRNA translatability.

A number of issues must also be considered in order to quantitate the amount of a specific mRNA using cell-free translation. We have previously described the translational properties of *Drosophila* RNA in a rabbit reticulocyte cell-free translation system (47). We have shown that the incorporation of [^{35}S]methionine in this system has a linear response within a range of RNA amounts and that individual mRNAs are translated with the same relative efficiency at different subsaturating RNA concentrations. The presence of calf liver tRNA carrier, which we use as a carrier in ethanol precipitations to recover RNA from gradients, has no effect on the spectrum of translation products synthesized by total RNA. In addition, the fraction of embryonic RNA that persists in larvae is unusual in that it is depleted of mRNA, since rRNA is more stable than mRNA. Therefore, we have done reconstruction experiments in which excess rRNA is added to total RNA preparations to duplicate this condition. Again, we see no differences in the spectrum of translation products synthesized, though at very high rRNA concentrations, total translational stimulation is inhibited. Finally, to determine if specific mRNA sequences were being selectively lost, modified, or degraded during centrifugation in KI/NaI, we compared the translation products synthesized by control embryonic RNA and the same RNA that had been incubated in our standard KI/NaI gradient solution. In general, the spectrum of polypeptides synthesized by control RNA and KI/NaI-treated RNA were very similar. However, the translation products synthesized by KI/NaI-treated RNA contained a relatively higher proportion of radioactivity in low molecular weight polypeptides, indicating that there is a small amount of inactivation or degradation of mRNA in KI/NaI gradients.

Stability of Embryonic mRNA during Early Larval Development

In this series of experiments, female flies were fed a yeast paste containing [^{3}H]uridine for 67 h. The embryos laid during the last 12 h of this adult labeling period were then collected, some were immediately frozen, and the remainder were surface-sterilized and placed into an axenic medium that contained density-labeled algae and [^{14}C]uridine. First instar larvae were collected from the medium at larval age times (and chase times) of 9, 20, and 33 h. Larval chase times were estimated by assuming that the average age of collected embryos was one-half the length of the collection period, that the duration of embryonic development was 23 h (35), and that larvae begin feeding immediately after hatching. Total RNA from embryos and larvae was extracted and centrifuged to equilibrium in KI/NaI gradients; the results are shown in Fig. 1. Since the half-life of embryonic total RNA during these larval growth conditions is 115 h (49), very little of the light [^{3}H]RNA has decayed during these early stages of larval development. However, even after only 9 h of larval feeding the dense chase was effective; most of the newly synthesized larval RNA (^{14}C labeled) had a buoyant density greater than did the persistent embryonic [^{3}H]RNA (Fig. 1 *B*).

Heavy and light RNAs were recovered from the appropriate gradient regions and translated in vitro. Radiolabeled translation products were electrophoresed on a 14% SDS polyacrylamide gel and visualized by fluorography; the fluorogram is shown in Fig. 2. There are three major results illustrated in this figure. First, in comparison with uncentrifuged RNA, the RNA recovered from KI/NaI gradients contained fewer mRNAs encoding high molecular weight polypeptides (com-
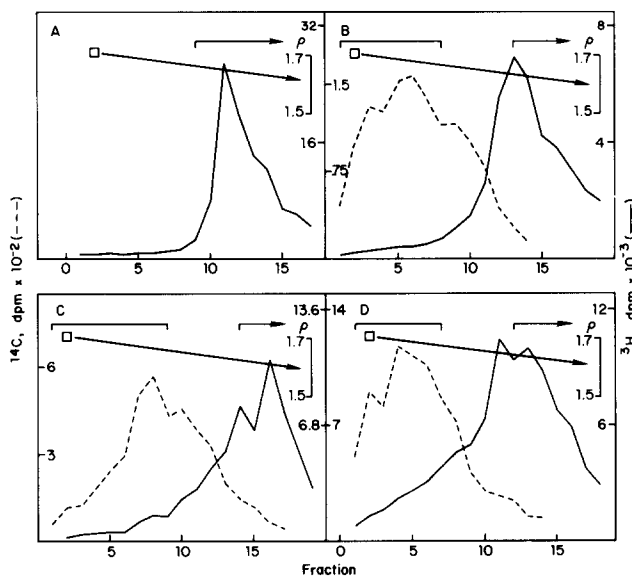

