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Distribution of Messenger Ribonucleic Acid in Polysomes and Nonpolysomal Particles of Sea Urchin Embryos: Translational Control of Actin Synthesis†

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ABSTRACT: We have used cell-free translation and two-dimensional gel electrophoresis to examine the complexities of the polysomal and cytoplasmic nonpolysomal [ribonucleoprotein (free RNP)] messenger ribonucleic acid (mRNA) populations of sea urchin eggs and embryos. We show that all species of mRNA detected by this method are represented in both the polysomes and free RNPs; essentially all messages present in polysomes are also in the free RNP fraction. However, the cytoplasmic distribution is clearly nonrandom since some templates are relatively concentrated in the free RNPs and others are predominantly in the polysomes. The polypeptides synthesized under the direction of unfertilized egg mRNA are qualitatively indistinguishable from those made by using embryonic mRNA, indicating that the complexity of the abundant class mRNA remains unchanged from egg through early development. However large changes in the abundancies of specific mRNAs occur, and changes are detected in the polysomal/free RNP distribution of some mRNAs through development. The differences in the relative abundancies of specific mRNAs between polysomes and free RNPs and the developmental changes that take place indicate significant cytoplasmic selection of mRNA for translation. Three different forms of actin (termed α, β, and γ) were identified among the translation products. Messages for all three are present in the unfertilized egg and early cleavage embryo, yet the γ form is preferentially located in the polysomes and α and β in the free RNPs. The relative concentrations of the three change greatly during development as do their relative distributions into polysomes and free RNPs. Examinations of in vivo labeled proteins largely support the in vitro findings. The results indicate that the synthesis of actin mRNAs increases greatly during development and that the expression of the actin mRNAs is partly controlled at the translation level during early development.

It is generally accepted that the unfertilized sea urchin egg contains mRNA[1] which, due to some unknown mechanism, is unavailable for translation. The rapid and large increase in the rate of protein synthesis elicited by fertilization is thought to involve a shift of this mRNA from ribosome-free cytoplasmic ribonucleoprotein complexes (free RNPs) into polysomes [for review, see Davidson (1976)]. It has further been suggested that the use of this stored mRNA may be regulated in a specific temporal sequence (Barret & Angelo, 1969; Ruderman & Gross, 1974). During development the maternal mRNA is gradually replaced by mRNA synthesized in the embryo, and this embryonic mRNA also is distributed into both the polysomes and free RNPs (Dworkin & Infante, 1976). It is clear that the free RNPs in this system may be playing a role in the posttranscriptional regulation of protein synthesis. Such a consideration is also evolving from studies in other systems (Alton & Lodish, 1977; Benecke et al., 1978; Jain & Sarkar, 1979; Doetschman et al., 1980). In the present study the possibility that there is extensive translational level control of protein synthesis through a specific selection of cytoplasmic mRNAs into polysomal and nonpolysomal RNPs was examined.

We have used cell-free translation and two-dimensional polyacrylamide gel electrophoresis to compare the "abundant-class" mRNA complexities and sequences of the polysomal and free RNP pools of early embryos. Galau et al. (1974) showed that hybridization–kinetic analysis operationally divides sea urchin mRNA into a complex class and an abundant class. While the complex class may constitute 90–95% of the sequence complexity, it comprises <10% of the total message present. A translation analysis as presented here examines primarily the abundant class sequences, which contain over 90% of the mRNA mass yet consist of, at most, a few hundred species. It is therefore possible, with two-dimensional gel electrophoresis, to detect the in vitro translation products of most of the abundant mRNAs. Mindful that only a small percentage of the total complexity is being detected,

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[1] Abbreviations used: mRNA, messenger ribonucleic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; Cl_3CCOOH, trichloroacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; DTT, dithiothreitol; RNase, ribonuclease; DNase, deoxyribonuclease; cDNA, complementary deoxyribonucleic acid.

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we have asked whether the expression of these abundant messages is developmentally regulated, and, in particular, if there is selective translation of mRNAs in the cytoplasm.

We report that virtually all the species of abundant mRNAs present in polysomes are also represented in the free RNPs and that the mRNA complexities are very similar. The results show that there is a nonrandom distribution of cytoplasmic messages between the polysomal and free RNP fractions, indicating significant regulation of RNA translation at the level of mRNA selection in the cytoplasm.

One group of translation products analyzed contains the actin isoforms. Actin is a protein which performs a variety of functions and is apparently ubiquitous in eukaryotic systems [for review, see Clarke & Spudich (1977)]. In line with its diverse functions, actin occurs in multiple forms, each with the same molecular weight, but some are separable on the basis of different isoelectric points. These actin isoforms appear to be synthesized in different ratios during differentiation of myotubes in muscle culture systems (Storti et al., 1978; Fyrberg & Donady, 1979) and in developing frog embryos (Ballantine et al., 1979). This paper will emphasize both the great changes in the expression of the actin mRNAs during sea urchin development and the specific cytoplasmic location of these and other mRNAs in polysomal and nonpolysomal compartments.

Experimental Procedures

Extraction of Polysomal and Free RNP RNA. Eggs were isolated from the sea urchin *Strongylocentrotus purpuratus* and fertilized, and the embryos were grown as previously described (Infante & Nemer, 1968). The embryos were lysed in TNM buffer (50 mM Tris, pH 7.8, at 4 °C, 240 mM NH_4Cl, 5 mM $Mg(AcO)_2$, and 250 mM sucrose) by homogenization in a Dounce homogenizer, and the polysomal and free RNP fractions were purified from sucrose gradients as detailed previously by Dworkin et al. (1977). RNA was extracted with phenol–chloroform, and the poly(A+) RNA was separated by using oligo(dT)–cellulose (Rudensey & Infante, 1979).

