Polypeptide Synthesis in Sea Urchin Embryogenesis: An Examination with Synthetic Polyribonucleotides

Abstract. *After fertilization of the sea urchin egg the rate of protein synthesis by a cell-free ribosomal system increases markedly. This increase can be attributed to newly synthesized messenger RNA, since (i) the rate of polypeptide synthesis elicited by synthetic messenger polyribonucleotides changes only slightly after fertilization, and (ii) the enzymes for the formation of amino acyl transfer RNA's of phenylalanine and leucine and the polymerization of polypeptide are in excess in the unfertilized egg. Polypeptide synthesis has been characterized in development to the gastrula stage.*

Changes in the components of a cell-free ribosomal preparation from sea urchin eggs have been studied in an effort to account for the rapid increase in protein synthesis after fertilization (*1*). The apparent check on protein synthesis in the unfertilized egg may result from limitations on the concentrations of enzymic components of either of two sequential sets of reactions, namely, the production of specific amino acyl sRNA's (for abbreviations, see *2*), or the assemblage of the amino acids into polypeptide chains specified by messenger RNA. The incorporation of a given amino acid in polypeptide can serve as a measure of the minimal capacity of the system to synthesize the prerequisite amino acyl sRNA (*3, 4*). The capacity of the sea urchin egg for assemblage of polypeptide can be compared to observed endogenous activities through incorporations in the presence and absence of messenger polyribonucleotides (*5*). The results of the present study are consistent with the hypothesis that the rate of protein synthesis before fertilization is limited not by the concentration of enzymic components, but by that of messenger RNA.

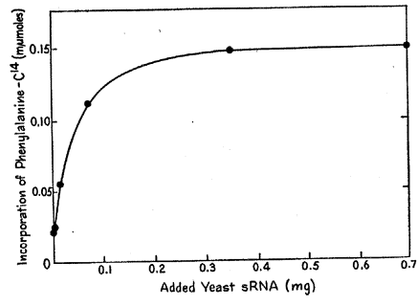

Fig. 1. Dependency of polyphenylalanine synthesis on sRNA in S-12 fraction of 1-hour fertilized eggs of *L. pictus*. The reaction mixture contained the following in μmoles/ml: 50 tris, *p*H 7.8; 10 $MgCl_2$; 240 KCl; 6 mercaptoethanol; 1 ATP; 5 PEP; 0.06 GTP; and 0.0035 L-phenylalanine-C^{14} (165 μc/μmole, Schwarz Bioresearch, Inc.). The total volume of 0.45 ml also included 20 μg PEP kinase, 100 μg polyU (Miles Chemical Co.) and various amounts of yeast sRNA as given on the abscissa. Incorporation was measured as mμmoles/hour per 100 mg S-12 RNA.

Eggs of *Lytechinus pictus* (*6*) or *Arbacia punctulata* (*7*) were fertilized and allowed to develop (*8*) in artificial sea water (*9*). At various stages of development the eggs were packed by light centrifugation in homogenization vessels, and an equal volume of homogenization medium (*1*), containing 0.006*M* mercaptoethanol, was added. Homogenization was effected with a Teflon pestle at 0° to 5°C. The homogenate was centrifuged for 10 minutes at 12,000*g*. The supernatant fluid (approximately 1 ml) was passed through a column (1.2 by 10 cm) of Sephadex G25, equilibrated with incubation medium, which was the same as the homogenization medium except that sucrose was omitted. The front of the effluent solution, which had been effectively dialyzed against incubation medium by this passage through Sephadex, was called the S-12 fraction, and was adjusted to contain 0.50 mg of RNA and approximately 5 mg of protein per milliliter (*10*). Incubations were performed with 0.2 ml of S-12 fraction, at 30°C for 45 or 60 minutes. The rate of incorporation of C^{14}-phenylalanine had been found to be constant up to 1 hour. The reactions were stopped with addition of TCA (to 5 percent). Samples were heated at 90°C for 20 minutes and the precipitates were washed and plated on glass-fiber disks. Radioactivity was measured in a gas-flow counter with a Micromil window and a counting efficiency of approximately 30 percent. Samples were counted at infinite thinness. All assays were in duplicate.