FIGURE 1   KI/NaI gradient series used for one-dimensional gel analysis of embryonic mRNAs that persist during early larval development. Embryos were collected from [^{3}H]uridine-labeled adults and transferred into a dense algal medium containing [^{14}C]uridine. RNA prepared from embryos and larvae was centrifuged to equilibrium in KI/NaI gradients. (A) Embryo RNA. (B) 9-h larva RNA. (C) 20-h larva RNA. (D) 33-h larva RNA. The RNA fractions pooled for translation analysis are marked with brackets.
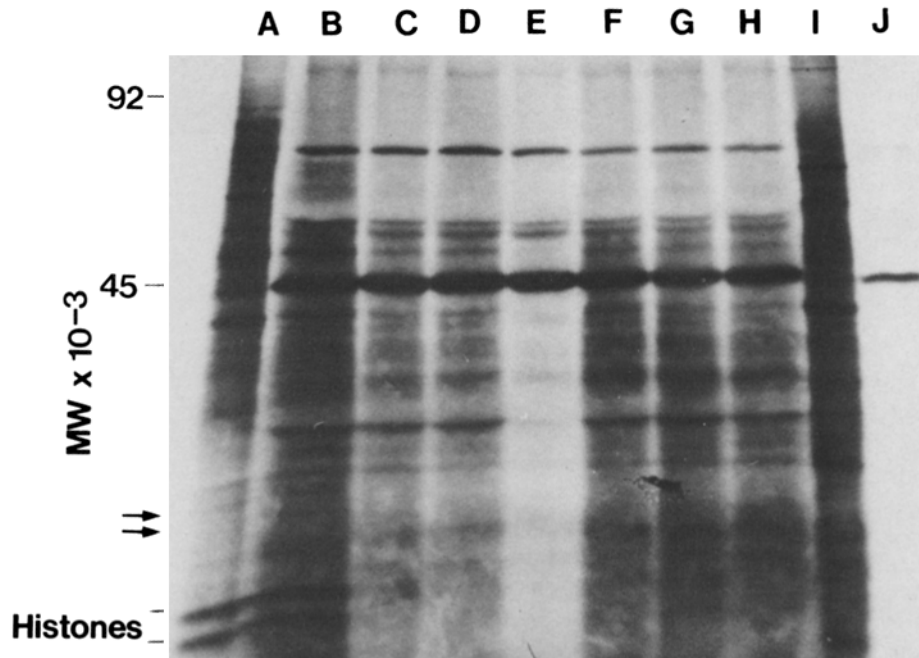
FIGURE 2 One-dimensional gel analysis of translation products synthesized by stable embryonic mRNA and newly synthesized larval mRNA recovered from developing larvae. RNA recovered from the bracketed regions in the Fig. 1 equilibrium gradients was translated in vitro. Translation products were electrophoresed on a 14% SDS polyacrylamide gel and detected by fluorography. Lane A, 0–12-h embryonic RNA (uncentrifuged). Light RNA: lane B, 0-h chase, lane C, 9-h chase, lane D, 20-h chase, lane E, 33-h chase. Heavy RNA: lane F, 9-h chase, lane G, 20-h chase, lane H, 33-h chase. Lane I, second instar larval RNA (uncentrifuged). Lane J, no RNA added. Lane E contains 60% of the radioactivity loaded into the other lanes. The black circles on the right side of the figure denote polypeptides that are radiolabeled in the translation system when no RNA is added. The histone polypeptides have been identified by co-migration with purified Drosophila histones. The arrows denote embryonic nonhistone polypeptides that are no longer found in the stable embryonic RNA fraction recovered from 9-h larvae.

