

STABILITIES OF NUCLEAR AND MESSENGER RNA MOLECULES IN SEA URCHIN EMBRYOS

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ABSTRACT

The kinetics of accumulation of radioactive adenosine in adenosine triphosphate and in RNA of nuclear, cytoplasmic, and polysomal fractions of sea urchin embryos have been analyzed. 85% of the RNA synthesized decays in the nucleus with an apparently uniform half-life of about 7 min. The remaining 15% goes to the cytoplasm, mostly entering polysomes, and decays with a quite uniform half-life of about 75 min. The nuclear RNA accounts for one-third and the cytoplasmic RNA accounts for two-thirds of the total unstable RNA which accumulates at steady state in the embryo. The size distribution of short-labeled nuclear RNA is very similar to that of long-labeled messenger RNA, when both are extracted directly from the cells without a previous cell fractionation.

INTRODUCTION

Heterogeneous, DNA-like RNA is the principal gene product in eukaryotic cells. This RNA has been separated into at least two major classes, nuclear and cytoplasmic messenger. Nuclear RNA is rapidly degraded with a half-life estimated variously at 3–30 min (1–5). On the basis of the decay of protein synthesis in the presence of the inhibitor of RNA synthesis, actinomycin D, most messenger RNA decays with a half-life of several hours (6). Some specific messenger RNA molecules appear to be highly stable (7). These investigations of DNA-like RNA have provided neither a quantitative measurement of the rate of synthesis of either class of DNA-like RNA, nor consistent information on the stabilities of DNA-like RNA, nor a precise determination of the relative amounts of nuclear RNA and messenger RNA. Such quantitative information would be useful in any consideration of the unknown role of nuclear RNA and its relationship to messenger RNA.

We recently described a method for estimating the range of stabilities of newly synthesized RNA in cells of sea urchin embryos (8), which are especially favorable for the analysis of DNA-like RNA because, unlike that in most animal cells, nearly all the RNA synthesized is DNA-like (9). The method is based on an analysis of the kinetics of entry of tritiated adenosine into adenosine triphosphate (ATP) and RNA. This analysis suggested two classes of newly synthesized, unstable RNA (8). One class, representing about 85% of the synthesis and a third of the steady-state level of unstable RNA, had a half-life of 5–10 min, whereas the other class, comprising 15% of the synthesis and two-thirds of the steady-state level, had a half-life of 60–90 min (8). The data, however, could not eliminate the existence of multiple classes of RNA with varied stabilities within these approximate limits. We have investigated this further by separating the RNA into nuclear,

cytoplasmic, and polysomal fractions and analyzing the kinetics of accumulation of RNA in these fractions. These fractionations indicate that the more rapidly decaying RNA is restricted to the nucleus whereas the other class is mostly polysomal messenger RNA.

MATERIALS AND METHODS

Culturing and Labeling of Embryos

In each experiment, embryos from a single female of *Lytechinus pictus* were incubated at 18°C as a 0.5% suspension (v/v) in artificial seawater with antibiotics (8). For labeling DNA, the embryos were suspended at 10% from 30 to 90 min after fertilization and incubated with 2 $\mu\text{Ci/ml}$ thymidine-2- ^{14}C (45.5 Ci/mole) to incorporate radioactivity into the DNA precursor pools. The embryos were washed by settling through seawater and resuspended at 0.5% until RNA labeling experiments began. By this time, all free precursor was incorporated and there was a constant amount of radioactive DNA per embryo throughout the course of the analysis of RNA synthesis in mesenchyme blastulae; thus, the radioactivity in DNA can be used to follow and quantitate the nuclei during fractionation procedures. No detectable ^{14}C radioactivity enters RNA. For experiments on the kinetics of labeling of RNA, 14-hr mesenchyme blastulae were suspended at 10% and immediately incubated with 10 $\mu\text{Ci/ml}$ adenosine-8- ^3H (25 Ci/mole) for 5 min and then diluted to a 1% suspension. Samples were taken at appropriate times and processed. The kinetics of accumulation of radioactive RNA are the same as in experiments carried out on suspensions of 1% or less (8). The labeled embryos form normal plutei when allowed to develop under these conditions.

