STIMULATION OF PHENYLALANINE INCORPORATION BY POLYURIDYLIC ACID IN HOMOGENATES OF SEA URCHIN EGGS

F.H. Wilt* and T. Hultin

The Wenner-Gren Institute, University of Stockholm, Sweden

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Amino acid incorporation by cell-free systems of sea urchin eggs increases abruptly after fertilization. This increase is a changing property of the ribosomes of the homogenate (Hultin, 1961). The experiments to be described demonstrate that poly U**can stimulate incorporation of phenylalanine by homogenates of sea urchin eggs, both before and after fertilization.

The experiments were performed on eggs of Psammechinus miliaris (Gmelin). Preparations of 12,000 x g supernatants, microsomes, and cell sap were made essentially as previously described (Hultin, 1961). The incorporation system contained 0.007 M $MgCl_2$, 0.2 M KCl, 0.15 - 0.25 M sucrose, 0.05 M Tris pH 7.8, 0.001 M ATP, 0.01 M PEP, 0.051 mM L-[^{14}C] phenylalanine (9.8 mC/mmole), and approximately 8 mg/ml of protein. Incubations were carried out at 20^{0}C for 1 hour, the reaction stopped by addition of trichloroacetic acid, and the proteins prepared for counting as described previously.

The 12,000 x g supernatant of unfertilized Psammechinus eggs incorporated very little phenylalanine, but 30 min after fertilization a 6-10 fold increase in incorporation activity was observed. After 2 hrs the incorporation was even more substantial. Addition of poly U

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* Present address: Dept. of Biology, Purdue University, Lafayette, Ind.

** Abbreviations: Poly A, polyadenylic acid; poly U, polyuridylic acid; PEP, phosphoenol pyruvate.

resulted in a marked rise in the rate of phenylalanine incorporation at all three stages (Fig. 1). At low poly U concentration (0.1 mg/ml) the relative stimulation was greater for homogenates from unfertilized eggs. At higher concentrations all three stages attained a roughly similar incorporation maximum.

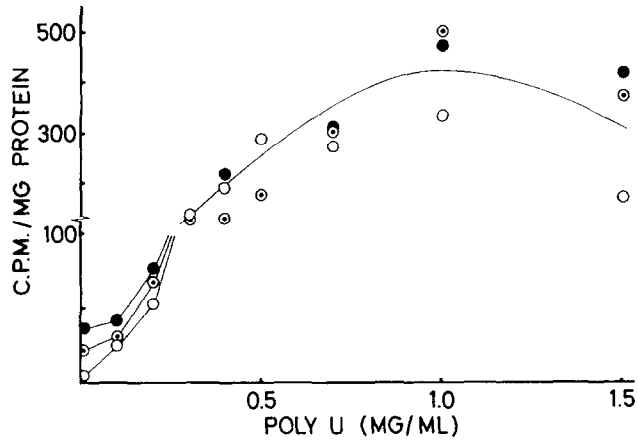

Fig. 1. Incorporation of L-[^{14}C] phenylalanine into protein by 12,000 x g supernatant fractions from Psammechinus eggs at varied concentrations of poly U. ○, unfertilized eggs; ⊙, fertilized eggs, 30 min; ● , fertilized eggs, 2 hrs.

The poly U stimulation was energy dependent (Table I), and inhibited by an excess of poly A (Miles Chem. Co., Clifton, N.J.). Leucine incorporation was not markedly stimulated, nor did other polynucleotides (poly A, E. coli S-RNA or Psammechinus RNA) markedly stimulate phenylalanine incorporation. The poly U stimulation was dependent on the presence of particles sedimentable at 105,000 x g.

The interaction between poly U and the stimulated microsomes was evidently not characterized by a tight binding. Homogenates of unfertilized or fertilized eggs were centrifuged at 12,000 x g and

TABLE I

SPECIFICITY OF THE POLY U EFFECT

Clarified homogenates from (A) unfertilized eggs, (B) eggs 20 min after fertilization.

	cpm/mg protein
A. Complete system	3
+ 0.25 mg/ml poly U	41
- PEP + 0.25 mg/ml poly U	3
- microsomes + 0.4 mg/ml poly U	1
Complete system*	4
+ 0.25 mg/ml poly U*	4
B. Complete system	28
+ 0.5 mg/ml poly U	90
+ 1.0 mg/ml poly A	32
+ 0.5 mg/ml poly U + 1.0 mg/ml poly A	37

* L-[^{14}C] phenylalanine replaced by L-[^{14}C] leucine (0.084 mM, 3 mC/mmole).

preincubated for 1-10 minutes with poly U (0.5 mg/ml) in the presence or absence of ATP and PEP. Microsomes were prepared from the preincubated suspensions by centrifugation through a density gradient, essentially as described previously (Hultin, 1961). As a result of this simple purification, the particles were deprived of most of their poly U-induced incorporation activity (Table II). The loss of activity was not prevented by increasing the Mg^{++} concentration to 0.015 M during the gradient centrifugation. When poly U was included in the incorporation system with the preincubated and purified particles, the incorporation activity was restored. The loss of activity was not due to an extensive breakdown of poly U during preincubation, since a rapid incorporation was observed when [^{14}C] phenylalanine and energy were added directly to the preincubated 12,000 x g supernatant. Despite the indication of a fairly loose association of poly U with the microsomes, the incorporated phenylalanine was not extensively detached from the microsomal fraction in the course of gradient centrifugation (Table III).

TABLE II

REVERSIBILITY OF POLY U ACTIVATION

The 12,000 x g supernatant from eggs homogenized 30 min after fertilization was either:

A. Used directly in incorporation experiments, or

B. Preincubated with poly U for 10 min. Microsomes were subsequently prepared from the preincubated system by gradient centrifugation and recombined with freshly prepared cell sap. Poly U, 0.5 mg/ml.

	cpm/mg protein
A. Complete system	38
+ Poly U	167
B. Preincubation with Poly U, PEP, ATP	
Complete system	21
+ Poly U	140
Preincubation with Poly U	
Complete system	15
+ Poly U	131
Preincubation with Poly U, centrifugation in medium with 0.015 M $MgCl_2$	
Complete system	18
+ Poly U	141

TABLE III

DISTRIBUTION OF PROTEIN-BOUND ISOTOPE BETWEEN PARTICULATE AND SOLUBLE FRACTIONS

The 12,000 x g supernatant from eggs homogenized 2 hours after fertilization was incubated under standard conditions. Microsomes were then isolated by centrifugation through a layer of heavy medium as in Table II.

A: Incubation with 0.5 mg/ml of Poly U.

B: No Poly U.

	cpm/mg protein
A. Microsomes	898
Soluble fraction, pH 5 sediment	206
Soluble fraction, pH 5 supernatant	22
B. Microsomes	66
Soluble fraction	12

The results demonstrate that there is probably no inherent inability of microsomes from unfertilized sea urchin eggs to incorporate amino acids into protein. The hypothesis that during normal development amino acid incorporation is initiated and stimulated by the presentation to the ribosomes of new RNA is under investigation. The amounts of poly U required for the stimulation of phenylalanine incorporation were considerably greater than that required in the E. coli system (Nierenberg and Matthaei, 1961). This was probably not due to RNAase activity since poly U was not very rapidly inactivated during preincubation. The data in Table II suggest that poly U had a relatively moderate affinity to the microsomes. The general relation between the effectiveness of poly U stimulation and the state of differentiation or rate of growth of a tissue deserves further exploration. Our results are compatible with the notion that details of the amino acid code in animals can be compared with results obtained on bacteria.

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