ACTIVATION OF PROTEIN BIOSYNTHESIS IN NON-NUCLEATE FRAGMENTS OF SEA URCHIN EGGS*

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Among various mechanisms that can be formulated (cf. Brachet, 1962; Tyler, 1963) to account for the initiation of active protein synthesis upon fertilization two favored possibilities are (1) the neutralization of an inhibitor of the ribosome-messenger RNA (mRNA) system assuming adequate mRNA to be present in the unfertilized egg, and (2) the production of mRNA, presumably by the nucleus. The former, with reference to the ribosomes, is supported particularly by Hultin's (1961) experiments showing that the microsomal fraction of unfertilized egg remains relatively inactive with fertilized egg supernatants whereas the reciprocal combination is active; also, that "unfertilized" microsomes could be activated by butyric acid treatment of homogenates. With reference to mRNA it is favored by experiments of Gross and Cousineau (1963) showing activation of protein synthesis upon fertilization in presence of actinomycin D.

The second possibility is supported mainly by the findings (Tyler, 1962,1963; Nemer, 1962; Nemer and Bard, 1963; Wilt and Hultin, 1962) that ribosomal preparations from unfertilized eggs can be stimulated by polyuridylic acid (poly U) to incorporate phenylalanine into protein and that the stimulation is of the same order of magnitude as obtained with preparations from fertilized eggs.

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The present experiments with artificially activated non-nucleate egg-fragments, to which reference has been made earlier (Tyler, 1962, 1963), oppose the proposition that nuclear production of messenger RNA is responsible for the increased protein synthesis upon fertilization. In an article in press, a preprint of which we recently received, Brachet, Ficq and Tencer (1963) report similar experiments, independently performed, with similar results.

MATERIALS AND METHODS. The sea urchins Lytechinus pictus and Strongylocentrotus purpuratus were used. An artificial sea water (Tyler, 1953) was used for culturing the eggs and for maintaining the animals.

Separation of the eggs into non-nucleate and nucleate fragments was done by a modification of E. B. Harvey's (1936) method. Eggs, deprived of their gelatinous coats by slight acidification (Tyler, 1949) or preliminary centrifugation, were centrifuged in the cold at 10,000 rpm for 15 minutes in a Spinco Model L centrifuge (SW 25.1 rotor) in tubes containing two layers of sucrose-sea water of graded density and a top layer of sea water. For L. pictus the lowest layer was 2 ml of a 3:1 solution of 1.1 molar sucrose and sea water, the latter containing a dense suspension of the eggs. The second layer was 6 ml of a 3:2 solution of 1.1 molar sucrose and sea water. For S. purpuratus the second layer was a 6.3:3.7 solution of 1.1 molar sucrose and sea water. Upon centrifugation the nucleated parts and the whole eggs banded just below the sea water layer and the non-nucleate parts banded at the boundary of the two sucrose solutions. The top band was generally removed first. Contamination of the non-nucleate with nucleate fragments was less than 0.2% in the present experiments.

The fragments were washed with ice cold sea water, allowed to warm to 20°C, and aliquots activated by 1/2 to 1 1/2 minute treatment with 0.004 or 0.005 M butyric acid in sea water. Incorporation of C^{14}-amino acids into protein, in vivo and in vitro, was determined by a modification

of the filter paper method of Mans and Novelli (1961) as previously described (Tyler, 1963).

EXPERIMENTS. The effect of butyric acid treatment on incorporation of amino acid into protein by homogenates prepared from non-nucleate egg-fragments was examined in three sets of experiments (Table I) and by the intact fragments in eight others (Table II). In the three homogenate experiments the increases in activity were 4.6-, 3.0- and 5.3-fold. Estimates of the percentage of eggs that are visibly parthenogenetically activated (membrane elevation) are more readily made with S. purpuratus, used in experiment 3. It approximated 50% in this experiment. In parallel tests (not listed) the untreated nucleate fragments, in this experiment gave incorporation values (19, 14 cpm per 0.075 ml) similar to the non-nucleate, as did also the butyric acid treated samples (94, 91 cpm). The latter, however, showed 100% membrane elevation. In experiment no. 1 incorporation values (not listed) for the untreated nucleate egg-fragments are much lower (3.6, 5.0 cpm) than for the non-nucleate.