Fractionation of Embryonic Cells

The development of this method was facilitated by adaptation of parts of other methods (10–12). All operations were carried out at 0°–4°C. Embryos (5×10^4) were washed through seawater and layered in seawater over 10 ml 1.5 M dextrose. A portion of each sample was held aside and processed as whole embryos as previously described (8). The remaining embryos were collected through dextrose at top speed in an International Clinical Centrifuge (International Equipment Co., Needham Heights, Mass.) and resuspended in 2 ml of 1.5 M dextrose. They were washed through dextrose 2–3 times until they began to dissociate, and then were suspended in 1.5 ml TNM buffer (0.24 M NH_4Cl , 5 mM MgCl_2 , 10 mM Tris-HCl, pH 7.8) made 0.5% in Triton X-100. The embryos were intermittently drawn gently through

a Pasteur pipette until the cells were fully lysed (by 5 min). The lysate was layered over 2 ml 1 M sucrose in 5 mM MgCl_2 and centrifuged for 5 min at 4250 rpm in a Sorvall swinging bucket rotor HB-4 (Ivan Sorvall, Inc., Norwalk, Conn.). The pellet is referred to as the nuclear fraction and the supernatant above the sucrose as the cytoplasmic fraction (all the radioactive RNA and DNA can be recovered in these two fractions). The fractions were then used for determinations of radioactivity in RNA and DNA. For polysomes, the cytoplasmic fraction was brought to 0.5% NP-40, layered over 17 ml gradients of 10–35% sucrose in TNM, and centrifuged for 7.5 hr at 24,000 rpm at 4°C in a Beckman SW27 rotor (Beckman Instruments, Inc., Fullerton, Calif.). The pellet, containing most of the radioactive RNA, and the fractions sedimenting more rapidly than single ribosomes were pooled as polysomes.

The isolated nuclei have smooth outlines, with few visible cytoplasmic tags. An average of only 6% of the total embryonic RNA is associated with the nuclear fraction; it has a broad size distribution and contains less than 1% of the total cellular 28S and 18S ribosomal RNA (13).

The isolated polysomes are active in protein synthesis. Most of the radioactive amino acids incorporated into nascent proteins sediment with polysomes rather than with single ribosomes (Fig. 1a), indicating that polysomes are virtually intact. This protein as well as the radioactive RNA sedimenting with polysomes is completely removed by mild ribonuclease. Most of the radioactive RNA is removed from the polysome region by EDTA (ethylenediaminetetraacetic acid) (Fig. 1). As evidenced by the peak of radioactive RNA associated with the monosomes, detectable amounts of newly synthesized ribosomal RNA are present in these extracts. The specific activity of the ribosomal RNA in ribosomes and polysomes is identical, indicating a random distribution of the radioactive ribosomes. We conclude that, except for this ribosomal RNA, virtually all the radioactive RNA sedimenting in the polysome region of cytoplasmic extracts prepared by this procedure is messenger RNA (3). That very small amounts of EDTA-insensitive RNA sediment with polysomes indicates that nuclear breakage or leakage is not extensive in our procedure, since the EDTA-insensitive RNA that sediments with polysomes is greatly reduced or eliminated in procedures in which nuclear breakage or leakage is reduced or eliminated (12).

