Cytoplasmic nonpolysomal ribonucleoprotein particles in sea urchin embryos and their relationship to protein synthesis

[informosomes/translational control/poly(A)/mRNA metabolism]

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ABSTRACT We have examined the relationship between the newly synthesized mRNA that enters polysomes in sea urchin embryos and the messengerlike RNA that enters the pool of ribosome-free ribonucleoprotein particles (free RNPs or informosomes). Although the RNA in the free RNPs turns over 25% more rapidly than in the polysomes, labeling kinetics indicate that the RNA containing poly(A) [poly(A)(+)RNA] and the RNA not containing poly(A) [poly(A)(-)RNA] within each cytoplasmic compartment have very similar half-lives. The poly(A)(+)RNA from both free RNPs and polysomes binds ribosomes almost equally well in a reticulocyte lysate, and this binding is sensitive to inhibitors of initiation. The poly(A)(-)RNA from polysomes initiates as well as poly(A)(+)RNA; however, poly(A)(-)RNA from free RNPs is only half as efficient in binding to ribosomes, and by this criterion is only 50% mRNA.

We have also examined the size and dynamics of shortening of the poly(A) tails of poly(A)(+)RNA from free RNPs and polysomes. Pulse-labeled poly(A) from both free RNPs and polysomes is about 180 nucleotides in length. Poly(A) shortening is very rapid in polysomes; steady-state labeled polysomal RNA is largely devoid of the 180-nucleotide-long poly(A) segments. Poly(A) shortening in free RNPs is slower; half of the poly(A) derived from steady-state free RNPs is still 180 nucleotides long. Despite this difference in the rates of poly(A) shortening, polysomes and free RNPs have very similar half-lives. There is, then, no obvious relationship between poly(A) shortening and turnover of mRNA in these embryos. The data are interpreted to mean that poly(A)(+)RNA from free RNPs is enriched for a class of mRNA that initiates less frequently *in vivo* than the bulk of the cellular mRNA.

Newly synthesized RNA from sea urchin embryos is found both associated with ribosomes and free of ribosomes in cytoplasmic extracts (1). The percentage of newly synthesized (nonribosomal) RNA that enters polysomes or the pool of ribosome-free ribonucleoprotein particles (free RNPs) is strongly dependent upon the stage of development. The RNA extracted from free RNPs and polysomes shows identical sedimentation patterns for the RNA that contains poly(A) [poly(A)(+)RNA]; this is also the case for the RNA that does not contain poly(A) [poly(A)(-)RNA], except for the absence of a prominent 9S (histone message) peak from the free RNPs. We have also shown that the polysomes are enriched for poly(A)(+)RNA species. Kinetic studies provide strong evidence against a simple precursor-product relationship between the free RNPs and all of the polysomes. Also, half-life determinations show that there is no class of particularly stable mRNA in either population (2). Thus, the free RNP particles do not represent a storage pool of cytoplasmic mRNA whose translation is delayed.

Based on these results we have suggested that there is an excess of potential mRNA transcribed in these embryonic cells and that RNA in excess of the capacity of the translational ap-

paratus results in the free RNP population. A prediction from these studies was that the RNA from free RNP should be capable of binding to ribosomes *in vitro* and forming initiation complexes. This is shown in the present report. It remained a question whether all of the free RNPs were always restricted to this pool or if a population of the free RNPs might cycle in and out of the polysome pool. We report here differences in the metabolism of the poly(A) tails derived from poly(A)(+)RNA of free RNPs and polysomes that support the latter possibility and provide further information concerning the nature of free RNPs.

METHODS

Growth of Embryos and Preparation of RNA. Gametes from the sea urchin *Strongylocentrotus purpuratus* or *Lytechinus pictus* were fertilized and grown as described (1). Embryos were labeled with [5-³H]uridine (26 Ci/mmol) or [2-³H]adenosine (10.8 Ci/mmol). Embryos were washed and lysed in 20 volumes of TNM homogenization medium (50 mM Tris, pH 7.8 at 4°/240 mM NH₄Cl/5 mM Mg(AcO)₂/250 mM sucrose) either by homogenization in a Dounce homogenizer (3) or detergent lysis in Triton X-100 (4) in the presence of 1.5 mg of bentonite per ml. Free RNPs and polysomes were isolated from sucrose gradients after sedimentation of postmitochondrial (Dounce) or postnuclear (Triton X-100) supernatants at 26,000 rpm in the Spinco SW 27 rotor for 12 hr (2). RNA extracted by phenol/CHCl₃ (2) was separated into poly(A)(+)RNA and poly(A)(-)RNA using oligo(dT)-cellulose (Collaborative Research, Waltham, MA) (5).