Cell-Free Translation. A nuclease-treated wheat germ system was used for the cell-free translations. Preparation of the system and the incubations were performed as described by Rudensey & Infante (1979) except that [^{35}S]methionine was the labeled amino acid. Incorporation of label was assayed by Cl_3CCOOH precipitation, and the samples were stored at −70 °C until electrophoresis.

In Vivo Labeling of Proteins. Eggs or embryos, at a concentration of 0.01 mL/mL of sea water, were incubated with 100 μCi of [^{35}S]methionine for 4 h (unfertilized eggs) or 30 min (embryos). After incubation the embryos or eggs were washed twice with ice-cold sea water and resuspended in 1 mL of lysis buffer (0.01 M Tris, pH 7.8, 0.001 M $MgCl_2$, and 0.001 M DTT) containing $NaDodSO_4$ at 2%. Embryos with intact fertilization membranes were disrupted by gentle homogenization in a Dounce homogenizer. After removal of aliquots for determination of Cl_3CCOOH-precipitable counts the lysate was precipitated with 2 volumes of cold ethanol.

Two-Dimensional Gel Electrophoresis. Two-dimensional polyacrylamide gel electrophoresis was performed essentially as described by O'Farrell (1975). Isoelectric focusing gels containing 9 M urea were prepared to give a pH gradient of 7–5. The second-dimension $NaDodSO_4$ slab gel was a 10–16% polyacrylamide gradient. Prior to isoelectric focusing the in vitro translation products were treated with RNase A (50 μg/mL for 10 min at 37 °C), solid urea was then added to 9 M, and an equal volume of lysis buffer (O'Farrell, 1975) containing 0.1% $NaDodSO_4$ was added. Aliquots (30 μL) of this mixture were layered onto the isoelectric focusing gel.

The ethanol-precipitated in vivo labeled proteins were collected by centrifugation and dissolved in 0.15 M NaCl, 0.015 M Tris, pH 7.6, and 0.005 M $MgCl_2$. The solution was treated with both RNase A and DNase I at 50 μg/mL each for 10 min at 37 °C. Solid urea was added to 9 M, followed by an equal volume of O'Farrell lysis buffer containing 0.1% $NaDodSO_4$.

Gels were stained in 0.2% Coomassie Blue R-250 in 30% methanol–10% acetic acid and destained in 30% methanol–10% acetic acid. They were then fluorographed by the method of Bonner & Laskey (1974), dried, and exposed to preflashed X-ray film (Laskey & Mills, 1975). The spots on the film corresponding to actin were quantitated relative to each other by scanning on an Ortec 4310 Densitometer. To eliminate artifacts due to saturation of the film, we scanned several early exposures of each gel.

Results

Translation Products of Polysomal and Nonpolysomal mRNA. Previous work utilized $NaDodSO_4$ gel electrophoresis to investigate the complexity of cell-free translation products of messages extracted from sea urchin embryos of various developmental stages (Ruderman & Pardue, 1977; Senger & Gross, 1978). Here we have used the superior resolving power of two-dimensional gel electrophoresis to compare the in vitro translation products of poly(A+) RNA from unfertilized eggs and of polysomal and nonpolysomal free RNP poly(A+) mRNA from three distinct early embryonic stages: 6 h (32–64 cell stage), 12 h (late morula), and 21 h (hatched blastula). Figure 1 shows these patterns, each of which contains over 400 spots derived from each RNA translated.

The patterns of the embryo polysomal (Figure 1b,d,f) and free RNP (Figure 1c,e,g) translation products are very similar to each other. By variation of the length of exposure times for autoradiography it can be determined that essentially all the spots found on gels of the polysomal products are also present on the gels of the free RNP mRNA directed products. We were unable to detect with certainty any qualitative differences between the two sets of translation products for the three stages shown and three others not shown. This finding indicates that, in this system at least, the free RNPs do not contain a distinctly restricted population of messages but probably contain some of all the mRNA species present in polysomes. The patterns in Figure 1 show, however, that there are many differences in the relative intensities of different spots, wherein some are more intense in the polysomal products and others are more intense in the patterns of the free RNP products. Arrows indicate some of the spots where differences between the two patterns exist. Thus, although it appears that all the mRNA species in the polysomes are represented in the free RNPs, there is clearly a nonrandom distribution of many mRNAs between the two cytoplasmic fractions.

Developmental Changes of mRNA in Polysomes and Free RNPs. A developing system, such as the sea urchin embryo, provides the opportunity to determine if there is a shift in the polysomal/free RNP distribution of an mRNA which might perhaps underlie its regulated translation. In these studies total poly(A+) RNA from eggs was used without fractionation into polysomal and free RNP RNA. Since most of the egg poly(A+) RNA is present in the nonpolysomal free RNP fraction, the egg pattern is essentially a reflection of the pool of messages in the maternal free RNPs. The pattern of unfertilized egg translation products (Figure 1a) shows that essentially all species of the abundant messages in the egg are present in both