Fig. 2 shows the cytoplasmic distribution of radioactive RNA labeled with tritiated adenosine under conditions approaching steady state for unstable RNA (8). Polysomal RNA accounts for 65% of the total radioactive RNA in the cytoplasm. The hatched area corresponds to radioactive RNA in these frac-

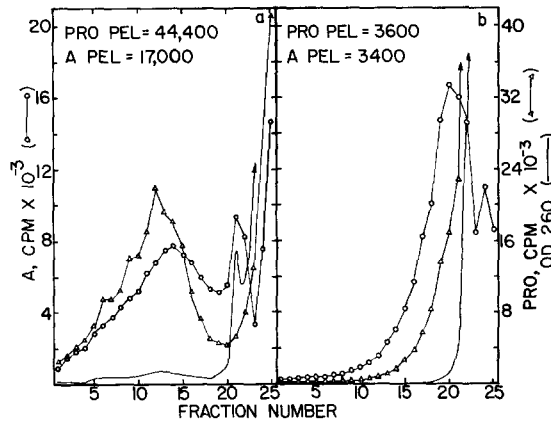


FIGURE 1 Polysome profile of cytoplasmic fractions labeled with adenosine (*A*) or proline (*PRO*). A 6.6% suspension of mesenchyme blastulae was labeled for 5 min with 10 $\mu\text{Ci/ml}$ adenosine- ^3H , diluted to 0.7%, and incubated for 6 hr. Another 6.6% suspension was labeled with 7 $\mu\text{Ci/ml}$ proline- ^3H (1.5 Ci/mole) for 3.5 min. Cytoplasmic fractions of both samples were prepared as described in Methods. Both samples were divided equally, with half brought to 0.15 M EDTA with a solution adjusted to maintain the pH at 7.8. All four samples were layered on linear gradients of 10–40% sucrose in TNM buffer, and centrifuged at 24,000 rpm for 1 hr at 4°C in a SW27 rotor. Fractions were precipitated with equal volumes of 1.5 N perchloric acid (PCA) and collected on Millipore filters. (a) Without EDTA; (b) with EDTA.

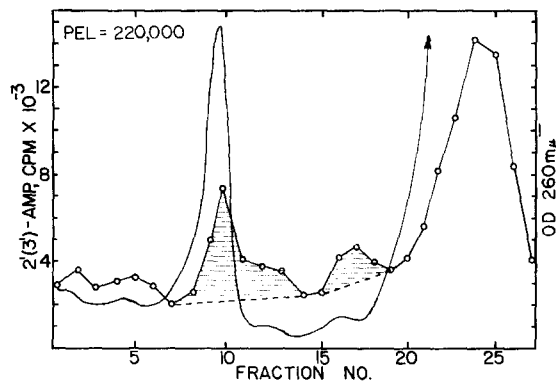


FIGURE 2 Distribution of radioactive RNA in subpolysomal structures. A 10% suspension of embryos was labeled for 5 min with 20 $\mu\text{Ci/ml}$ adenosine- ^3H and diluted to 0.8% for 6.5 hr before processing. A cytoplasmic fraction was prepared as described in Methods, divided equally, and layered on two 37 ml 15–35% sucrose gradients, which were centrifuged at 24,000 rpm for 8 hr at 4°C in an SW27 rotor. Fractions from both gradients were precipitated with equal volumes of 1.5 N PCA, and collected on Millipore filters. The filters from one gradient were immediately prepared for scintillation counting, while the filters from the other gradient were dried. RNA and DNA were dissolved off these dried filters with 2 ml of 0.1 N NH_4OH for 2 hr at room temperature and the filters were removed. The solutions were made 0.3 N in NaOH and incubated for 18 hr at 37°C. The nucleotides of the hydrolyzed RNA were separated as described previously (8). The percentage of the total acid-precipitable, alkaline-labile radioactivity in 2'(3')-AMP was calculated (about 95% for fractions 1–22, and about 70% for fractions 23–27). The radioactivity in 2'(3')-AMP of RNA is plotted for each fraction. The hatched areas, representing ribosomal RNA, were determined as described in Methods.

tions which sediments as 18S and 28S ribosomal RNA. Since radioactive ribosomal RNA is randomly distributed and since 45% of the ribosomes are in polysomes (13), one can calculate that about 9.5% of the

total radioactive RNA in the cytoplasm is ribosomal RNA. The peak of radioactive RNA near the top of the gradient accounts for all of the 4-5S (probably transfer) RNA accumulated in the embryos. If the

radioactivity in this small RNA and ribosomal RNA is subtracted from the total radioactivity in the cytoplasm, then 88% of the remaining cytoplasmic radioactivity is in heterogeneous DNA-like RNA on polysomes; this is probably messenger RNA.