Kinetic Studies. Hatched blastulae at 2 ml/100 ml of sea water were incubated with 500 μ Ci of [³H]uridine. Aliquots were taken at the appropriate times and radioactivity was determined for poly(A)(+) and poly(A)(-)RNA by trichloroacetic acid precipitation. Time points were standardized by A₂₆₀ measurements applied to each oligo(dT)-cellulose column. Details of procedures used are presented elsewhere (2, 4).

Ribosome Binding Studies. [³H]Uridine-labeled RNA was isolated from hatched blastulae and fractionated into poly(A)(+)RNA and poly(A)(-)RNA through oligo(dT)-cellulose. At this embryonic stage 30–40% of the polysomal mRNA and 15–25% of the free RNP RNA is polyadenylated (2). A reticulocyte lysate was prepared according to Godchaux *et al.* (6), and the cell-free conditions were according to Villa-Komaroff *et al.* (7) with KCl at 110 mM and MgCl₂ at 1.2 mM. Incubations were routinely in the presence of 1.6 μ g of cycloheximide per ml for 4 min at 30°. The reaction was terminated by the addition of 0.2 ml of ice-cold buffer (50 mM Tris, pH 7.5 at 4°/100 mM KCl/5 mM Mg(AcO)₂/250 mM sucrose). The mixture was layered on a 5-ml 15–30% sucrose gradient made up in the same buffer and centrifuged at 39,000 rpm for 80 min in the Spinco SW50.1 rotor. Ten-drop fractions were collected from the top of the gradient and radioactivity in each fraction was determined in Econofluor (New England Nuclear) after trichloroacetic acid precipitation.

Abbreviations: RNPs, ribonucleoprotein particles; poly(A)(+)RNA, RNA that contains poly(A); poly(A)(-)RNA, RNA that does not contain poly(A).

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It was not possible to quantitate the amount of poly(A)(+)RNA that was used by A_{260} units. In comparing poly(A)(+)RNA from different cellular compartments we used equal amounts of radioactivity. The amount of poly(A)(-)RNA used was measured by A_{260} . Nonlimiting levels of RNA were used in all assays.

Poly(A) Analysis. Five milliliters of eggs were grown to 16 hr (blastulae) and then divided into two equal parts in 125 ml of sea water each. One part was labeled for either 20 or 60 min with 1 mCi of [3 H]adenosine and the other part was labeled for 5 hr (to steady-state) with 500 μ Ci of [3 H]adenosine. Poly(A)(+)RNA was then prepared from free RNPs and polysomes for each time point. Unlabeled poly(A) (25 μ g) was added to each preparation, which was then treated with nucleases under conditions where poly(A) is not degraded (8, 9). The nucleases were removed by phenol/ CHCl_3 extraction. The poly(A) was finally purified through oligo(dT)-cellulose and its size was determined by electrophoresis on 9% polyacrylamide gels in 99% formamide (10). The 10-cm gels were cut into 50 2-mm slices directly into scintillation vials using a Gilson gel fractionator. Water (0.5 ml) was added and the vials were incubated at 30° overnight. Radioactivity was determined in a scintillation mixture that contained Triton X-100. Standards run on parallel gels were yeast tRNA stained with methylene blue and L cell poly(A) assayed by radioactivity.