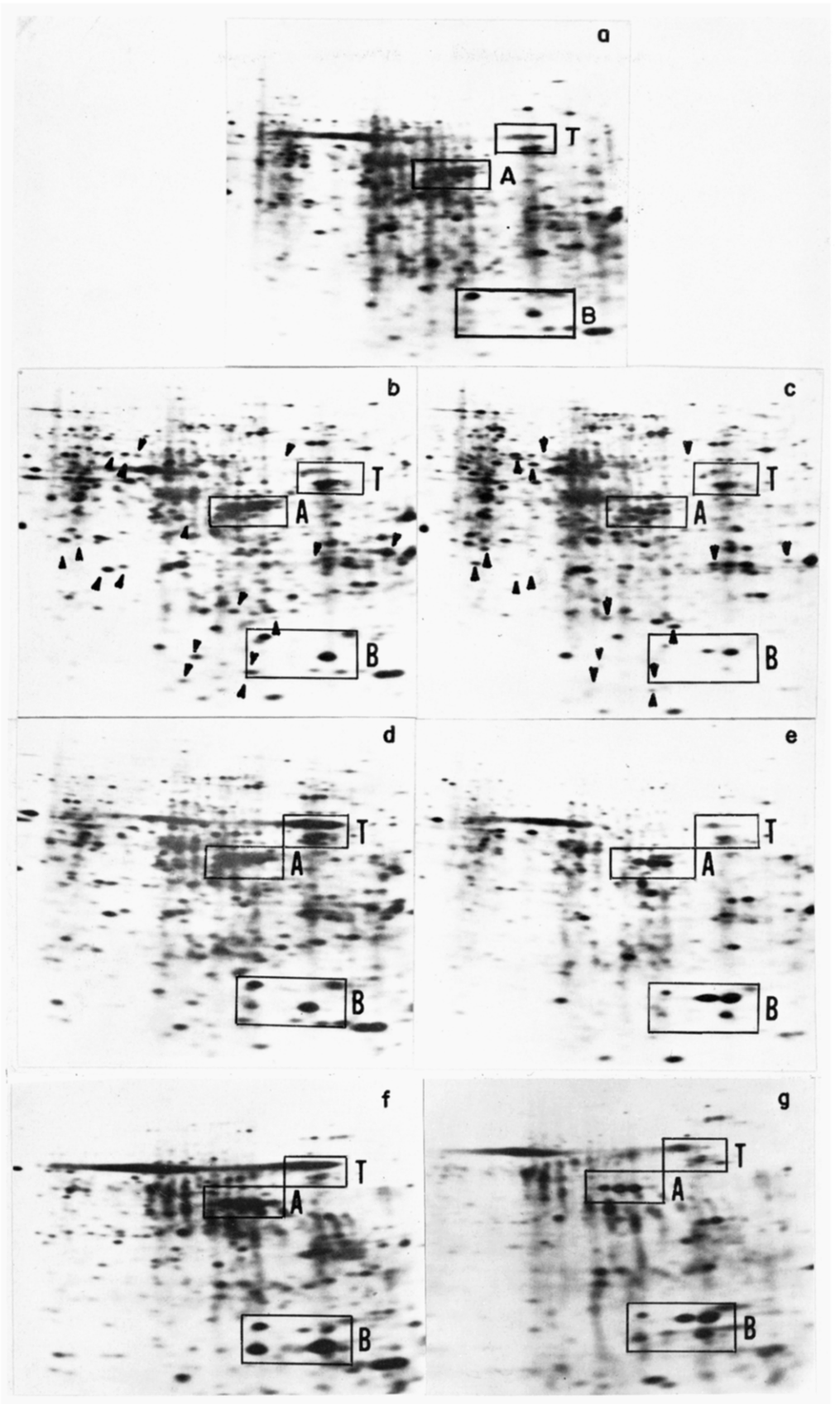

FIGURE 1: Two-dimensional polyacrylamide gels of cell-free translation products of polysomal and free RNP RNAs from different stages of development. Cell-free translation with [^{35}S]methionine and two-dimensional electrophoresis was done as described under Experimental Procedures with 500 000 cpm applied to each gel. The pH gradient was 7–5 from left to right. Length of exposure to X-ray film was regulated to give resolution of a maximum number of spots. The boxed-in areas refer to areas of specific interest: T (tubulin), A (actin, see Figure 3), and B (see Figure 2). Arrows point out other spots which show differences through development or between polysomal and free RNP translation products. RNA used: (a) egg; (b) 6-h polysomal; (c) 6-h RNP; (d) 12-h polysomal; (e) 12-h RNP; (f) 21-h polysomal; (g) 21-h RNP. (a–e) were exposed for 30 days, (f) for 8 days, and (g) for 14 days.

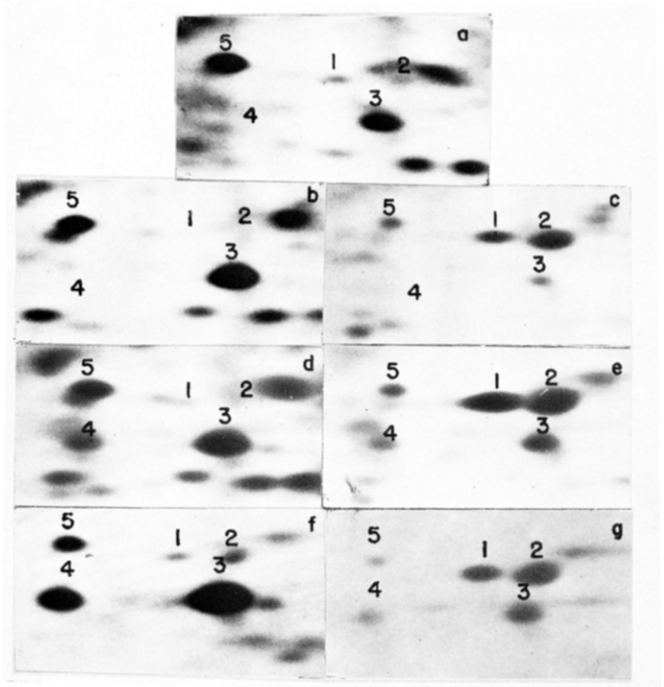

FIGURE 2: Differences between cell-free translation products of polysomal and free RNP RNAs. The area labeled B in Figure 1 was enlarged. RNA translated: (a) egg; (b) 6-h polysomal; (c) 6-h RNP; (d) 12-h polysomal; (e) 12-h RNP; (f) 21-h polysomal; (g) 21-h RNP. Exposure times were regulated to give maximum resolution: (a–e) 30 days; (f) 2 days; (g) 4 days. The numbered spots are referred to in the text.