Determination of Specific Radioactivities of RNA and ATP

The methods have been described and justified previously (8, 14). Briefly, each fraction was homogenized in 0.75 N HClO₄ and the precipitate was collected by centrifugation and washed with 0.5 N HClO₄. The ATP was isolated from the acid extract of whole embryos, assayed by means of the luciferin-luciferase system (15, 16), and counted for radioactivity. The acid-precipitable RNA was hydrolyzed with NaOH, and the alkaline-labile radioactivity in RNA and the stable radioactivity in DNA were determined (correcting for tritium exchange in base [8, 17]). The fraction of RNA radioactivity in 2'(3')-adenosine monophosphate was determined by separating the ribonucleoside monophosphates of a hydrolysate by thin-layer chromatography. This also resolves the residual radioactive ATP trapped in the acid-precipitated RNA. Contamination by radioactive ATP of cytoplasmic fractions of embryos labeled with tritiated adenosine for only a few minutes is substantial and is corrected for in this way. Scintillation counting was done as previously described (8).

Size Distribution of RNA

Late mesenchyme blastulae 16 hr after fertilization were suspended at 10% and incubated for 5 min with 0.2 μ Ci/ml adenosine-8-¹⁴C (47.2 mCi/mmol) and diluted to 1%. Under these conditions the specific radioactivity of the total ATP pool reaches a constant maximum by 45 min and steady-state labeling of unstable RNA, mostly cytoplasmic RNA, is reached by 6 hr. After 6 hr the embryos were suspended at 10% and incubated for 5 min with 10 μ Ci/ml adenosine-³H to label predominately nuclear RNA. The embryos were collected through cold seawater, and the RNA was extracted directly with phenol as previously described (9). The extracted RNA was centrifuged through a 17 ml 15–35% sucrose gradient at 26,000 rpm for 9 hr at 25°C in a Beckman SW27 rotor. Acid-precipitable, alkaline-labile radioactivity for each fraction of the gradient was determined.

RESULTS

The kinetics of entry of radioactive adenosine into ATP and into adenosine monophosphate (AMP) of RNA isolated from nuclear, polysomal, and whole cell fractions are shown in Fig. 3. Radio-

activity enters and equilibrates rapidly with the ATP pool (Fig. 3 *d*). A rapid incorporation of radioactivity into total RNA begins immediately but slows and finally approaches a maximum. The kinetics of incorporation are characteristic for unstable RNA (8) and are analyzed quantitatively below. The incorporation curves for nuclear RNA and cytoplasmic or polysomal RNA show two significant differences. First, nuclear RNA reaches a maximum level of incorporation much sooner than cytoplasmic RNA, indicating that it is much more unstable or transient. Second, the rapid initial rate of incorporation into total RNA is mainly accounted for by nuclear RNA, there being a lag in the attainment of a maximal rate of incorporation into cytoplasmic RNA.

The nature and length of this cytoplasmic lag have not been precisely determined. A constant 2–3% of the radioactive DNA is in the cytoplasmic fraction, indicating at least this much leakage of nuclear materials. About 5–10% of the radioactive RNA appears in the cytoplasm after a 5 or 10 min labeling period (see Fig. 6). This relatively con-