Maximal activities of the enzymic components of the S-12 fraction were measured under conditions in which concentrations of nonenzymic components were in excess. Incorporation of phenylalanine in polypeptide in the presence of polyU was substantially stimulated by yeast sRNA. Hence excess concentrations of both polyU and yeast sRNA (*11*) were determined for a system synthesizing PPA. In the presence of polyU at 0.3 mg/ml, with labeled phenylalanine the only amino acid present, a plateau value for incorporation was reached (Fig. 1) on increasing sRNA beyond 0.2 mg/ml. If the process is similar to that observed in *Escherichia coli* (*4*), the stimulation of incorporation in PPA by sRNA entails the intermediate formation of a phenylalanyl sRNA.

Table 1. Interrelation of protein and polyphenylalanine synthesis in S-12 fraction from 1-hour fertilized eggs of *A. punctulata*.*

Conditions			Incorporation of C^{14}-phenylalanine per 5 mg protein (count/min)
Poly U	sRNA	Amino acids	
+	−	+	4,340
+	−	−	3,525
−	−	+	935
−	−	−	55
+	+	−	15,200

* The reaction mixture was the same as that described in Fig. 1, except: S-12 fraction containing 6.6 mg of protein was incubated in a total volume of 0.85 ml. A mixture of 20 L-amino acids minus phenylalanine was used, each 22 μmole/ml; polyU, 0.6 mg/ml; yeast sRNA, 0.6 mg/ml; and 0.7 mμmole of L-phenylalanine-C^{14} (150,000 count/min). Incubations were at 30°C for 60 minutes.

The maximal velocities (V_{max}) of PPA synthesis were determined in the presence of excess yeast sRNA in S-12 fractions prepared at different stages

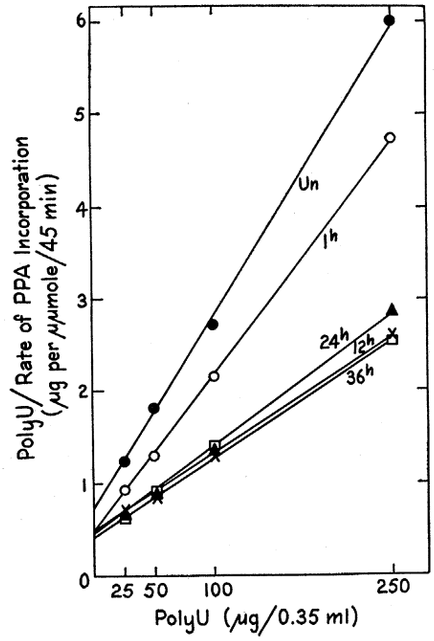

Fig. 2. Effect of concentration of polyU on polyphenylalanine synthesis by the S-12 fraction of *L. pictus* at various stages of development: ● Unfertilized egg; ○ 1-hour zygote; × 12-hour blastula; ▲ 24-hour gastrula; □ 36-hour gastrula. The reaction mixture was the same as that of Fig. 1, except the concentration of yeast sRNA was 0.7 mg/ml and polyU was varied as indicated on the abscissa, and a volume of 0.35 ml was incubated for 45 min.

of development. Figure 2 shows the ratio of polyU concentration to PPA incorporation plotted against polyU concentration, in accordance with the Lineweaver-Burk modification of the Michaelis-Menten relation (*12*). The calculated V_{max} increased from 50 $\mu\mu$mole of phenylalanine incorporated per 45 minutes in the unfertilized egg to 60 in the 1-hour zygote, to approximately 100 in the 12-hour blastula, where it remained constant through the 24-hour and 36-hour gastrula stages. Thus the capacity of the system for PPA synthesis increased with development to a value twice that of the unfertilized egg. Michaelis-Menten constants, calculated from experiments on three batches of eggs and a single batch of polyU, were essentially the same for the several stages of development (110 $\pm$ 20 μg/ml), indicating that the change in V_{max} probably reflected a change in one component or a single set of components.