RESULTS

Ribosome Binding Studies of RNA from Free RNPs and Polysomes. In order to test if RNA from the free RNP has message potential we have used the rabbit reticulocyte cell-free protein-synthesizing system, and assayed the ability of [3 H]nucleoside-labeled RNA to bind ribosomes and to form polysomes. In Fig. 1, poly(A)(+)RNA from either free RNPs or polysomes was incubated in the reticulocyte lysate as described in *Methods*. After incubation the mixture was sedimented on sucrose gradients. Parallel controls were done with aurintricarboxylic acid, a potent inhibitor of initiation. Labeled RNA from both polysomes and free RNPs binds to ribosomes, and a high yield of polysomes composed of two to five ribosomes are formed. The binding of RNA to ribosomes is strongly inhibited by aurintricarboxylic acid and heparin (the latter result is not shown). The RNA concentration may be varied over a 10-fold range with the same qualitative and quantitative results. An estimate of the free RNP poly(A)(+)RNA that has the ability to bind to ribosomes may be made by comparing the proportion of this RNA that binds to ribosomes with that of poly(A)(+)RNA from polysomes. The latter may be taken as entirely composed of mRNA. The important result is that the poly(A)(+)RNA from the free RNPs binds ribosomes to nearly the same extent as that from polysomes. Thus, using poly(A)(+)RNA from polysomes as the standard, we can conclude that the poly(A)(+)RNA of free RNPs has the potential for message function *in vivo*.

A similar study is shown with the poly(A)(-)RNA from free RNPs and polysomes in Fig. 2. The polysomal poly(A)(-)RNA is just as efficient as poly(A)(+)RNA in the ribosome binding assay. In contrast to poly(A)(+)RNA, the poly(A)(-)RNA component of free RNPs binds to ribosomes in this system only about one-half as well as the polysomal RNA (Fig. 2B). To show that there is not an inhibitor of initiation being extracted with the free RNP RNA, unlabeled free RNP poly(A)(-)RNA in an amount equivalent to that used in Fig. 2B, was added to [3 H]uridine-labeled polysomal poly(A)(-)RNA. There is no inhibition of the ability of polysomal poly(A)(-)RNA to bind ribosomes in the presence of poly(A)(-)RNA from free RNPs (Fig. 2C).

FIG. 1. Ribosome-binding assay for [3 H]uridine-labeled poly(A)(+)RNA from polysomes (A) and free RNPs (B). 5,000 cpm of poly(A)(+)RNA derived from either polysomes or free RNPs in 15 μ l of water was added to 100 μ l of reticulocyte lysate in the presence of 1.6 μ g of cycloheximide per ml. Incubations were as described in *Methods*. The arrows refer to absorbance peaks (A_{260}) that are due mainly to globin polysomes. More than 80% of the radioactive RNA was recovered from the gradients in all of these experiments. Purified rRNA did not bind to structures at 80 S or larger, and there was no binding of [3 H]RNA when the incubation was done at 0°. Treatment of the mixtures with 10 mM EDTA prior to centrifugation eliminated all the radioactivity from the polysome region. Sedimentation is from left to right; the ordinate is percent of cpm in that fraction. (●) Without aurintricarboxylic acid; (○) in the presence of 0.1 mM aurintricarboxylic acid. Heparin at 40 μ g/ml gave the same results as aurintricarboxylic acid (26).

Table 1A presents a summary of six separate experiments as given in Figs. 1 and 2. These data, obtained with steady-state-labeled RNA, show clearly the near equality of poly(A)(+)RNA from free RNPs and polysomes in the binding assay and the reduced efficiency of poly(A)(-)RNA from free RNPs to form polysomes. Table 1B shows that the same results are obtained for 1-hr "pulse"-labeled RNA from free RNPs and polysomes. Free RNPs and polysomes can also be prepared from a detergent lysate, which also solubilizes membrane-bound material. Table 1C shows no differences between the binding properties of RNA prepared this way and RNA prepared by Dounce homogenization. Thus, it appears in sea urchins that free RNPs are composed of poly(A)(+)RNA, which is mRNA, and poly(A)(-)RNA, only half of which is mRNA. Our further results will deal primarily with the poly(A)(+)RNA of free RNPs and polysomes.

Kinetics of Labeling and Half-Life of Poly(A)(+)RNA. In some systems it has been shown that one can experimentally manipulate cells and move either whole populations of mRNA or specific messages between the free RNP pool and polysomes (11-15). The question we would like to address is whether the embryo normally makes use of the mRNA in the free RNPs for protein synthesis or whether the mRNA in the free RNP pool is restricted to this pool for the duration of its lifetime. One approach is to determine if the kinetics of accumulation of RNA into polysomes and free RNPs supports the possibility of a dynamic equilibrium between the free RNPs and polysomes. When the total RNA in each pool is examined, the kinetic relationships do not support such an equilibrium (2). However, since it was shown above that poly(A)(+)RNA derived from free RNPs is indeed mRNA, we reexamined the kinetic relationship between free RNPs and polysomes using this pure mRNA preparation.