pare Fig. 2, lanes A and B or H and I), indicating, as discussed previously, that there is some mRNA degradation during KI/NaI centrifugation. Second, the similar polypeptide pattern observed in Fig. 2, lanes B–D indicates that most of the very abundant embryonic mRNAs survive through the first 20 h of larval development. However, some translation products synthesized by embryo RNA (Fig. 2, lane B) are absent from stable embryo RNA recovered from 9-h larvae (lane C). We have identified the low molecular weight translation products that are no longer detectable at 9 h as histones. Therefore, histone mRNA sequences, which are very abundant in Drosophila embryos (2, 48), and a few nonhistone abundant mRNAs are relatively short-lived. Finally, except for the absence of histones, the polypeptide pattern present in the larval RNA translation lanes is similar to that in the embryonic RNA translation lanes. Since two-dimensional gel analyses of translation products have indicated that the abundant nonhistone mRNA populations in embryos and larvae are virtually identical (48), this result is not surprising. Nevertheless, the similarity in this experiment could result from cross-contamination between the pooled heavy and light RNA fractions. As discussed later in Results, however, there is no significant RNA cross-contamination in these experiments.

To assess more accurately the turnover characteristics of specific embryonic nonhistone mRNAs during early larval development, we examined translation products using a two-dimensional gel analysis. In this experiment, larvae were collected after a chase period of 41 h. Embryonic and larval RNA were prepared and centrifuged in KI/NaI gradients. Heavy and light RNA samples recovered from these two gradients were translated; fluorograms displaying the radiolabeled translation products are shown in Fig. 3. If the translation products of total embryonic RNA (Fig. 3A) are compared with the translation products of embryonic RNA recovered from 41-h larvae (Fig. 3B), it is clear that the relative intensity of many polypeptide spots is lower in Fig. 3B. This

indicates that the relative stabilities of the embryonic mRNAs are different. A few examples of polypeptides synthesized by mRNAs of differing stabilities are denoted by arrows in Fig. 3,A and B. We have tentatively identified two of the noted translation products by comparison with published two-dimensional gel analyses of Drosophila proteins; they are the 70-kD heat-shock protein and an α-tubulin. To approximate whether the embryonic mRNAs were decaying with discrete turnover rates or as members of half-life classes, we measured, using computerized scanning densitometry (18), the optical density of 18 embryonic RNA translation products present in both the 0- and 41-h-chase fluorograms. To reduce the potential for scanning inaccuracies, we chose polypeptide spots that were readily identifiable on both films (Fig. 3,A and B) and well separated from other spots and high background areas of the film. For each film, the density of 17 spots was normalized to the density of an actin isoform known to be encoded by a very stable mRNA (see below). This normalization controls for possible variability in RNA recovery and inequality in film exposures. The results of this analysis are shown in Table I. That the normalized density ratios cannot be grouped into obvious half-life classes argues that the embryonic mRNAs are decaying with a continuum of half-lives.

