EVIDENCE FOR TRANSLATIONAL LEVEL CONTROL OF PROTEIN SYNTHESIS IN THE DEVELOPMENT OF SEA URCHIN EGGS*

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Control of protein synthesis in the mature sea urchin egg before and after fertilization is thought to occur in the absence of concurrent m-RNA synthesis (2,12,6,7). This is based primarily on the findings that protein synthesis occurs in parthenogenetically-activated, enucleated egg fragments (3), actinomycin-D does not adversely affect the rate of protein synthesis during the early division stages (9), and that both the unfertilized and fertilized egg contains similar species of m-RNA (18). This evidence suggests that protein synthesis in the sea urchin egg during this time is regulated at the translational level. In support of this suggestion, we report results obtained from in vitro experiments which show that the polysomes and the 105,000xg supernatants of unfertilized eggs are not functionally equivalent to comparable fractions from fertilized eggs.

METHODS

Gametes of the sea urchins Lytechinus variegatus and Echinometra lucunter were shed, washed, and incubated as previously described (4,16). Thirty minutes after fertilization the eggs, and also the unfertilized eggs, were separately washed three times in isotonic NaCl:KCl (5), twice in homogenizing medium (0.24 M KCl, 0.01 M $MgCl_2$, 0.01 M Tris, pH 7.6), resuspended in homogenizing medium, and homogenized in a Duall homogenizer (Kontes Glass Co., Vineland, New Jersey). Following centrifugation of the

crude homogenate at 12,000xg for 30 minutes, the upper two-thirds of the supernatant below the buffy layer was removed. The 12,000xg supernatants from fertilized and unfertilized eggs were either recentrifuged at 105,000xg for 2 hours or layered on sucrose density gradients. The linear 15-30% sucrose gradients, with a 5 ml cushion of 50% sucrose, were centrifuged for 2 hours at 24,000 rpm in the Spinco SW-25 rotor. The 105,000xg pellets were either resuspended in homogenizing medium and used directly for the in vitro studies on protein synthesis or subjected to further washings and recentrifugation at 105,000xg.

RESULTS AND DISCUSSION

The 12,000xg supernatant obtained from unfertilized sea urchin eggs was not as capable of in vitro protein synthesis (3.8 cpm/mg protein) as the 12,000xg supernatant derived from fertilized eggs (12.8 cpm/mg protein). The addition of the energy generating system (cofactors) stimulated the in vitro synthesis of proteins by the 12,000xg supernatants from fertilized eggs (183.7 cpm/mg protein) more than of unfertilized eggs (4.1 cpm/mg protein). The capacity for in vitro protein synthesis was retained in the 105,000xg pellet from fertilized eggs (Table 1) and also required the addition of cofactors for maximum incorporation of amino acids. After two washings with homogenizing medium, the 105,000xg pellet from fertilized eggs lost its capacity to incorporate labeled amino acids into proteins in vitro (Table 2). The washed 105,000xg pellet from fertilized eggs regained its ability to synthesize proteins upon the addition of the 105,000xg supernatant from fertilized eggs but not of unfertilized eggs. The fractions from the polysomal region (16) of the sucrose density gradients were inactive for in vitro protein synthesis (Table 3). Only the sample obtained from the polysomal region of the sucrose gradient upon which supernatants from fertilized eggs had been layered was capable of protein synthesis after the addition of the 105,000xg supernatant from fertilized eggs. When the mate-

TABLE I

Incorporation of leucine-C^{14} by 105,000xg pellets and supernatants*

	cpm/mg protein
(a) 105,000xg pellets from:	
Unfertilized eggs	9.9
Unfertilized eggs + cofactors	15.1
Fertilized eggs	18.2
Fertilized eggs + cofactors	147.2
(b) 105,000xg supernatants from:	
Unfertilized eggs	6.2
Unfertilized eggs + cofactors	8.7
Fertilized eggs	3.6
Fertilized eggs + cofactors	6.2

*The in vitro incubation system has been described (10) and contained 10 μM phosphoenol pyruvate, 1 μM ATP, 0.2 μM GTP, and 50 μg pyruvate kinase. The OD_{260} of the 105,000xg pellets from unfertilized and fertilized eggs were equal, as were the 105,000xg supernatants to each other. After incubation for 90 min. at 30° C, an equal volume of 10% TCA was added, the precipitate was washed 3 times in 5% TCA by centrifugation, heated to 90° C for 20 min. in 5% TCA,, washed 3 more times with 5% TCA, and extracted 3 times with isopropyl alcohol. The moist protein precipitate was then dissolved in formic acid and plated on preweighed aluminum planchets for counting and determination of mg of protein.