The addition of a full complement of amino acids to these systems resulted in additional incorporation of phenylalanine, whether polyU was present or absent (Table 1). In both cases the additional incorporation could be interpreted as protein synthesis. The increase in PPA synthesis upon the addition of yeast sRNA (Table 1, lines 1 and 5) implies that the concentration of endogenous sRNA was rate limiting for this synthesis. Then in the absence of added yeast sRNA and in the presence of polyU, the additional incorporation of phenylalanine (lines 1 and 2) must have been mediated by an sRNA source not available to PPA synthesis. Such a source might be sRNA linked to ribosomes and possibly already in combination with endogenous messenger RNA.

In the unfertilized egg there can be a 200-fold increase in phenylalanine incorporation in polypeptide through the addition of polyU (Fig. 3 and Table 2). Since this incorporation is coupled to the formation of phenylalanyl sRNA (*4*) through activating and transfer enzymes, the activities of these enzymes are apparently in considerable excess of the needs of protein synthesis. An excess in enzymic activity for the synthesis of leucyl sRNA can also be inferred from the stimulation of leucine incorporation in the presence of polyUG (Table 4).

Incorporation of a mixture of labeled amino acids from a yeast protein hydrolysate was reduced 5 percent by the presence of unlabeled phenylalanine. Thus phenylalanine constitutes 5 percent of the total amino acid residues incorporated in protein. Protein synthesis has been represented in Fig. 3 and Table 2 by values of phenylalanine incorporation multiplied by 20. Hence the unfertilized egg is capable of incorporating over 10-fold more amino acid residues into PPA than into protein. As for the incorporation of leucine into polypeptide in the presence and absence of polyUG (5:1), there is an 11-fold stimulation (Table 4). Less than 10 percent of polypeptide synthesized on polyUG (5:1) template is leucine (*13*) and 12 percent of the protein may be leucine. The unfertilized egg can then synthesize approximately 10 times more polypeptide, directed by polyUG, than protein. If activities tested with synthetic messenger polyribonucleotides are comparable to endogenous protein synthesis, then the polypeptide synthetic capacity of the enzymes and ribosomes of the unfertilized egg exceeds the observed amount of protein synthesis, and it cannot be a limiting factor.

The rate of protein synthesis in the S-12 fraction of *Arbacia punctulata* increased 14-fold during the first 60 minutes after fertilization; at the same time PPA synthesis increased less than 1.5-fold (Fig. 3). These two types of polypeptide synthesis, the one elicited, presumably, by endogenous messenger RNA, the other by exogenous messenger polyribonucleotide, respond differently upon fertilization. Either they represent separate classes of ribosomes in the unfertilized egg which become activated differentially, or synthesis of messenger RNA after fertilization accounts for the greater increase in the rate of synthesis of protein.

In development of *Lytechinus pictus* to blastulae the rate of protein synthesis increased sharply, but further development to gastrulae took place with much less increase (Table 2). In this second phase the ratios of PPA to pro-

Table 2. Incorporation in polypeptide by S-12 fractions of various embryonic stages (hours of development) of *L. pictus*.*

Polypeptide	Incorporation in polypeptide ($\mu\mu$mole/100 μg S-12 RNA)			
	0 hr	1 hr	12 hr	24 hr
	Batch No. 1			
Protein	5	24	50	
PPA	51	65	75	
	Batch No. 2			
Protein	5	16	63	58
PPA	49	59	106	96
	Batch No. 3			
Protein	2	9		40
PPA	21	51		82
Average ratio, PPA protein	10	3.6	1.6	1.8

* S-12 fractions from three batches of eggs were assayed for PPA synthesis according to conditions in Fig. 2, except the yeast sRNA concentration was 0.5 mg/ml and the polyU was 0.5 mg/ml. For protein synthesis a mixture of 20 L-amino acids minus the labeled amino acids was present at 0.22 μmole/ml. Protein synthesis for batch 2 was measured by incorporation of L-leucine-C^{14}, 1 mμmole/ml. The ratio of incorporation of leucine to phenylalanine was 2.5; hence phenylalanine incorporation in protein was multiplied by 20, and leucine incorporation by 8.