FIG. 2. Ribosome-binding assay for $[^3\text{H}]$ uridine-labeled poly(A)(-)RNA from polysomes (A) and free RNPs (B). Eight micrograms of poly(A)(-)RNA from polysomes or free RNPs was analyzed in the reticulocyte assay (see legend of Fig. 1). (A) Polysomal RNA; (B) free RNP RNA; (C) 8 μg of $[^3\text{H}]$ uridine-labeled polysomal RNA in the presence of 8 μg of unlabeled free RNP RNA. Use of 40 μg of unlabeled free RNP RNA gave the same result. (●) Without aurintricarboxylic acid; (○) in the presence of 0.1 mM aurintricarboxylic acid.

Fig. 3 shows the kinetics of accumulation of $[^3\text{H}]$ uridine into total RNA, poly(A)(+)RNA, and poly(A)(-)RNA of polysomes (Fig. 3A) and of free RNPs (Fig. 3B). The times required to achieve steady-state labeling are indistinguishable for the poly(A)(+)RNA and poly(A)(-)RNA within each population of RNP, but they are different between the polysomes and free RNPs. These data provide a measure of the half-life of each RNA class (4), and they are consistent with the report of Nemer *et al.* (16), who showed by pulse-chase kinetics that poly(A)(+)RNA and poly(A)(-)RNA derived from polysomes decay at the same rate. The half-time for turnover in these experiments is about 60 min for free RNP RNA and 80 min for polysome RNA, which yields half-lives of about 50 min and 70 min for the RNA of free RNPs and polysomes, respectively, provided that the cellular $[^3\text{H}]$ UTP pool reaches its maximum specific activity similarly to the $[^3\text{H}]$ ATP pool (2). Of particular importance, the poly(A)(+)RNA from free RNPs and polysomes can be distinguished by their different half-lives. This is good evidence against an equilibrium between the poly(A)(-)RNA of free RNPs and the total class of polysomes. However, these data cannot eliminate the possibility that the free RNPs may be in equilibrium with a subclass of polysomes that does not equilibrate with the bulk of the polysomes. This subclass of polysomes would necessarily be a small part of the total, which would not be detected as a separate kinetic component. Thus, the polysomes could contain RNA of different half-lives whose rates of accumulation overlap sufficiently to blend into the apparently single-kinetic-component saturation curves

shown in Fig. 3. Indeed, the kinetics of accumulation of the polysomal 9S histone message is different from that of the total polysomal RNA and the nonhistone poly(A)(-)RNA (Fig. 4). In these studies the embryos were exposed to $[^3\text{H}]$ uridine for various times and the poly(A)(-)RNA from polysomes was centrifuged on sucrose gradients. The 9S histone mRNA is distinguished from the bulk of the polysomal RNA because it reaches steady state with a halftime of 60 min, whereas the rest of the poly(A)(-)RNA displays a halftime of 85 min. Thus, although the RNA of polysomes becomes labeled as a single kinetic component (Fig. 3) (2), the polysomes do contain subpopulations of RNA with different half-lives, and the free RNPs might contribute to one such subpopulation. To test further the possibility that the poly(A)(+)RNA of free RNPs may cycle in and out of the polysome pool, we have examined the dynamics of poly(A) metabolism in the free RNPs and polysomes.

Poly(A) Shortening in Free RNPs and Polysomes. It has been reported that poly(A) sequences located at the 3'-end of mRNA shorten with time (17-19). It has been difficult to associate poly(A) shortening with any physiological processes. However, differences in the metabolism of poly(A) from free RNPs and polysomes would support the conclusion from the kinetic analysis that the two populations are indeed autonomous.