Translation products synthesized by larval RNA accumulating during the first 41 h of larval development are shown in Fig. 3C. Comparison of this polypeptide pattern with that seen in Fig. 3B indicates that many of the decaying embryonic mRNAs are also being newly synthesized in larvae and that the translation products seen in Fig. 3B are not due to contamination of the pooled light fractions of the gradient by larval RNAs. Several of the abundant products seen in larval RNA are not seen, or are greatly reduced, in the stable embryonic mRNA fraction. If there were substantial contamination, all of these abundant products would be seen in the light peak.
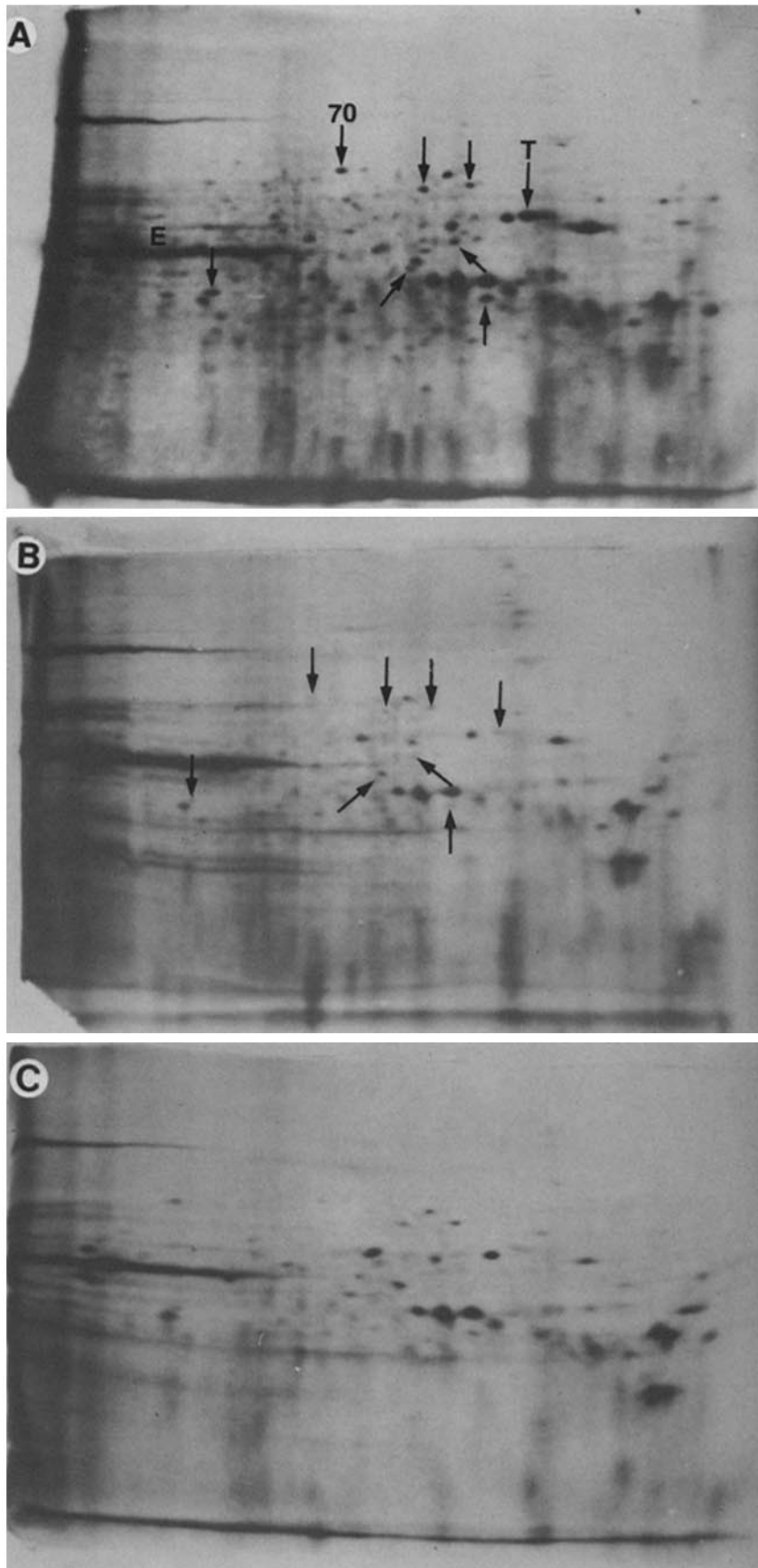
FIGURE 3   Two-dimensional gel analysis of translation products synthesized by stable embryonic mRNA and newly synthesized larval mRNA recovered from developing larvae. RNA recovered from the light and heavy regions of KI/NaI equilibrium gradients was translated in vitro. Translation products were electrophoresed on pH 5–7 isoelectric focusing tube gels and then on 10% SDS polyacrylamide slab gels. Slab gel electrophoresis was from top to bottom; the basic end of the pH gradient is on the left side of each gel. Polypeptides were visualized using fluorography. Translation products were synthesized by (A) embryonic RNA, 0-h chase; (B) stable embryonic RNA, 41-h chase; (C) newly synthesized larval RNA, 41-h chase. In A, the horizontal streak labeled E is a polypeptide that becomes radiolabeled in the translation system even when no RNA is added. In A and B polypeptides encoded by embryonic mRNAs that have dramatically decreased in concentration by 41 h of larval development have been denoted with arrows. Tentative polypeptide identifications: 70, 70-kD heat-shock polypeptide; T, α-tubulin.