Table 3. Dependency on added yeast sRNA for protein and polyphenylalanine synthesis at different stages of development in *L. pictus*.*

Stage		Leucine incorporation in protein ($\mu\mu$mole/100 μg S-12 RNA)			Phenylalanine incorporation in polyphenylalanine ($\mu\mu$mole/100 μg S-12 RNA)		
		−sRNA	+sRNA	Capacity (%)	−sRNA	+sRNA	Capacity (%)
	Unfertilized	0.56	0.64	87	3.9	42	9
1-hr	Zygote	1.83	2.07	88	4.3	53	8
12-hr	Blastula	5.48	7.85	70	14.7	96	15
24-hr	Gastrula	6.74	7.25	93	22.2	90	25

* Conditions were the same as those for Table 2. A mixture of 20 L-amino acids minus leucine, 0.22 μmole/ml; L-leucine-C^{14}, 1 mμmole/ml; polyU, 0.7 mg/ml in incubations with L-phenylalanine-C^{14}. Yeast sRNA, 0.7 mg/ml.

Table 4. Dependency on added yeast sRNA for incorporation of leucine in polypeptide in presence and absence of polyUG in *A. punctulata*.*

Stage		Leucine incorporation in polypeptide ($\mu\mu$moles/100 μg S-12 RNA)					
		−PolyUG			+PolyUG		
		−sRNA	+sRNA	Capacity (%)	−sRNA	+sRNA	Capacity (%)
	Unfertilized	0.41	0.46	91	2.93	5.00	59
1-hr	Zygote	2.43	2.58	94	4.28	6.58	65
12-hr	Blastula, late	3.93	3.85	102	6.18	6.28	98

* Conditions were the same as those of Table 3. PolyUG (5:1), 0.6 mg/ml.

tein synthesis are essentially constant (Table 2), indicating that the two types of synthesis may eventually become regulated by a common determinant.

Protein synthesis and the synthesis of polypeptide directed by synthetic polyribonucleotides were studied in the presence of an excess of yeast sRNA or in the absence of added sRNA in S-12 fractions derived at various stages of development. In the presence of an excess of polyU, the concentration of endogenous sRNA in the S-12 fraction of unfertilized eggs of *L. pictus* could support only 9 percent of the PPA synthesis that could be achieved in the presence of an excess of yeast sRNA (Table 3). In the course of development (Table 3) there was a substantial increase in the synthetic capacity determined by the concentration of endogenous sRNA. In the presence of polyUG the S-12 fraction from *A. punctulata* allowed 59 percent of the incorporation of leucine in polypeptide attained with added yeast sRNA (Table 4). At the blastula stage the endogenous concentration of sRNA was completely adequate to support the requirements of leucine incorporation in the presence of polyUG. PolyUG presents much less demand upon the supply of leucyl sRNA than polyU upon the supply of phenylalanyl sRNA. In both cases the supply of endogenous sRNA appears to increase with development.