the polysomes and free RNPs of early embryos. However, there are major quantitative changes (e.g., area labeled T) in some translation products during early development. There are many such changes, and they occur in both polysomal and free RNP patterns, with many taking place after 12 h of development. Specific details of two groups of translation products (labeled A and B in Figure 1) will be presented (Figures 2 and 3) to describe the kinds of developmental changes which occur and the variations seen in the distribution of some mRNAs between polysomes and free RNPs.

The gel area labeled B in Figure 1 is enlarged in Figure 2. Here a marked difference between the patterns of five labeled protein products obtained from polysomal and free RNP RNA is evident. In the translation products of 6-h polysomal mRNA (Figure 2b), the spots designated 3 and 5 are predominant, while 1, 2, and 4 become detectable only after long times of autoradiography. But the 6-h free RNP mRNA translation products (Figure 2c) show a different pattern with spots 1 and 2 predominating and 3, 4, and 5 being very faint, although 5 is more evident than 3 and 4. This difference in pattern between free RNP and polysomal RNA translation products persists through early development. By the 21-h stage all five products are clearly present in both polysomal and RNP patterns, and the differences are most apparent.

Proteins synthesized in a 30-min labeling period in vivo were also analyzed by two-dimensional polyacrylamide gel electrophoresis (data not presented). In general, the in vivo and in vitro derived patterns are qualitatively very similar with most of the spots being common to both. The areas in the in vivo labeled protein pattern corresponding to the gels in Figure 2 showed that spots 3, 4, and 5 become clearly visible at ~15 h of development and are prominent thereafter. In vivo products comigrating with spots 1 and 2 are first clearly detectable at ~20 h of development, but they are comparatively minor up to at least 24 h.

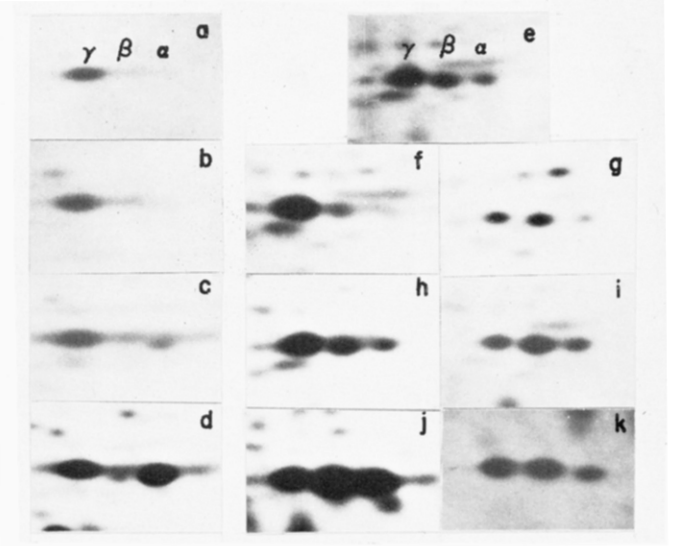

FIGURE 3: Synthesis of actin in vivo and in vitro. The area labeled A in Figure 1 was enlarged. Corresponding areas from two-dimensional gels of in vivo labeled proteins were also enlarged. (a) Egg in vivo; (b) 6-h in vivo; (c) 12-h in vivo; (d) 21-h in vivo. (e–k) are in vitro translation products. RNA used: (e) egg in vitro; (f) 6-h polysomal; (g) 6-h RNP; (h) 12-h polysomal; (i) 12-h RNP; (j) 21-h polysomal; (k) 21-h RNP. Exposure times were regulated to give maximum exposure: (a and b) 5×10^5 cpm applied to gels, 14-day exposure; (c) 3×10^5 cpm, 22 days; (d) 3×10^5 cpm, 7 days; (e–k) 5×10^5 cpm; (e–i) 10-day exposure; (j) 2 days; (k) 4 days.

The unfertilized egg contains the messages for these five proteins (Figure 2a), yet the dramatic increase in the relative intensities of these five translation products through early development indicates that the amounts of their specific mRNAs available in the cytoplasm must also increase. This is most likely the result of transcription of new templates by the embryo, although other possibilities exist. In any case it would appear from these data that the vast majority of the mRNA coding for the proteins in spots 3 and 4 becomes associated with ribosomes and is translated, whereas the messages for proteins 1 and 2 are almost totally shunted into translationally inactive free RNPs. Synthesis of these proteins is therefore controlled, at least partially, at the translation level.

The isoforms of actin present another set of templates whose cellular content increases during development and which are distributed in both the free RNPs and polysomes. We will show below that major changes occur in the polysomal/free RNP distribution of the actin mRNAs through development. Before describing the nature of this distribution and the developmental changes, some characteristics of sea urchin actin and its synthesis in vivo will be given.