TABLE I. Relative Decay Rates of Embryonic mRNAs in Larvae

Spot No. (tentative identification)	Normalized optical density*		Normalized density ratio (b/a)
	(a) 0 h	(b) 41 h	
1	0.61	0.44	0.72
2	0.38	0.01	0.01
3	0.43	0.26	0.60
4	0.74	0.10	0.13
5	0.36	0.01	0.03
6	0.35	0.07	0.20
7 (muscle-specific actin)	0.60	0.31	0.52
8 (actin)	1.00	1.00	1.00
9 (actin)	0.76	0.67	0.88
10	0.79	0.36	0.46
11	0.38	0.28	0.74
12 (β-tubulin)	1.20	0.65	0.54
13	0.18	0.17	0.94
14	0.49	0.34	0.69
15	0.91	0.32	0.35
16	0.47	0.20	0.43
17 (muscle-specific tropomyosin)	1.12	1.10	0.98
18 (α-tubulin)	0.99	0.01	0.01

* The two-dimensional gel films shown in Fig. 3, A and B were scanned using computerized densitometry (18). For each individual film, the actual optical density values were normalized to the optical density of spot 8.

Stability of Embryonic mRNA during Later Larval Development

To determine how far into larval development the most stable class of embryonic mRNA persisted, we translated embryonic RNA recovered from much older larvae. Larvae were collected from the dense chase medium at larval age (and chase) times of 91 h (first and second instar larvae) and 127 h (second and third instar larvae). KI/NaI gradients of RNA from these samples (Fig. 4) clearly show the turnover of [³H]RNA, as expected, since the half-life of embryonic rRNA under these larval growth conditions is 115 h (49). The translation products directed by light embryonic RNA recovered from these three gradients are shown in Fig. 5. Although the dense RNA recovered from 127-h-old larvae stimulated protein synthesis and produced a complex set of translation products (data not shown), we did not detect any translation products from light RNA. The radiolabeled polypeptides observed in Fig. 5 C were also present in similar film exposures of gels containing translation products from reactions with no added RNA. The many background radiolabeled polypeptides are visible due to the very long fluorographic exposures necessary for this analysis. The complete absence of light RNA translation products and the complex translation pattern directed by heavy RNA from the same gradient again demonstrates the absence of significant cross-contamination between heavy and light RNA in our experiments.

As seen in Fig. 5B, a small group of embryonic mRNAs, representing a subset of those mRNAs detected at 41 h of larval development (Fig. 3B), appear to survive at least until 91 h of larval development. Some of the polypeptides encoded by these mRNAs are marked by arrows in Fig. 5,A and B. By comparing our gels with previously published Drosophila two-dimensional gel patterns, we have tentatively identified five

of these polypeptides: three actin isoforms, muscle-specific tropomyosin, and a β-tubulin.

DISCUSSION

The Utility of Density Labeling for Measuring RNA Stability

We have measured the stability of Drosophila embryonic mRNAs using an approach that employs both density labeling and cell-free translation. As mentioned in the introduction, the two most common methods used to measure the stability of individual mRNAs are assay of the decay of specific mRNAs after transcriptional inhibition, and measurement of the rate of radiolabel accumulation into specific mRNA species. Unlike the first method, our approach does not rely on drug treatment; therefore, we do not have to contend with the possibility that such treatment could artifactually alter
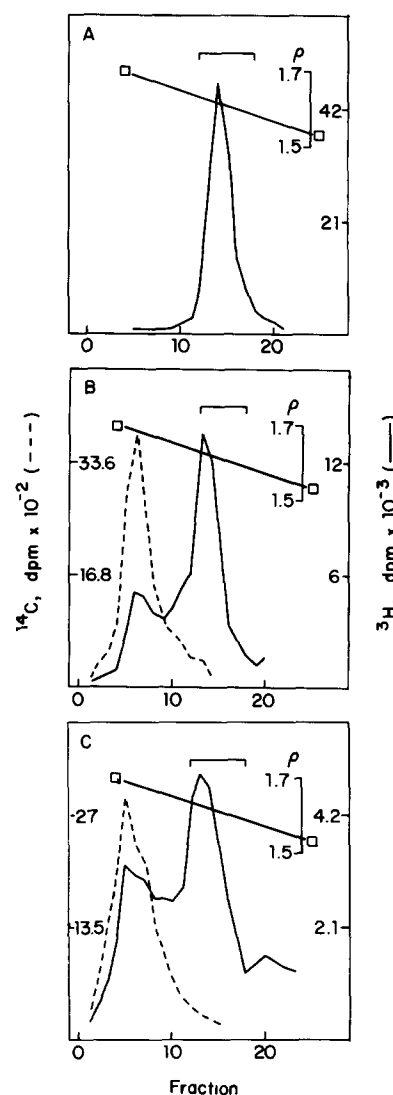

