# INTERRELATION OF MESSENGER POLYRIBONUCLEOTIDES AND RIBOSOMES IN THE SEA URCHIN EGG DURING EMBRYONIC DEVELOPMENT

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Polypeptide synthesis has been shown to be dependent upon the interaction of messenger polyribonucleotides and ribosomes derived from E. coli (Nirenberg and Mattaei, 1961). The relationships involved have not yet been demonstrable in other than bacterial systems. In this communication a microsomal preparation from sea urchin eggs is shown to respond sensitively to the addition of synthetic polyribonucleotides, with an enhancement of amino acid incorporation into polypeptides. Ribosomal activity without such additions is very small in unfertilized eggs, but increases markedly after fertilization (Hultin, 1961). Hultin has suggested that an activation of ribosomes accounts for this change. However, results presented below show that ribosomal activity in the presence of added messenger polyribonucleotides does not increase upon fertilization. Indeed, egg ribosomes experience a decrease in ability to respond to such additions after fertilization and during the course of subsequent embryonic development. It is proposed that the increase in ribosomal activity upon fertilization, reported by Hultin (1961), is due to the synthesis and attachment of endogenous messenger RNA.

Microsomes were prepared from eggs of the sea urchin Arbacia punctulata. Eggs were homogenized in the medium of Hultin (1961), containing 0.005 M mercaptoethanol, and centrifuged at 12,000 x g for 10 minutes. The 12,000 x g supernatant material was centrifuged for 90 minutes at 100,000 x g. The "microsomal" pellets were suspended in the incubation medium of Hultin (1961), containing 0.005 M mercaptoethanol. Microsomal protein concentrations of these suspensions were adjusted after protein determination (Lowry et al., 1915).

Following incubation, incorporation in protein was measured according to the method of Siekevitz (1952). Self absorption corrections were made to 3.25 $mg/cm^2$.

The synthetic polyribonucleotides, poly UC (uridylic-cytidylic copolymer) and poly U (polyridylate), elicited great increases in the measurable activity of unfertilized egg ribosomes (Tables I and II). The incorporation of leucine-$C^{14}$ in protein was enhanced 24 fold by poly UC, but less than three fold by poly U. That of phenylalanine-$C^{14}$ was enhanced as much as 173 fold by poly U. The stimulation of incorporation by the respective synthetic polyribonucleotides is in accordance with the code letters of the two amino acids (Nirenberg and Matthaei, 1961; Lengyel et al., 1961). The dependency of polypeptide synthesis in this egg ribosomal system on messenger polyribonucleotide is similar to that demonstrated by Nirenberg and Matthaei (1961) in E. coli. As opposed to the case of E. coli ribosomes, it is not necessary to have a pre-incubation period, in which protein synthesis must occur in order to expend endogenous messenger RNA. It is likely that ribosomes of the unfertilized egg are naturally stripped of such messenger RNA. The interrelation of messenger RNA and ribosomal particle may now be studied in a system of a higher order than that of bacteria, and the universality of proposed amino acid codes may be tested with another type of ribosome. The same possibility has been afforded by the recent demonstration of a response to poly U by the ribosomes of rabbit reticulocytes (Arnstein et al., 1962).

At the two-cell stage there is a five fold increase in basal ribosomal activity over that of the unfertilized egg. However, in the presence of poly U or poly UC the incorporation levels of phenylalanine or leucine, respectively, show little difference between unfertilized and fertilized ribosomes. Those of the unfertilized ribosomes are slightly higher. It can be concluded that fertilized egg ribosomes have no greater innate ability to incorporate amino acids into protein than do those of unfertilized eggs. The increase upon fertilization in activity without added messenger polyribonucleotide seen here in Arbacia and also seen in Paracentrotus lividus by Hultin (1961) may

not be the result of ribosomal activation per se, but of the formation and attachment of endogenous messenger RNA.

Table I

Stimulation by Poly UC of L-Leucine-$C^{14}$ Incorporation with Microsomes from Unfertilized and Fertilized Eggs.

| Microsomal Source | Additions | L-Leucine Incorporation (C.p.m./mg protein) | Ratio of Incorporations: Stimulated / Control |
|---|---|---|---|
| Unfertilized Egg | None | 15 | |
| | Poly UC | 367 | 24 |
| | Poly U | 39 | 2.6* |
| 2-Cell Stage (90 min.) | None | 103 | |
| | Poly UC | 297 | 3 |

The incubation system of 0.9 ml contained 4.0 mg microsomal protein, 0.5 mg yeast s-RNA, 10 µg PEP kinase, 0.25 µcurie L-Leucine-u.l.-$C^{14}$ (138 µc/µM), and the following in µmoles: 5.0 PEP, $K^+$ salt, 1.0 ATP, $K^+$ salt, 0.06 GTP, 215 $K^+$, 10 $MgCl_2$, 6.0 mercaptoethanol, 10 of each amino acid except leucine, but 100 each of serine and proline. Where indicated, 90 µg poly UC (5:1), and 70 µg poly U. Mixtures incubated 90 min. at 30°C. Values for incorporation are averages of duplicate determinations.

*As compared to 16 for phenylalanine at the same concentration of poly U.

Table II

Stimulation by Poly U of L-Phenylalanine-$C^{14}$ Incorporation with Microsomes from Embryos of Various Stages of Development.

| Microsomal Source | Additions | L-Phenylalanine Incorporation (C.p.m./mg protein) | Ratio of Incorporations: Stimulated / Control |
|---|---|---|---|
| Unfertilized Egg | None | 21 | |
| | Poly U | 3615 | 173 |
| 2-Cell Stage (90 min.) | None | 113 | |
| | Poly U | 3010 | 27 |
| Blastula (12 hr.) | None | 117 | |
| | Poly U | 1600 | 14 |

The incubation system was the same as that of Table I, except that it contained 5.0 mg of microsomal protein, 1.0 µcurie L-phenylalanine-u.l.-$C^{14}$ (30 µc/µM), 10 µmole of each amino acid except phenylalanine, and 0.50 mg poly U, where indicated. Duration of incubation was 120 min.

In Table II, after fertilization the stimulation of egg ribosomal activity by polyribonucleotides diminishes from 173 fold in the unfertilized egg to 27 fold at the two-cell stage and 14 fold in the swimming blastula (12 hours). Variations in the degree of response have been encountered in different experiments. The absolute level of response seems to depend upon the batch of unfertilized eggs used. However, the relative decreases in stimulation upon fertilization and development occur regularly, regardless of batch. The reason for variations among batches is not known. They may be related to differences in degree of maturation. The progressive decreases in degree of ribosomal response to added messenger polyribonucleotide needs further examination. This decrease may be due to a change in the nature of responsive ribosomes or to the formation of new types. On the other hand much of the reservoir of ribosomes in the unfertilized egg may be free of messenger RNA, but as development proceeds an increasing proportion of these become pre-empted by new messenger RNA, thus leaving fewer and fewer of them receptive to added messenger polyribonucleotides.

In summary, microsomal preparations from sea urchin eggs can respond to the addition of synthetic polyribonucleotides with marked stimulation of amino acid incorporation in polypeptides. Furthermore, the ability of these microsomes to respond to such additions decreases upon fertilization and during the course of subsequent embryonic development.

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