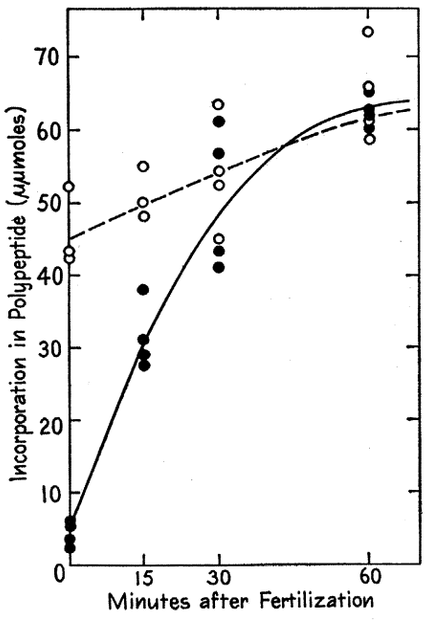

Fig. 3. Polyphenylalanine and protein synthesis by the S-12 fraction of *A. punctulata* before and after fertilization. Incorporation in protein is ●——●, PPA is o·····o. Conditions were the same as in Table 1, except that 3 mg of S-12 protein were present. Incorporation of phenylalanine in protein (in absence of polyU) has been multiplied by 20.

In both species the addition of yeast sRNA had little effect on the incorporation of leucine or phenylalanine in protein. Unless yeast sRNA is lacking in transfer RNA's specific for the sea urchin, endogenous levels of sRNA are adequate at all stages to the needs of protein synthesis (*14*).

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References and Notes

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2. Abbreviations: sRNA, transfer ribonucleic acid; TCA, trichloroacetic acid; PPA, polyphenylalanine; polyU, polyuridylic acid; polyUG, polyuridylic-guanylic acid; ATP, adenosine triphosphate; PEP, phosphoenolpyruvate; GTP, guanosine triphosphate; tris, tris (hydroxymethyl) aminomethane.
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Polymorphism and Population Density in the African Land Snail, Limicolaria martensiana

Abstract. *In natural populations of the African land snail,* Limicolaria martensiana, *the degree of polymorphism in color and pattern may vary with the density of the population. This could occur because predators eat the snails selectively and use past experience as a guide in finding further prey. Hence contrasting color forms may be at an advantage in dense populations where predators would have ample opportunity to learn to recognize prey.*

Distinct sympatric color forms, with few or no intermediates, occur in a wide variety of species of animals, especially in the tropics. Such genetic polymorphisms may be in stable equilibrium because the fitness of the heterozygotes is greater than that of the homozygotes or because the fitness of the forms varies with their frequency in the population. In this report I suggest that in a polymorphic land snail the fitness of several color forms may vary with the density of the population.

Limicolaria martensiana (Sm.) (Achatinidae) (*1*) is a highly sedentary land snail occurring in well defined, isolated populations in many parts of Uganda, and presumably elsewhere in East Africa. The size of the full-grown snail varies in different populations, but 35 mm would be an average length. Where it occurs, it is one of the most conspicuous members of the fauna. The snails live chiefly on or near the ground and feed on both living and rotting vegetation. After heavy rains they may climb several meters up shrubs and trees.

In the Kampala area there is a common form in which the shell is pale buff, heavily and intricately streaked with dark brown. There is much (continuous) variation within this form; indeed it is difficult to find two individuals alike. In some populations pallid forms also occur. In these pallid forms the dark brown streaks of the streaked form are usually just discernible as faint lines; the general appearance of these snails is an overall dilution of the streaked form so that at a distance they appear more or less uniformly buffish-pink, pale buff, or pale yellow. Three distinct pallid forms occur, apparently with no intergrades. In pallid 1 the shell is pale yellow or pale buff with streaks just discernible except at the upper edge of the whorl where for a few millimeters they are as dark as in the streaked form. The columella is dark brown. In pallid 2 the shell is uniformly buffish-pink, with streaks indistinct and columella dark brown. In pallid 3 the shell is uniformly buffish-pink with streaks indistinct and columella unpigmented.

Table 1 shows the relative frequency of these color forms in four populations, all occurring within a mile of each other in the Kampala area. The density of the population is also given. Each population is completely isolated from the others by major ecological and minor geographical barriers. In all populations the streaked form predominates, and at Kololo it is the only form. At Makerere, all three pallid forms occur and together comprise 38.6 percent of the population. At