FIGURE 4 KI/NaI gradient series used for two-dimensional gel analysis of embryonic mRNAs that persist during late larval development. Embryos were collected from [³H]uridine-labeled adults and transferred into a dense algal medium containing [¹⁴C]uridine. RNA prepared from embryos and larvae was centrifuged to equilibrium in KI/NaI gradients. (A) Embryo RNA. (B) 91-h larva RNA. (C) 127-h larva RNA. The RNA fractions pooled for translation analysis are marked with brackets.
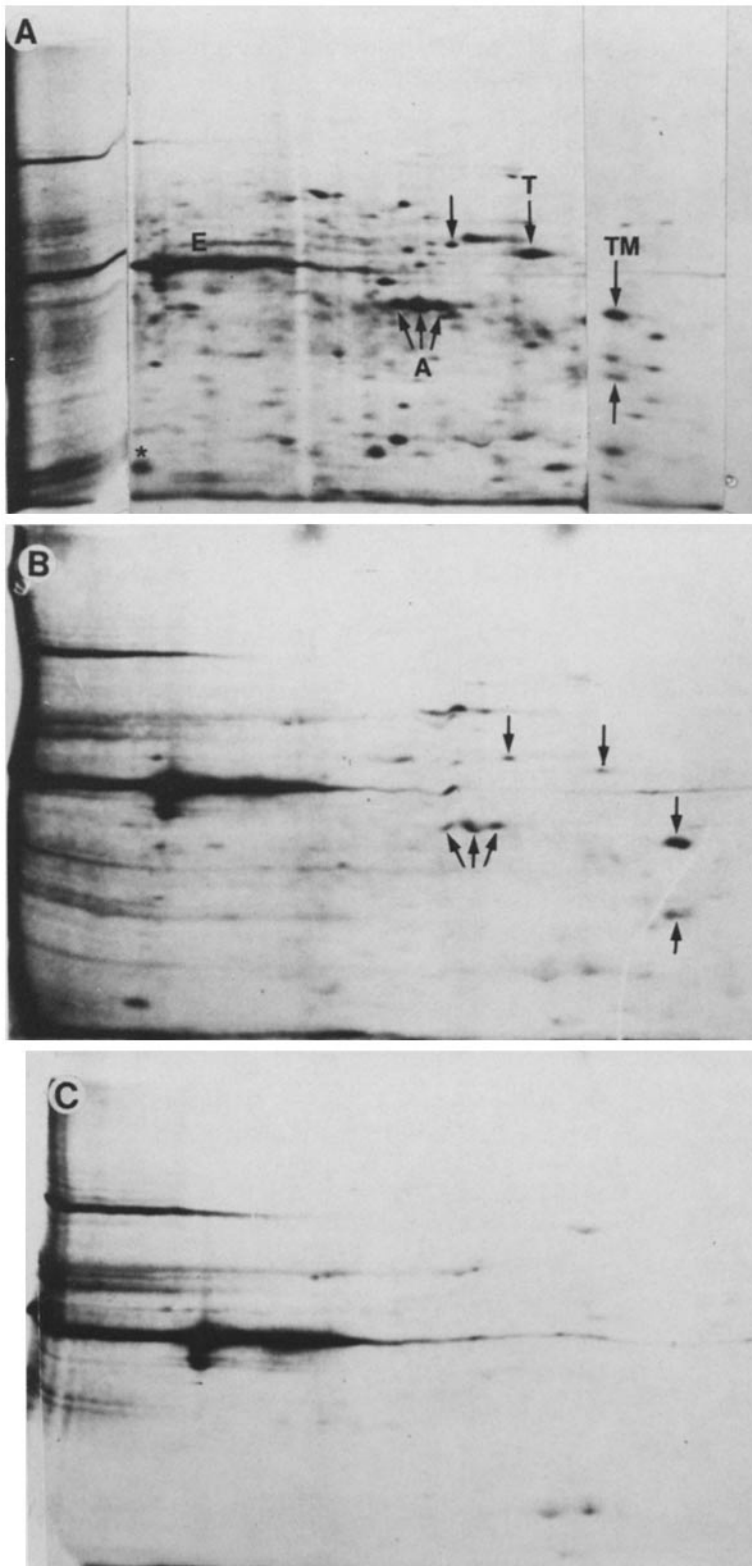
FIGURE 5 Two-dimensional gel analysis of translation products synthesized by stable embryonic mRNA recovered from developing larvae. RNA recovered from the light regions in the equilibrium gradients shown in Fig. 4 was translated in vitro. Translation products were electrophoresed and visualized using fluorography as described in the legend to Fig. 3. Translation products were synthesized by (A) embryonic RNA, 0-h chase; (B) stable embryonic RNA, 91-h chase; (C) stable embryonic RNA, 127-h chase. In A, the horizontal streak labeled E is the major radiolabeled polypeptide that is endogenous to the translation system. The asterisk denotes a polypeptide that is not reproducibly observed in all translations of gradient-recovered embryo RNA. Polypeptides encoded by extremely stable embryonic mRNAs are denoted by arrows in A and B. Tentative polypeptide identifications: A, actins, TM, tropomyosin, T, β-tubulin.