Table I

Effect of treatment with butyric acid (0.005 M) on incorporation of C^{14}-valine by homogenates from non-nucleate fragments of eggs of L. pictus (Exper. 1 and 2) and S. purpuratus (Exper. 3)

Experiment	Treatment time	Counts per minute		Ratio
		Untreated (U)	Treated (T)	T/U
1	1/2 min.	16.3, 14.3	68.4, 72.8	4.6
2	1 1/2 min.	2.6, 1.4	6.6, 5.6	3.0
3	1 min.	19 , 17	98 , 92	5.3

The amounts of egg material are the same for treated and untreated samples in each experiment; in no. 1 and no. 2 values are per mg protein; in no. 3 values are per 0.075 ml of packed egg-fragments. Incubation mixture: 9 volumes homogenate:1 volume reaction mixture (0.8 ml M/8 PEP; 0.1 ml 0.0038 M C^{14}-L-valine at 4.8 curies/mole; 0.1 ml M/10 ATP).

A similar difference was noted in the experiments with the intact egg-fragments when the values were calculated on the basis of the same

amount of egg-protein. Thus for experiment no. 1 (Table II) the corresponding values for the untreated nucleate fragments are less than half (10,450; 9,745 cpm) of those for the non-nucleate. Treated nucleate fragments in this experiment also gave values less than half (26,835; 22,390 cpm) of those for the non-nucleate.

Table II

Effect of treatment with butyric acid (0.004 M, 1 min.) on incorporation of C^{14}-amino acids by intact non-nucleate fragments of eggs of L. pictus

Experiment	Counts per minute						Ratios	
	Untreated (U)		Treated (T)		Fertilized (F)		T/U	F/U
1	25,650;	25,230	72,750;	72,300			2.8	
2	1,040;	920	3,340;	2,663			3.1	
3	5,861;	5,584	8,877;	8,669			1.5	
4	1,475;	1,281	3,384;	3,017			2.3	
5	9,540;	6,700	20,753;	17,467	19,348;	18,451	2.3	2.3
6	247;	220	788;	769	665;	663	3.3	2.8
7	1,005;	833	8,885;	7,526	8,969;	8,122	8.9	9.3
8	999;	879	4,627;	4,307	7,645;	7,404	4.8	8.0

Values for exper. 1 are per mg protein; for the other experiments quantities of egg fragments were not determined but amounts were the same for treated as for untreated samples in each experiment. Incubations were for 1/4 to 2 hours at 20°C in 50 mm^3 of a 2.5 μc/ml sea water solution of C^{14}-valine per ml of egg-fragment suspension, except for exper. 5 in which C^{14}-phenylalanine was used.

The effect of fertilization on amino acid incorporation by non-nucleate fragments was also determined in four of the experiments listed in Table II. In these the values are of the same order as obtained by butyric acid treatment, except for one (no. 8) in which fertilization gave distinctly higher values. The data show, then, that artificial activation of non-nucleate fragments can result in as much increase in protein-synthesis as is obtained upon fertilization.

DISCUSSION. The ability of non-nucleate sea urchin egg-fragments to synthesize protein has been demonstrated earlier (Malkin, 1954). The present experiments, and those by Brachet et al. (1963), show that the

great increase in protein synthesis that normally occurs upon fertilization can take place in absence of a nucleus, and is thus not due to production or release of new mRNA by the nucleus. Brachet et al. (1963) point out that a change in permeability to amino acid is not excluded as a basis for their results. The present homogenate-experiments rule out this possibility.

Among the possibilities previously considered (cf. Brachet, 1962; Tyler, 1963) an "unmasking" of blocked cytoplasmic mRNA is favored by these results in addition to the "chemical enucleation" experiments of Gross and Cousineau (1963). Since there is evidence (Monroy and Tyler, 1963) that polysomes form following fertilization it would appear that the blocked mRNA is not attached to the ribosomes. One reservation that applies to these conclusions is the possibility of production of mRNA from a cytoplasmic DNA. However, there is considerable uncertainty (cf. Brachet, 1962) about the amount, nature and activity of the so-called cytoplasmic DNA of unfertilized eggs.

SUMMARY. Incorporation of amino acid into protein by non-nucleate fragments of sea urchin eggs can be stimulated by artificial activation to the same extent as by fertilization.

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