Newly labeled mRNA begins to enter the cytoplasm maximally under our labeling conditions after 10-15 min (2). The 20-min labeling period used in Fig. 5A shows what is essentially, therefore, the instantaneous size of the poly(A) tails from

Table 1. Comparison of $[^3\text{H}]$ uridine-labeled RNA from free RNPs and polysomes that is active in the ribosome binding assay

		Poly(A)(+)RNA			Poly(A)(-)RNA		
		<80 S	80-100 S	> 100 S	<80 S	80-100 S	>100 S
A	Polysomes	19-30	10-15	56-69	15-18	15-18	62-70
	Free RNPs	22-37	10-14	53-64	44-56	10-15	32-38
B	Polysomes	15	16	69	22	20	58
	Free RNPs	26	14	60	50	13	37
C	Polysomes	30	13	57	30	8	62
	Free RNPs	31	14	55	52	12	36

$[^3\text{H}]$ Uridine-labeled RNA from polysomes and free RNPs was assayed in the reticulocyte lysate described in the legend of Fig. 1 and *Methods*. The table shows the percent of RNA that remained unassociated with ribosomes (<80 S), associated with the monosome (80-100 S), and associated with polysomes (>100 S). (A) Steady-state-labeled RNA (4-5 hr of labeling) prepared from Dounce homogenates and compiled from several experiments. (B) One-hour pulse-labeled RNA prepared from a Dounce homogenate. (C) Steady-state-labeled RNA from a Triton X-100 detergent lysate.

FIG. 3. Steady-state kinetics of accumulation of [³H]uridine into total RNA, poly(A)(+)RNA, and poly(A)(-)RNA from polysomes (A), and free RNPs (B). Twelve-hour *L. pictus* embryos were labeled as described in *Methods* and fractionated into free RNPs and polysomes. Poly(A)(+)RNA and poly(A)(-)RNA were isolated by chromatography through oligo(dT)-cellulose (see *Methods*). The absolute levels of radioactivity in free RNPs and polysomes are not strictly comparable because of the correction factors (absorbance at 260 nm in each preparation). (X) Total RNA; (●) poly(A)(+)RNA; (○) poly(A)(-)RNA.

poly(A)(+)RNA of free RNPs and polysomes. They are nearly identical in size and migrate similarly to pulse-labeled mammalian cell poly(A), which is about 180 nucleotides long (20). Fig. 5B shows the profiles of 1-hr and 5-hr labeled poly(A) from polysomes. Poly(A) shortening is extremely rapid; within 1 hr there is a large reduction in 180-nucleotide poly(A) segments. The 5-hr steady-state distribution of poly(A) shows that only 10% of the segments contain poly(A) of the original size. Poly(A) shortening must begin very rapidly upon entrance of the polysomal mRNA in the cytoplasm. Fig. 5C shows the identical experiment for poly(A) from free RNPs isolated from the same

FIG. 4. Steady-state kinetics of accumulation of [³H]uridine into 9S histone mRNA. Poly(A)(-)RNA, prepared as in the legend of Fig. 3, was sedimented on sucrose gradients (2) and the radioactivity under the 9S peak was determined. The data were analyzed as in Fig. 3 and standardized for A₂₆₀ of the 18S rRNA peak. (X) Total poly(A)(-)RNA; (○) 9S RNA.

FIG. 5. Poly(A) shortening in poly(A)(+)RNA from polysomes and free RNPs. Poly(A)(+)RNA was isolated from [³H]adenosine-labeled polysomes and free RNPs from *S. purpuratus* embryos labeled from the 16th hr of development (mesenchyme blastulae). Poly(A) was isolated and electrophoresed on 9% polyacrylamide gels in 99% formamide (see *Methods*). Electrophoresis is from right to left. The arrow indicates the position of poly(A) derived from L cells labeled for 1 hr. Yeast tRNA migrated to the midpoint of each gel. (A) Poly(A) from free RNPs (○) and polysomes (●) labeled for 20 min. (B) Poly(A) from polysomes (●) labeled for 1 hr, (○) labeled for 5 hr. (C) Poly(A) from free RNPs (●) labeled for 1 hr, (○) labeled for 5 hr.

lysate. The 1-hr poly(A) profile of this RNA is very similar to the 20-min pulse-labeled poly(A), even after 5 hr, more than 50% of the poly(A) is of the original length. The dynamics of poly(A) shortening on free RNPs and polysomes are clearly different, providing further evidence that the poly(A)(+)RNA from free RNPs is different from that of polysomes.