Developmental Changes in Actin Synthesis. We found that at all stages analyzed one of the major products, in vivo and in vitro, was actin. Work with other systems, such as chick muscle (Storti et al., 1978), mouse L6 cells (Hunter & Garrels, 1977), and *Drosophila* myoblast cultures (Storti et al., 1978; Fyrberg & Donady, 1979), has shown that actin can be separated on the basis of isoelectric point into multiple forms. We report here that sea urchin actin can also be separated into at least three distinct forms. We have designated these as α, β, and γ in order of increasing isoelectric point. Actin was identified by comigration with rabbit skeletal muscle actin and by chromatography on DNase–cellulose columns (Lazarides & Lindberg, 1974).

Figure 3 shows enlarged areas of two-dimensional polyacrylamide gel electrophoresis patterns which contain actin (area indicated as A in Figure 1). In parts a–d are shown the proteins synthesized in vivo. The first interesting finding is

Table I: Developmental Changes in Cell-Free Synthesis of α-, β-, and γ-Actin Isoforms Directed by RNA from Polysomes and Free RNPs[a]

		6 h			12 h			21 h		
	egg (%)	poly (%)	RNP (%)	poly/RNP	poly (%)	RNP (%)	poly/RNP	poly (%)	RNP (%)	poly/RNP
α	20	7	18	0.4	19	23	0.8	35	40	0.9
β	30	21	46	0.5	34	45	0.8	43	43	1.0
γ	50	72	36	2.0	47	32	1.4	21	17	1.2

[a] The α, β, and γ spots in Figure 3e-k were quantitated relative to each other as described under Experimental Procedures. The total intensity of the three actin spots on each gel was set at 100% and the relative amount of each isoform present calculated relative to this.

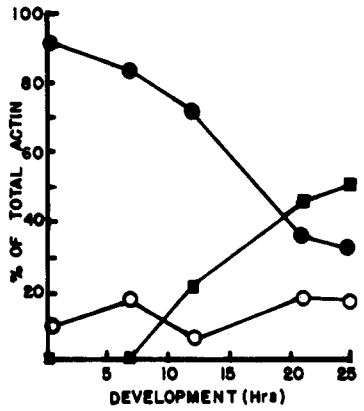

FIGURE 4: Changes in relative synthesis of the α-, β-, and γ-actin isoforms during early development in vivo. The α, β, and γ spots shown in Figure 3a–d were quantitated relative to each other as described under Experimental Procedures. (■) α-Actin; (○) β-actin; (●) γ-actin.

that actin is being synthesized in the unfertilized egg. Secondly (taking into account different exposure times), there is a great increase in the overall intensity of the actin spots on the gels during early development indicating an increased expression of these templates in the early embryo. Thirdly, the patterns in Figure 3a–d show that the synthesis of the three actins change relative to each other. This change has been quantitated and is presented in Figure 4. In the egg the predominant species being synthesized is the γ form which represents >90% of the three actin isoforms. This is also true for the 6-h embryo. However, an increase in the α form relative to γ and β is seen in the 12-h stage, and by the 21-h stage the α form predominates, being ~50% of the three actins. Through this time period β-actin remains relatively constant at ~15% of the total actin synthesized. A more extensive report of these changes beyond 24 h will be presented elsewhere (L. J. Heilmann and A. A. Infante, unpublished experiments). Of importance here are the findings that actin synthesis in this system is regulated both quantitatively and qualitatively. We have examined the distribution of actin mRNAs in the free RNPs by in vitro translation analysis to determine any role this compartment may play in these levels of regulation.

Actin mRNA in Polysomes and Free RNPs. Figure 3e–k shows the actin regions in two-dimensional gel patterns of in vitro translation products using egg RNA or embryonic polysomal and free RNP poly(A+) RNA. The proportions of the three actin isoforms in these gel patterns have been calculated for each developmental stage and are given in Table I.

Figure 3e shows that the translation products of total egg poly(A+) mRNA contain all three actin forms, although they are present in unequal amounts. From this analysis it appears that, of the three isoforms, the message for γ-actin is present in the highest concentration (50%) in the egg, with β and α forms being only 30% and 19% of the total, respectively (Table I).

A major difference exists between the actins specified by the poly(A+) mRNA of the 6-h embryonic polysomal (Figure 3f) and free RNP fractions (Figure 3g). In the polysomal gels the spot corresponding to γ-actin is by far the most intense, representing ~72% of the total actin synthesized (Table I). The β-actin is distinctly present but at a relatively much lower concentration, being ~21% of the three isoforms. Extended exposure of the X-ray film is necessary to detect α-actin. The pattern of the RNP poly(A+) RNA translation products (Figure 3g) is quite different. In this case the γ-actin spot is much less intense, and, in fact, the β-actin spot is the most intense, amounting to 46% of the total actin synthesized. α-Actin is again the least of the three, but its relative intensity (18% of total actin) is much greater than in the polysomal gels. Thus, early cleavage polysomes are enriched for γ-actin messages over those for α and β. Interestingly, if one superimposes the polysomal and RNP gel patterns (equal counts were applied to each gel and the exposure times were the same) of the 6-h embryos, the resultant image is nearly identical with the unfertilized egg pattern.

To determine if the preferential location of the γ-actin message in the polysomal fraction of 6-h embryos is an artifact of the fractionation procedure, we compared the cell-free translation products with in vivo labeled proteins. The unfertilized egg, which contains the messages for all three actins, actively synthesizes almost entirely γ-actin (Figures 3a and 4). The same is seen with the proteins being synthesized in vivo by the 6-h embryo (Figures 3b and 4), in agreement with the results of the translation of 6-h polysomal mRNA (Figure 3f). This implies that egg polysomes are also enriched for γ-actin messages over those for α and β. These data suggest the presence of a translational control mechanism in the unfertilized egg and early cleavage stage embryo which selectively activates and translates specific messages stored in the maternal RNP fraction.