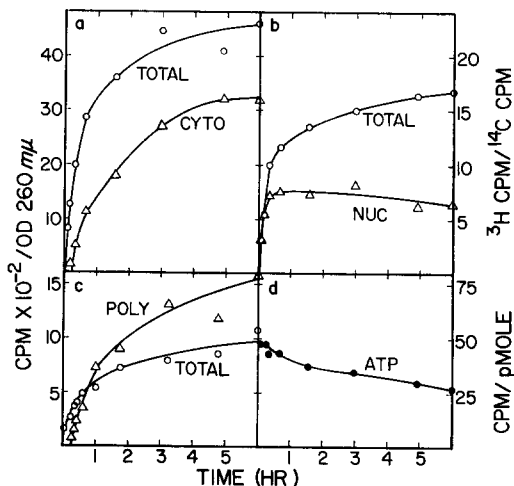


FIGURE 3 Entry of tritiated adenosine into RNA and ATP. Mesenchyme blastulae were labeled and fractionated as described in Methods. The accumulation of radioactivity in 2'(3')-AMP of RNA in whole embryos and cytoplasm is normalized by the OD₂₆₀ of RNA in *a*; it is normalized with DNA-¹⁴C in *b*. The specific radioactivities of ATP in *d* are from the same experiment as *a* and *b*. The accumulation of radioactivity in RNA of whole embryos and polysomes (*POLY*) of another experiment is shown in *c*; the two curves are on arbitrary scales and cannot be compared quantitatively. *NUC*, nuclear fraction; *CYTO*, cytoplasmic fraction.

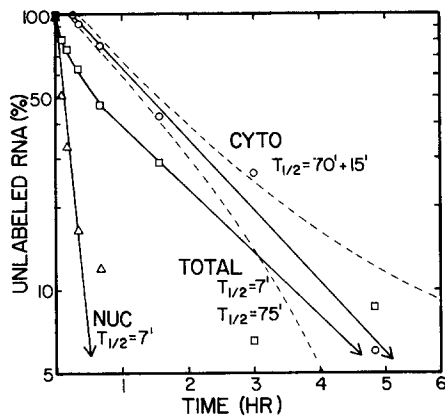


FIGURE 4 First order decay analysis of the molar accumulation of radioactive RNA in whole embryo, nuclear (NUC), and cytoplasmic (CYTO) fractions. The logarithm of the percentage of the steady-state level of radioactive, unstable RNA remaining unlabeled is plotted against time. The experimental data for whole cells and cytoplasmic fractions are from Fig. 3 a and for the nuclear fraction from Fig. 3 b. The 95% confidence limits of the mean decay curve for cytoplasmic RNA are indicated by the broken lines.

stant level may represent nuclear contamination. Most of this RNA sediments more slowly than polysomes, whereas after 15 min the majority of the newly accumulated cytoplasmic RNA sediments with polysomes (13). In the subsequent analyses, we have subtracted the amount of radioactive RNA in cytoplasmic fractions by 10 min as a background of nuclear contaminants and used 15 min as an absolute lag time. This value agrees with previous estimates of a lag for sea urchin embryos (5, 18) and HeLa cells (3). The parameters established in the following kinetic analyses are only slightly altered by assuming there is no lag and/or not subtracting a background.

The molar accumulation curves of radioactive RNA for each fraction (not shown) were calculated as previously described (8) from the specific radioactivities of the RNA and the ATP pool, assuming that the ATP pool is not compartmentalized. If, as it appears, there is a steady state amount of unstable RNA during an experiment, the curve for the decay of unlabeled, unstable RNA is the inverse of the molar accumulation curve for radioactive, unstable RNA (8). If it is assumed that the decay of RNA follows first order reaction kinetics, this decay curve can be subjected to standard decay analysis as shown in Fig. 4 (8).

The decay curve for total RNA of whole embryos is complex and can be resolved into two straight lines defining two classes of RNA with half-lives of 8 and 75 min. The decay curves for RNA in the separated nuclear and cytoplasmic fractions can both be described by simple straight lines. The stabilities calculated for these two fractions of RNA are the same as the two stabilities calculated for total RNA. The straightness of the lines suggests that the RNA in each fraction has a uniform stability.

At steady state, 88% of the radioactive cytoplasmic RNA which is not ribosomal or 4-5S RNA is associated with polysomes (Fig. 2). A decay analysis of the accumulation of radioactive RNA in polysomes shown in Fig. 3 c is plotted in Fig. 5. The polysomal RNA has an apparently uniform stability of 80 min.