normal mRNA decay rates. For the second method to be valid, the specific activity of the precursor pool must be measured during the labeling period. In contrast, the effectiveness of our light pulse–dense chase labeling strategy is easily monitored by including two different radiolabels in the pulse and chase media. The distribution of the chase radiolabel between the light and dense RNA fractions indicates the effectiveness of the chase.

The density labeling methodology presented here can be

applied to a variety of experimental systems. Although we have used dense algae as the source of label in our *Drosophila* experiments, RNA can also be density labeled by including dense ribonucleosides in culture media (5, 20). In another variation of the strategy applied in this report, one can also assay RNA levels using DNA/RNA hybridization instead of in vitro translation (49). However, unlike in vitro translation, a hybridization procedure using cloned DNA fragments will not discriminate between intact and partially degraded RNA

species and does not allow one to examine a large number of specific mRNAs.

Drosophila Embryonic mRNAs Decay with a Continuum of Half-Lives

Different *Drosophila* embryonic mRNAs probably decay with a continuum of half-lives (Table I). However, for the purposes of this discussion, the embryo mRNAs can be divided into three broad classes of different relative half-lives: those decaying with fast, intermediate, or slow turnover kinetics. The major unstable mRNAs are the histone mRNAs, although a few nonhistone mRNAs also appear to decay rapidly. Approximately 90% of the nonhistone mRNAs are members of the intermediate stability class. This class may include the mRNAs that code for the 70-kD heat-shock protein and an α-tubulin. Approximately 10 mRNAs are present in the slow decay class of embryonic mRNA. Actin, tropomyosin, and β-tubulin mRNAs may be representatives of this stability class.

The persistent embryonic mRNAs whose stability we have measured have been recovered from larvae that are being fed algae and are therefore developing at a relatively slow rate. We have previously shown that the stability of inherited embryonic rRNA increases under slow growth conditions (49). We do not know if the stability of the inherited embryonic mRNA sequences also changes with larval growth rate. However, previous investigations have determined that polyadenylated RNA is equally stable in resting and growing tissue culture cells (1, 32, 37) and that the half-life of specific mRNA species does not change as a function of *Escherichia coli* growth rate (11, 19). In any case our major concern is the relative stability of mRNAs in this system.

Embryonic Histone mRNAs Are Relatively Unstable and Decay Coordinately

At 9 h of larval development, we cannot detect the presence of embryonically synthesized histone mRNA, although most of the other embryonic abundant mRNAs are still present. If we assume first-order degradation kinetics and that no more than 5% of the initial amount of histone mRNA is present after a 9-h chase, then histone mRNA has a maximum half-life of 2 h. This result agrees with that of Anderson and Lengyel (2), who calculated the rate of histone mRNA synthesis and accumulation and determined that histone mRNAs decay during *Drosophila* embryogenesis with a maximum half-life of 2.5 h. Histone mRNA is unstable in amphibian (52) and sea urchin (31, 45) embryos as well.