At ~12 h postfertilization major changes in actin mRNA distribution and translation begin (Figure 3h,i). The relative intensity of the in vitro synthesized actin spots on the gels increases dramatically. Since the increase occurs in both free RNP and polysomal translation products, it must be due to a relative increase in cytoplasmic actin mRNA which could be the basis for the observed increase of actin synthesis in vivo (Figure 3a–d). Accompanying this overall quantitative change, there are apparent changes in the distribution of the different actin messages between the polysomal and RNP fractions. At 12 h the major translation product of polysomal actin mRNA is still γ-actin, but the proportions of α and β are much higher than at 6 h, being 19 and 34% of the total actin, respectively (Table I). This is a sudden change since 9- and 10-h embryos yield patterns similar to the 6-h embryos (data not shown). The 12-h free RNPs contain mRNA which specifies predominantly the β species as in the earlier embryonic stages.

By 21 h after fertilization the proportions of the three actin messages have dramatically changed from those in the earlier RNP and polysome fractions (Figure 3j,k). β-Actin has now become the major isoform in the polysomal as well as the free RNP translation products, and γ-actin is the least, although

it is still one of the major translation products. Also, both the polysomes and free RNPs now contain all three messages in large amounts and in similar proportions. Table I shows that the ratios of $\alpha/\beta/\gamma$ in polysomal RNA translation products are similar to the ratios found in free RNP RNA translation products. Thus, at this embryonic stage, in contrast to the 6-h embryo, the relative proportion of each actin mRNA in polysomes and free RNPs is the same, with the distribution of actin mRNAs between polysomes and free RNPs evidently reflecting the actual ratios of these mRNAs in the cytoplasm.

As shown above in Figure 4, 12 h also marks the beginning of changes in the pattern of actin synthesis in vivo. The change in the expression of the α- and γ-actin messages in vivo correlates very well with the changes observed in vitro. However, although β-actin is the major actin product of 21-h embryonic polysomal mRNA, this isoform is not detected in like quantities in vivo (cf. Figure 3d,j). This could reflect properties inherent to the in vitro system which might more efficiently translate the β-actin mRNA or posttranslationally modify the product in some way as has been shown to occur with actin by Garrels & Hunter (1979). The use of a cell-free system from sea urchins may help resolve this possibility. Another possible explanation is that the β-actin message may be more extensively polyadenylated than either the α or γ messages and thereby becomes enriched in the poly(A+) mRNA preparations used for translation [cf. Hunter & Garrels (1977)]. However, translation of polysomal poly(A−) RNA of 21-hour embryos where the in vitro-in vivo difference is greatest yielded an actin pattern essentially identical with the poly(A+) RNA pattern (L. J. Heilmann and A. A. Infante, unpublished results). This agrees with reports by Kaufmann et al. (1977) for HeLa cell actin and by Brandhorst et al. for sea urchin RNA.

Discussion

For all cases examined, the patterns of polysomal RNA translation products were very similar to the free RNP patterns, indicating a similarity in the complexities and sequences of the mRNAs present in the two compartments. There is no evidence for the absolute restriction of an abundant mRNA to either polysomes or free RNPs. Thus some of all the cytoplasmic mRNAs are present in the free RNPs. This finding speaks against the free RNPs functioning to completely sequester a given species of mRNA from the translation apparatus as is implied in the "informosome" model presented by Spirin (1966). However, although the total mRNA complexities and sequences are very similar, some messages are more likely to be present in the polysomes while others are more likely to be in the free RNPs. There is definitely not a general random distribution of all the cytoplasmic mRNAs between the polysomal and nonpolysomal fractions. This difference in the relative abundancies of specific mRNAs in the RNPs and polysomes indicates an extensive degree of selection in the use of individual species of cytoplasmic mRNA.

Dworkin & Infante (1976) have shown that the free RNPs synthesized by the embryo are not in a simple precursor–product relationship with polysomes, nor do free RNPs result from polysome disassembly. It would appear, therefore, as if there is some equilibrium distribution of an mRNA between the polysomes and a cytoplasmic nontranslating condition, and whatever factors are involved in determining this distribution they can discriminate between different mRNAs.

The results very likely reflect meaningful differences in the utilization of cytoplasmic mRNA species and are not due to anomolies in the vitro translation system. The same results (but not as extensive) have been obtained by using the reticulocyte–lysate system (L. M. Rudensey, unpublished observations), and almost all of the in vitro products have corresponding counterparts in proteins synthesized in vivo. Furthermore, an earlier translation analysis suggested that the message for the histone H1 is enriched in the free RNPs compared to the other histone mRNAs (Rudensey & Infante, 1979); this has recently been substantiated through direct measurements of the histone mRNAs which show that 30% more H1 mRNA is present in the free RNPs compared to the nucleosomal histone mRNAs (E. Baker and A. A. Infante, unpublished experiments). Our results also agree with those of Walters et al. (1979) who have recently determined by using a cDNA hybridization analysis that the free RNPs of CHO cells contain all the sequences present in the polysomes but at different relative frequencies.