DISCUSSION

The data presented indicate that there is considerable mRNA present in the pool of free RNPs in embryonic sea urchin cells. Poly(A)(+)RNA in the free RNPs constitutes 10-15% of the poly(A)(+)RNA of these cells, and this RNA is competent in the ribosome binding assay. It is surprising that a considerable amount of the poly(A)(-)RNA from free RNPs is not active since poly(A)(-)RNA from polysomes is completely active in the assay. It is unlikely that a large amount of the poly(A)(-)RNA from free RNPs is a result of random nuclear leakage since sedimentation profiles, half-lives, and translation efficiencies (unpublished data) are different for poly(A)(-)RNA from free RNPs and nuclei. Rather, this RNA may have a function other than in protein synthesis. It is possible that this RNA does have message potential, but this potential cannot be realized in our assay, perhaps, for example, because the RNA is not properly methylated.

In this report we have examined the kinetics of labeling of

free RNPs and polysomes by separating the RNA into poly(A)(+) and poly(A)(-) classes. Both poly(A)(+)RNA and poly(A)(-)RNA from free RNPs turn over more rapidly than polysomal mRNA. These kinetics eliminate the possibility that the free RNPs equilibrate with the bulk of the polysomes; that is, the free RNPs cannot contain transcripts that cycle in and out of the polysome pool and equilibrate completely with that pool. These kinetics, however, cannot eliminate the possibility that free RNPs may be in equilibrium with a subclass of polysomes. Evidence for the existence of kinetic subpopulations of mRNA in polysomes is presented in Fig. 4, where the histone (9S) mRNA of polysomes is shown to turn over differently from the bulk of the polysomal RNA.

This problem was addressed by examining the metabolism of the poly(A) tails of RNA from free RNPs and polysomes to see whether free RNPs and polysomes could be distinguished on this basis. It is clear from Fig. 5 that although the original lengths of the poly(A) segments from free RNPs and polysomes are the same, their metabolism is apparently quite different. The steady-state-labeled poly(A) from polysomes is almost devoid of the original long segments, whereas about 50% of the steady-state poly(A) from free RNPs is still the original length of approximately 180 nucleotides. This could indicate the presence of a subpopulation of poly(A) that does not shorten with age in the free RNPs, in contrast to polysomal poly(A), which quantitatively shortens. There is, however, another more plausible explanation which we favor. Since the half-life of the free RNPs is less than an hour (50 min), over 50% of a steady-state-labeled population would be less than 1 hr old and should therefore display a poly(A) profile similar to poly(A) labeled for 1 hr. Since the 1-hr profile still displays a predominate peak at about 180 nucleotides, half of the steady-state-labeled poly(A) will also have a modal value of 180 nucleotides even though the poly(A) is quantitatively shortening with age. The polysomal poly(A), which shortens more rapidly [compare the 1-hr-labeled poly(A) of Fig. 5B with the pulse-labeled poly(A) of Fig. 5A], will have very few 180-nucleotide-long segments in a steady-state population. Thus, the steady-state-labeled poly(A) profiles can be explained on the basis of a quantitative shortening of the free RNP poly(A), albeit at a much slower rate than polysomal derived poly(A).

Despite this difference in poly(A) shortening, polysomes and free RNPs have very similar half-lives. There is, then, no obvious relationship between poly(A) shortening and turnover of mRNA in these embryos. Since shortening occurs on polysomes immediately upon their entrance into the cytoplasm but not immediately on the free RNPs, it seems reasonable to associate poly(A) shortening with initiation in this system. Based on this possible association, we suggest that poly(A) shortening on free RNPs is the result of their involvement in protein synthesis and their low rate of shortening is because free RNPs initiate infrequently. Free RNPs, then, would be in a dynamic equilibrium with a special subclass of polysomes that does not equilibrate with the bulk of the polysomes. This would also be consistent with the kinetic data presented above.

Cytoplasmic mRNA distributions have been determined in several systems. In some cells there is very little mRNA present as free RNP, and polysomes accumulate as a direct result of increased rates of transcription (21–23). In other systems, including the unfertilized sea urchin egg, there appears to be a store of mRNA sequences in the free RNPs preceding the accumulation of the messages in polysomes (13–15, 24, 25). In these systems mRNA is apparently not effectively utilized by

the translational apparatus until there is some form of stimulation. Our data suggest that these free RNPs may contain a class of mRNA that initiates infrequently. In the sea urchin this mRNA may become available to the developing embryo as initiation factor activity increases or in response to specific stimulation. Thus, free RNP mRNA may exist in the cytoplasm of cells not as a stable, "masked" message, but rather as a rapidly turning over, underutilized population of mRNA.

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