The kinetic analysis of isolated fractions of RNA described above defined two major stability classes of RNA, nuclear and cytoplasmic. An analysis of the kinetics of accumulation of total RNA had led to our proposal of a simple two-class model in which 85% of the RNA synthesized had a short half-life and 15% had a long half-life; by this model the more stable RNA accumulated slowly until at steady state it accounted for two-thirds of the unstable RNA (8). The validity of the kinetic analysis depends upon the validity of the assumptions made, and the accuracy of the parameters established by the kinetic analysis depend upon the accuracy of the approximations used in calculating the molar accumulation curve (8). If the less stable RNA is nuclear and the more stable RNA is cytoplasmic, the relative amounts of newly synthesized RNA in the cytoplasm and nucleus after

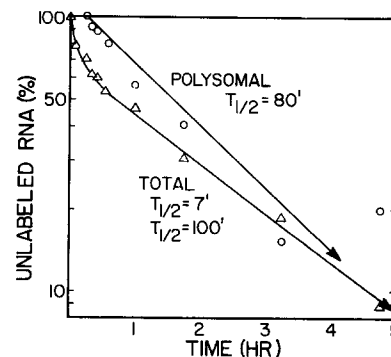


FIGURE 5 First order decay analysis of the molar accumulation of radioactive RNA in whole embryos and polysomes (from data in Fig. 3 c).

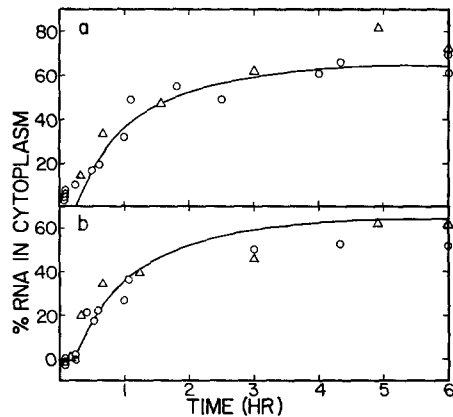


FIGURE 6 The percentage of the total radioactive RNA localized in the cytoplasm plotted *versus* time. Data in *a* are based on cytoplasmic fractions (see Results) and those in *b* are based on nuclear fractions. The solid lines describe the curve predicted from the parameters estimated in Fig. 4. The open triangles are from the experiment presented in Fig. 3 *a, b, d*; molar quantities were calculated using the average ATP specific activity, a 15 min lag, and a background subtracted from the cytoplasmic fraction as in Fig. 4. The other data points are from several experiments; they are the fractions of radioactivity in RNA, not corrected for ATP specific activities or background radioactivity.

various labeling periods can be predicted from this simple two-class model. These proportions can also be determined from direct measurements which are independent of the kinetic analysis and they provide a test of the validity of the parameters of the model derived from the kinetic analysis.

The fraction of newly synthesized RNA in the cytoplasm was determined in two ways: the radioactive RNA/OD260 in RNA for the cytoplasmic fraction was divided by the radioactive RNA/OD260 in RNA for whole embryos (Fig. 6 *a*); or the radioactive RNA/DNA (DNA measured by either thymidine-¹⁴C and/or adenosine-³H) in the nuclear fraction was divided by the radioactive RNA/DNA in whole embryos and subtracted from 1 (Fig. 6 *b*). In Fig. 6 the curve predicted by the kinetic analysis in Fig. 4 for the changing fraction of cytoplasmic RNA is described by the solid lines (using a value of 15 min for the lag between synthesis and appearance of RNA in the cytoplasm). The data points based on the two methods of calculation fall close to the predicted curve. This provides independent evi-

dence for the existence of the two classes of RNA accumulating in the nucleus and cytoplasm with the parameters estimated by the kinetic analysis.