We sought to determine if there is a fixed ratio of an mRNA between polysomes and free RNPs or if the subcellular distribution of an mRNA could vary. Studies using probes for specific mRNAs will be required to provide a conclusive answer to this; however, the analysis of translation products indicates that the distribution of each mRNA species may be differentially regulated. Precise quantitation is not possible, but it is clear that some translation products are always more intense in the polysomal products (e.g., spots 3–5 in Figure 2) while others are always more intense in the RNP mRNA products (e.g., spots 1 and 2 in Figure 2). A constant subcellular distribution is also indicated for the tubulin messages, where, despite an apparently great developmental increase in the cytoplasmic concentrations of these mRNAs, the mRNA is always much more abundant in the polysomes. Dworkin & Dawid (1980), using cloned cDNA probes prepared from *Xenopus laevis* embryos, have also recently shown that the polysomal/free RNP distributions of poly(A+) RNA species are not constant for all species.

The actins exemplify a quite different situation. There is not a constancy in the distribution of the three actin mRNAs relative to each other in the polysomes and free RNPs during development. In Table I, the relative amounts of each actin isoform synthesized by using polysomal RNA are compared to the relative amounts synthesized by using free RNP RNA. This comparison allows us to determine if the distributions of the three mRNAs between polysomes and free RNPs are the same or if there is a differential selection of any mRNA into one fraction. For the 6-h embryo, the relative amount of γ-actin synthesized by polysomal RNA is 2 times greater than the amount synthesized with free RNP RNA, whereas the α- and β-actins synthesized by polysomal RNA are about one-half (0.4 and 0.5, respectively) that synthesized by using free RNP RNA. There is clearly an unequal distribution of the three mRNAs at this stage, and the γ-actin mRNA appears to be disproportionally concentrated in the polysomes. This inequality is less evident in the 12-h embryo, and by the 21-h stage the relative amounts of each isoform synthesized with the two RNA preparations are very similar. At this stage, in contrast to the 6-h cleavage stage embryo, the relative abundancies of the three actin mRNAs in each cytoplasmic fraction are the same.

If the process of activation of stored maternal mRNA were a simple gross stimulation one would expect to find messages in the same relative abundancies in both embryo compartments as in the egg RNA. On the other hand, a specific directed activation of certain messages would tend to preferentially locate these messages on the embryonic polysomes. We speculate that the results with the 6-h stage indicate the presence of a translational control mechanism in the cleavage

stage embryo which operates to selectively activate and permit translation of specific templates (in this case the γ-actin mRNA) stored in the RNPs of the egg. This regulatory phenomenon, active in the unfertilized egg and during the cleavage stages, is not apparent later in development for the actins; a transition which suggests that the selection mechanisms involved in defining the cellular polysomal/free RNP distribution of these mRNAs changes during development. This implies that the mRNPs stored in the egg are regulated differently from those made by the embryo. At 6 h virtually all mRNA is of maternal origin (Davidson, 1976), but by 21-h the actin mRNA has very likely been replaced by newly made embryonic templates. Differences between maternal and embryonic free RNPs have been reported by Young & Raff (1979); thus, a difference in the free RNP itself may account for the observed differences in the regulated translation of the actin mRNAs.

The findings presented here are relevant to several developmental questions. First, it is clear that the translation products produced with egg mRNA are qualitatively indistinguishable from both polysomal and free RNP mRNA directed products of all embryonic stages examined. There is therefore no extensive transcription and accumulation of many new species of mRNA that are not already abundant in the unfertilized egg. Also, we could not document the total disappearance of any translation product, indicating that essentially all of the abundant maternal templates are either stable or are replenished by transcription in the early embryo. This conservation of RNA sequences also is true for the complex class messages for which Galau et al., (1976) have shown that all sequences present on gastrula polysomes are present in the total RNA of oocytes.

Another developmental aspect of this study is that despite an apparent lack of major qualitative changes in message composition between eggs and 21-h embryos, there occur dramatic changes in the cellular concentrations of many abundant mRNAs. Some of these changes are evident primarily in the polysomal RNA translation products as seen in the tubulin spots (box T in Figure 1) which increase greatly. Some occur primarily in the free RNP products (e.g., spots 1 and 2 in Figure 2), while other relative increases or decreases in mRNA concentrations take place in both polysomal and free RNP patterns simultaneously. Double-label gel analyses of proteins synthesized in vivo also show significant quantitative variations in the expression of individual templates brought about by fertilization and during development [M. B. Dworkin, P. Maguire, and A. A. Infante, unpublished observations; cf. Brandhorst (1976)]. The variations in frequencies of mRNAs within the abundant class sequences imply that these templates may code for proteins that perform other than merely housekeeping functions. We report that at least three actins are synthesized in the sea urchin [cf. Merlino et al., (1980)] and that the mRNAs for all three are present in the unfertilized egg. Recently Spudich & Spudich (1979) have reported that actin purified from sea urchin egg cortex contains only one isoform. We have likewise found that staining of two-dimensional polyacrylamide gels of total egg proteins shows only one isoform whose migration corresponds to what we have termed the α-actin (L. J. Heilmann and A. A. Infante, unpublished data). This is surprising since, as seen in Figure 3a, the egg synthesizes almost exclusively γ-actin. This situation might result from a very rapid turnover of egg γ-actin or some kind of precursor–product relationship.

Details of actin characterization and developmental changes will be presented elsewhere. What is shown here (Figure 4) is that the relative proportions of the different actins synthesized in vivo vary greatly during development, with the γ/α ratio changing from more than 100:1 in the egg and cleavage stage embryo to about 0.7:1 in the early mesenchyme blastula, a change that is the result not of a decrease in γ-actin synthesis but rather a dramatic increase in synthesis of α-actin. This shift is also seen in the in vitro translation products of mRNA from both the free RNP and polysomal compartments. All the actins increase greatly during development relative to other translation products, suggesting that, very likely, new transcription of these templates may be largely responsible for the in vivo and in vitro changes. Whether the actin mRNAs become a greater proportion of the abundant messages due to new synthesis is being tested through direct analysis of actin mRNA content using specific probes.