In agreement with other studies (24, 31, 50, 51), we have observed that the half-lives for those individual histone mRNAs whose translation products we can easily resolve from one another (H3 from H2A/H2B) are not significantly different. Therefore, different histone mRNA species must contain common recognition signals that control their degradation.

Putative Actin, Tropomyosin, and Tubulin Transcripts Are Examples of mRNAs Preferentially Conserved During Larval Development

Our experiments have indicated that a small set of mRNAs, tentatively identified as those encoding actin (cytoskeletal and muscle specific), tropomyosin (muscle specific), and β-tubu-

lin, are unusually long-lived. The preferential stability of these mRNAs is not simply due to the fact that they are some of the most abundant mRNAs in the embryo; other similarly abundant mRNAs (those we have tentatively identified as histone and α-tubulin, for example) are relatively unstable. The difference in the relative half-lives of two mRNAs that apparently encode tubulin subunits is quite large. This implies that the differing patterns of α- and β-tubulin accumulation during *Drosophila* development (33) probably result from both the transcriptional regulation of tubulin genes and the differential stability of tubulin mRNAs.

We can no longer detect the in vitro synthesis of those peptides believed to be actin, tropomyosin, or β-tubulin by embryonic RNA recovered from 127-h old larvae. If we assume first-order decay and that no more than 5% of the initial amount of these mRNAs is present after a 127-h chase, then the maximum half-life of these mRNAs is ~30 h. The stability of polyadenylated RNA or individual nonhistone mRNA species during *Drosophila* development has not been reported. However, the contractile protein mRNAs, including actin and tropomyosin, are also long-lived ($t_{1/2}$ = 20 h) in quail muscle fiber cells (5). In contrast to our results, experiments using drug treatment to inhibit RNA synthesis have indicated that *Dictyostelium* actin mRNA has a 3-h half-life (30) and that β-tubulin mRNA is unstable ($t_{1/2}$ = 2 h) in cultured mammalian cells (4).

Why would the embryonic mRNA sequences that code for actin, tropomyosin, and tubulin be preferentially conserved during larval development? During *Drosophila* late embryogenesis and early larval development, the larval visceral and hypodermal muscles form and orient along a scaffold of microtubules (for review see reference 14). Large amounts of actin, tropomyosin, and tubulin are undoubtedly required during larval muscle differentiation. Indeed, it has been shown that during late embryogenesis and/or early larval development, mRNAs that code for actin (17, 38), myosin heavy-chain (36), tropomyosin (3), and tubulin (26, 33) are transcribed and accumulated. If our polypeptide identifications are correct, then our experiments also indicate that newly synthesized actin, tropomyosin, and tubulin mRNAs are present as abundant mRNA species in young larvae (see Fig. 3 C). If we assume that the embryonic mRNAs that we have found to be functionally intact in vitro are participating in larval protein synthesis in vivo, then it appears that *Drosophila* larvae meet their requirement for large amounts of actin, tropomyosin, and tubulin by translating both newly synthesized larval mRNAs and preferentially conserved embryonic mRNAs.

Control of mRNA Half-Life

The mechanism(s) that control the stability of specific mRNA species is at present unknown. The mRNAs coding for the major proteins synthesized by terminally differentiated cells frequently have half-lives of 30 h or more (7, 21, 23). It is therefore possible that the stable subset of embryonic mRNAs inherited by larvae are compartmentalized into specific terminally differentiated cells, such as the muscle cells. This compartmentalization could in itself be responsible for the preferential stability of the embryonic actin, tropomyosin, and tubulin mRNAs.

Alternatively, the primary sequence of mRNAs, that is, their 5'- and 3'-terminal sequences, and their sequence-dependent secondary structure and protein composition, may

determine their half-life. In *Drosophila,* histone mRNAs are the only embryonic abundant mRNAs found exclusively in the nonpolyadenylated RNA class of mRNA (48). However, their short half-life cannot be attributable only to their lack of a 3'-polyadenylation tract; a class of polyadenylated RNA sequences found in *Drosophila* Schneider cells can apparently decay with a half-life similar to that we have observed for nonadenylated histone mRNA (16, 28). Our density labeling approach, which enables us to recover and thus characterize stable mRNAs, could provide a way to investigate whether the primary sequence of mRNA is altered during its lifetime.

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