During cellular fractionation, both nuclear RNA and cytoplasmic RNA are often subject to some nuclease degradation (13). Comparison of the size of RNA extracted from nuclear and cytoplasmic fractions after carrying them through such fractionation procedures is likely, then, to be misleading. In these embryos the size of the nuclear RNA and cytoplasmic RNA can be compared without fractionating the cells, since after a 5 min labeling period about 85% of the radioactive RNA is nuclear RNA destined to decay within the nucleus, whereas after a steady-state labeling about two-thirds of the radioactive RNA is cytoplasmic. A double-labeling experiment comparing predominantly nuclear RNA labeled for 5 min with adenosine-³H and predominantly cytoplasmic RNA labeled for 6 hr with adenosine-¹⁴C is shown in Fig. 7. There is no major difference in size distribution of short-labeled (mostly nuclear) and long-labeled (mostly cytoplasmic) RNA greater than 10S, suggesting that there is very little difference in the size of nuclear RNA and cytoplasmic RNA in these sea urchin embryos. The difference evident in Fig. 7 would be further minimized by taking into account the 18S and 28S ribosomal RNA which would account for about 5% of the radioactivity accumulated during the long labeling period.

DISCUSSION

The validity of assumptions made for the kinetic analysis has been discussed previously (8). Straight

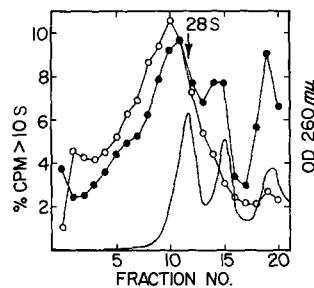


FIGURE 7 Size distribution of predominantly nuclear RNA labeled for 5 min with adenosine-³H (open circles) and predominantly cytoplasmic (polysomal) RNA labeled for 6 hr with adenosine-¹⁴C (closed circles). See Methods for details. The percentage of the total radioactivity in RNA greater than 10S is plotted for each fraction.

lines fit the first order decay graph of the data for the accumulation of RNA in nuclei, cytoplasm, and polysomes in several experiments. Straight lines indicate uniform stabilities for these classes of RNA. We have developed an error analysis for these data which indicates the statistical validity of this conclusion (13). The 95% confidence limits of the mean decay curves were established from the pooled data of several independent experiments; these limits are shown for cytoplasmic RNA in Fig. 4 and were also established for nuclear RNA. An analysis of these limits indicates that if all the RNA molecules in a class were equally stable, there is a 95% probability that the half-life is between 0.95 and 1.05 times the means determined in Fig. 4. This analysis also provides an estimate of the 95% confidence limits of possible amounts of RNA with half-lives different from the mean. The maximum possible fraction of stable RNA in either class is 6%. As much as one-fourth of the radioactive RNA could have a half-life 0.28 or 1.9 times the mean half-life, one-half could have a half-life 0.66 or 1.33 times the mean, and three-fourths could have a half-life 0.85 or 1.15 times the mean. Less than 7% of the nuclear or cytoplasmic RNA could have a half-life equal to the mean half-life of the other class. The accumulation of nuclear RNA is complicated by the accumulation of presumptive cytoplasmic RNA before its entry into the cytoplasm. By 10–15 min this RNA is just becoming detectible and may be responsible for the slight curvature indicated by the actual data points plotted for nuclear RNA in Fig. 4. We conclude that most of the molecules in the populations of both nuclear RNA and messenger RNA have an essentially uniform stability, although it is possible for a small portion of the molecules to have stabilities very different from the average.

Many experiments on animal cells have indicated that nuclear RNA turns over rapidly (1–5) and cytoplasmic (3, 5) or messenger RNA in polysomes (1, 5, 6) considerably more slowly. Most of these experiments (1–3, 6) have been based on the decay of radioactive RNA after the inhibition of RNA synthesis with actinomycin D, and none has been concerned with the range of stabilities of RNA. Estimates of the stability of DNA-like RNA of animal cells using actinomycin D give substantially greater values than other methods utilizing physiological conditions (4, 19, 20). Nuclear RNA in HeLa cells (3) and duck erythroblasts (1) decays with complex kinetics in the

presence of actinomycin D, suggesting a possible range of stabilities, but more likely an alteration of decay rates with time after drug treatment, or other derangement (3). Experiments on the decay of the capacity to synthesize specific proteins in the presence of actinomycin D in differentiated animal cells indicate that specific messenger RNA molecules being synthesized in the same cells at the same time have different stabilities (21). The sea urchin embryo may represent a special case in that the bulk of the messenger RNA has a narrow range of stabilities. This suggests that the stability of all messenger RNA molecules may be regulated by the same mechanism in the embryo. It will be interesting to apply our physiological methods for estimating the stabilities of RNA to other cell systems for comparison with the actinomycin D method.