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Stereochemical Control of Ribosomal Peptidyltransferase Reaction. Role of Amino Acid Side-Chain Orientation of Acceptor Substrate†

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ABSTRACT: The substrate specificity of the acceptor site of peptidyltransferase of *Escherichia coli* 70S ribosomes was investigated in the fMet-tRNA·A-U-G·70S ribosome and AcPhe-tRNA·poly(U)·70S ribosome systems by using a series of 2′- and 3′-aminoacyldinucleoside phosphates as acceptors. These chemically synthesized compounds are analogues of the 3′ termini of either 2′(3′)-, 2′-, or 3′-aminoacyl transfer ribonucleic acids (AA-tRNAs) of the types C-A-aa, C-2′-dA-aa, C-3′-dA-aa, C-3′-dA-3′-NH-aa, and C-2′-dA-2′-NH-aa (aa = Phe, D-Phe, Lys, Leu, Ala, Glu, Pro, Gly, Asp, Met, and α-aminoisobutyryl). It was found that the 3′-aminoacyl derivatives of optically active amino acids are much better acceptors of *N*-formyl-L-methionine (fMet) or *N*-acetyl-L-phenylalanine (AcPhe) residues than the isomeric 2′-aminoacyl derivatives with affinity constant ratios (K_M 2′/3′) > 100. Likewise, C-A(D-Phe) is a weaker acceptor than the corresponding L derivative C-A-Phe. In contrast, all glycyl derivatives (C-2′-dA-Gly, C-3′-dA-Gly, C-3′-dA-3′-NH-Gly and C-2′-dA-2′-NH-Gly) are good acceptors of the fMet residue, with ratios (K_M 2′/3′) of ~2. On the basis of these results, a model for the stereochemical control of the peptidyltransferase reaction is proposed. It assigns a major role to the orientation of the amino acid side chain in 2′- or 3′-AA-tRNA. A detailed model of the interaction of the acceptor terminus of 3′-AA-tRNA with the acceptor site of peptidyltransferase is also proposed. The model is strikingly similar to those for the active sites of proteolytic enzymes.

AA-tRNA[1] can exist as either a 2′ or 3′ isomer owing to rapid transacylation of the aminoacyl residue within the cis diol grouping of the 3′-terminal adenosine residue (Griffin et al., 1966). The physiological role of 2′- and 3′-AA-tRNA in enzymatic processes involved in protein biosynthesis has recently become the subject of considerable interest (Ofengand, 1977; Sprinzl & Cramer, 1979).

One of the important substeps of protein biosynthesis is peptide bond formation catalyzed by peptidyltransferase. It is, therefore, of considerable interest to study the specificity of this ribosomal enzyme toward isomeric 2′- and 3′-AA-tRNAs. Studies of the peptidyltransferase acceptor site are of particular significance because this is the site where the amino acid becomes incorporated into the growing peptide chain. Thus, the substrate specificity of the acceptor site may be a very important factor influencing selection of the proper AA-tRNAs for incorporation into protein (Sprinzl & Cramer, 1979). Modified AA-tRNAs or their 3′-terminal fragments in which the 2′⇄3′ transacylation cannot occur have recently been used for studies of isomer specificity of the acceptor site of peptidyltransferase. All of these studies used only derivatives of phenylalanine, and most of them concluded that 3′-Phe-tRNA is the acceptor in the peptide bond forming step (Ofengand, 1977; Sprinzl & Cramer, 1979). Quite surprisingly, Chinali et al. (1974) have also observed significant acceptor activity with Phe-tRNA-C-C-3′-dA ("nonisomerizable 2′-Phe-tRNA"). On the basis of these experiments, several important questions have arisen. (1) Is the peptidyltransferase acceptor site really specific for 3′-AA-tRNA, and is this specificity a general feature for all amino acids? (2) Is the peptidyltransferase acceptor site

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[1] Abbreviations used: AA-tRNA, aminoacyl transfer ribonucleic acid; EF-T_u, elongation factor T_u; Tris, tris(hydroxymethyl)aminomethane; PEP, phosphoenolpyruvate; S-150, the supernatant of the cell extract after centrifugation at 150000*g* (Chládek et al., 1974); AcPhe, *N*-acetyl-L-phenylalanine; fMet, *N*-formyl-L-methionine; Me_2Gly, α-aminoisobutyric acid; Z, benzyloxycarbonyl; Bzl, benzyl; Boc, *tert*-butyloxycarbonyl; DCC, dicyclolexylcarbodiimide; Me_4Si, tetramethylsilane; DSS, 4,4-dimethyl-4-silapentane-5-sulfonate; tRNA-C-C-3′-dA, tRNA with 3′-deoxyadenosine incorporated at the 3′ end; tRNA-C-C-2′-dA, tRNA with 2′-deoxyadenosine incorporated at the 3′ end; A-Phe, 2′-(3′)-*O*-L-phenylalanyladenosine; 2′-dA-Phe, 2′-deoxy-3′-*O*-L-phenylalanyladenosine, 3′-dA-Phe, 3′-deoxy-2′-*O*-L-phenylalanyladenosine; 3′-dA-3′-NH-Gly, 3′-deoxy-3′-glycylamidoadenosine; 2′-dA-2′-NH-Gly, 2′-deoxy-2′-glycylamidoadenosine; similar abbreviations are used for dinucleotide derivatives and other aminoacyl derivatives; TLC, thin-layer chromatography; RNase, ribonuclease; EDTA, ethylenediaminetetraacetic acid.

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