TABLE 2

Effect of washing the 105,000xg pellets and the subsequent addition of 105,000xg supernatants on the incorporation of leucine-C^{14} into protein*

105,000xg pellet from:	No. of washings	Addition of 105,000xg supernatant from:	cpm/mg protein
a. Unfertilized eggs	none	none	15.6
b. Unfertilized eggs	two	none	4.7
c. Unfertilized eggs	two	fertilized eggs	7.9
d. Unfertilized eggs	two	unfertilized eggs	3.9
e. ---	---	unfertilized eggs	4.6
f. Fertilized eggs	none	none	120.6
g. Fertilized eggs	two	none	26.4
h. Fertilized eggs	two	fertilized eggs	133.0
i. Fertilized eggs	two	unfertilized eggs	26.0
j. ---	---	fertilized eggs	5.2

*Cofactors were present in all samples. 105,000xg pellets were washed by two repeated resuspensions in homogenizing medium and repelleting at 105,000xg; the methods of incubation and washings are indicated in the legend of Table 1.

TABLE 3

Incorporation of leucine-C^{14} by fractions obtained from sucrose density gradients*

Polysome fractions from:	Addition of 105,000xg supernatant from:	cpm/mg protein
a. Unfertilized eggs	none	10.4
b. Unfertilized eggs	fertilized eggs	5.8
c. Unfertilized eggs	unfertilized eggs	4.8
d. Fertilized eggs	none	4.8
e. Fertilized eggs	fertilized eggs	153.4
f. Fertilized eggs	unfertilized eggs	29.3

*See Methods and legend of Table 1 for experimental details. Fractions isolated from the polysome region of a sucrose density gradient were pooled, and the polysome pellet obtained by centrifugation at 105,000xg for 3 hours. Cofactors present in all samples.

rial collected from the polysomal region of the sucrose density gradient was used directly for in vitro protein synthesis (15), omitting the pelleting at 105,000xg, similar results were obtained (Iverson, unpublished).

The results obtained with the in vitro protein synthesizing systems are comparable to those obtained with unfertilized or fertilized eggs. The 105,000xg pellet isolated from fertilized eggs contains, loosely associated with it, the necessary amino acid-activating enzymes and other factors required for protein synthesis. The addition of an energy generating system and of the 105,000xg supernatant from fertilized eggs is required for in vitro protein synthesis if the polysomes had been washed or obtained from a sucrose gradient (Tables 2 and 3).

The addition of the 105,000xg supernatant from unfertilized eggs to polysome preparations from fertilized eggs does not stimulate in vitro protein synthesis as much as the 105,000xg supernatant obtained from fertilized eggs. These results suggest that a factor (or factors) for translational control of protein synthesis may be present in the 105,000xg supernatant derived from unfertilized sea urchin eggs. It is also possible that an activator of protein synthesis may exist in the fertilized eggs (11). In addition to being deficient in polysomes, the un-

fertilized eggs may also have another component (s), such as tRNA, of those required for protein synthesis which are not as competent as when isolated from fertilized eggs. Hultin (10) has reported that the 105,000xg supernatants from unfertilized and fertilized eggs were equally effective in stimulating protein synthesis by the whole microsomes obtained by his technique.

The means by which translational control of protein synthesis is accomplished in the unfertilized sea urchin egg (8,10,13,14,17) is unclear at the present time, but two mechanisms appear feasible. One is by a factor(s) in the 105,000xg supernatan as suggested by the results of this paper and the other by a conformational alteration of the polysome. The latter might be achieved either by a configurational change (1) or by a coating of the polysome (ribosome) in the mature sea urchin egg as has been suggested on the basis of biochemical (11) and electron microscopy (4,19) evidence.

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