In similarly conceived experiments, Aronson and Wilt (5) determined the kinetics of accumulation of radioactive RNA in nuclei and cytoplasm of sea urchin embryos. On the basis of limited data they proposed that most of the RNA turned over in the nucleus and that some stable RNA accumulated in the cytoplasm. Their data fit our predicted curve in Fig. 6, but our cell fractionation procedure has made possible this more complete kinetic analysis.

Messenger RNA released from HeLa cell polysomes under conditions which release poliovirus RNA intact (22) is predominantly between 12S and 30S in size (23). This is considerably smaller than RNA extracted from nuclei of HeLa cells (3, 4). This difference in size distribution of RNA extracted from isolated fractions has been observed in other cells as well (1, 24, 25), and it is commonly accepted that messenger RNA is smaller than nuclear RNA. We have found that there can be considerable degradation of RNA in whole cell, nuclear, or cytoplasmic fractions during the lysis procedure used for cell fractionation. Our analysis of the size distribution of nuclear RNA and cytoplasmic RNA which does not involve cell fractionation should be less sensitive to differences in lability after cell lysis since the cells are homogenized in sodium dodecyl sulfate (SDS) and immediately extracted with phenol. Even so, we have had preparations in which long-labeled (cytoplasmic) RNA was smaller than usual when the phenol extraction procedure was not done carefully with freshly distilled phenol (13). DNA-like RNA labeled for short or long periods in the

pluteus has a size distribution very similar to that of the blastula (9, 13). Messenger RNA with sedimentation properties similar to those of whole cell RNA in Fig. 7 has been extracted from undegraded polysomes (18) and total cytoplasm (29) isolated from sea urchin embryos. Our results suggest that there is little difference in size distribution of nuclear RNA and messenger RNA in sea urchin embryos. Alternatively, nuclear RNA may be preferentially degraded in our extraction procedure. We are currently preparing similar experiments on mammalian cellular RNA.

The long-labeled RNA has a peak size of 31–34S; a 32S molecule has a molecular weight of at least 2.5×10^6 , and could code for protein with a molecular weight of 2.5×10^5 . This is 5–10 times larger than the mean molecular weight of newly synthesized polypeptides in urchin embryos (B. Brandhorst, unpublished observations). The significance of the large size of the messenger RNA molecule has not been determined.

The amount of messenger RNA can be estimated from our data as the amount of radioactive heterogeneous RNA associated with polysomes at steady state, 9.4×10^{-3} g/g total RNA. Since about 80% of the total cellular RNA is ribosomal and since 45% of the ribosomes are in polysomes, this radioactive messenger RNA is 2.6% of the polysomal RNA. This amount compares with the values of 1.7% to 2.9% calculated by several independent studies which compare the size of total cellular messenger RNA (4), myosin messenger (26), or hemoglobin messenger (27), with the average number of ribosomes observed in these respective polysomes. Relatively stable messenger RNA synthesized before the beginning of the incorporation labeling period may exist in these embryos (28), and would not be detected. The 2.6% value, then, is a lower limit for the fraction of polysomal RNA which is messenger RNA.

We have shown that the rate of synthesis per nucleus of both stability classes of DNA-like RNA are similarly reduced between blastula and pluteus stages (8). Since the two stability classes correspond to nuclear RNA and messenger RNA, there is a coordinate regulation of the rates of synthesis of these two classes. This observation may be important for models of the role of nuclear RNA and its relationship to messenger RNA.

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