IN VITRO INCORPORATION OF AMINO ACIDS INTO PROTEINS STIMULATED BY RNA FROM UNFERTILIZED SEA URCHIN EGGS (°)

R.Maggio, M.L.Vittorelli, A.M.Rinaldi & A.Monroy
Laboratory of Comparative Anatomy,
The University of Palermo, Italy.

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Ribosomes of unfertilized eggs are unable to carry out *in vitro* incorporation of amino acids into proteins but acquire this property within a few minutes after fertilization (Hultin, 1961).However, addition of poly-U confers to the ribosomes of the unfertilized eggs the ability to synthesize polyphenylalanine (Nemer, 1962; Tyler, 1962, 1963; Wilt and Hultin, 1962). It is likely then that the inactivity of the ribosomes of the unfertilized eggs depends on unavailability of messenger RNA (mRNA). This conclusion is supported by the finding that in the unfertilized egg ribosomes exist largely as monosomes, the formation of polysomes beginning after fertilization (Monroy and Tyler, 1963). The important question is: does the cytoplasm of the unfertilized egg contain a store of mRNA to which ribosomes have no access; or does its synthesis begin only after fertilization ? Recent work has given cogent, albeit indirect, evidence in favour of the former alternative (Gross and Cousineau, 1963; Gross et al., 1963; Brachet et al., 1963; Denny and

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Tyler, 1964). The results reported here indicate the presence in the unfertilized egg of RNA which, in vitro, can act as a template for protein synthesis.

Methods.

Our test system consisted of the 30,000 x g supernatant (S-30) fraction of rat liver, prepared in the medium A of Robinson and Novelli (1962) with the addition of 5.10^{-3}M of mercaptoethanol. RNA from unfertilized eggs and developmental stages of Paracentrotus lividus was prepared and purified by a combination of the methods of Hiatt (1962) and of Barondes et al. (1962). Ribosomes of unfertilized eggs and developmental stages were prepared from homogenates in the same medium enriched with 0.25 M KCl. In experiments with sea urchin ribosomes cell sap of rat liver was used. Incubation was carried out in a system as described by Cammarano et al. (1963). The reaction was stopped by addition of 100 µM of ^{12}C amino acids and of TCA, 10 per cent final concentration. The precipitate was extracted with cold and hot TCA, alcohol-ether and finally dissolved in ammonia, dried and counted on an EKCO scintillation counter at 40 per cent efficiency. Proteins were determined by the method of Lowry et al. (1951) and RNA by the orcinol reaction and in some cases also from the O.D.

Results.

Table I shows that the total RNA prepared from unfertilized eggs of Paracentrotus stimulates incorporation of labelled amino acids into proteins in the rat liver test system. The stimulation is proportional to the amount of RNA added (Fig.1). The stimulating efficiency of RNA from unfertilized eggs was compared to that of RNA from the nuclear and ribosomal fractions of rat liver (Fig.2). While the highest stimulation was obtained with nuclear RNA (see Barondes et al., 1962), RNA from unfertilized

Table I

Stimulation of ^{14}C amino acids incorporation into protein by RNA and RNA fractions from unfertilized eggs and blastulae of Paracentrotus lividus.
Abbreviations for the ^{14}C amino acids used: V = L-valine; A = L-alanine; Ph = L-phenylalanine; L = L-lysine; A H = algal protein hydrolysate. Incubation was carried for 20 minutes except in exp.20 with the sea urchin test system which was incubated for 60 minutes. Activity is expressed in c.p.m./mg ribosomal proteins.

Exp. no.	Test system	Amino acids used	RNA added +	c.p.m.
3++	S-30 rat liver fraction	3 µC Ph+A+L	none	176
			Tot.350 µg	440
			Ribosom.362 µg	1233
			Soluble+++ 268 µg	340
20++	S-30 rat liver fraction	V - 1 µC	none	905
			Tot.1100 µg	1402
			Tot.mes.blast.990 µg	1128
20	Ribosomes unf.eggs	AH-0.5 µC	none	11
			Tot.1100 µg	124
			Tot.mes.blast.990 µg	67
20	Ribosomes mes.blast.	AH-0.5 µC	none	895
			Tot.1100 µg	1050
			Tot.mes.blast.990 µg	1030

+ When not otherwise specified, the RNA used is from unfertilized eggs.

++ In these experiments the RNA was added after a preincubation of 8 minutes.

+++ Contained 63,5 per cent of non-4S RNA as determined by sucrose density analysis.

eggs caused a considerably higher stimulation than RNA from liver ribosomes. No significant difference was found between RNA from unfertilized eggs and from mesenchyme blastulae. Fractionation experiments showed that the RNA extracted from the 105,000 g microsomal pellet was considerably more active than that from the supernatant fraction. Incorporation of phenylalanine in a system

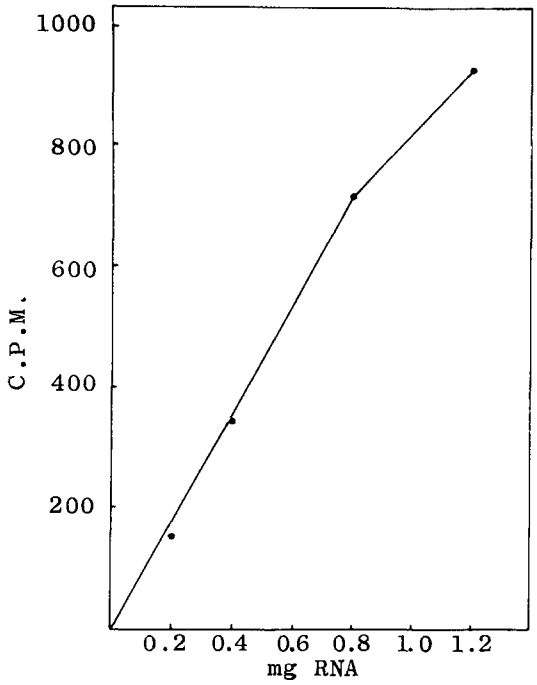

Fig.1 - Incorporation into proteins of amino acids from ^{14}C algal protein hydrolysate stimulated by various amounts of total RNA from unfertilized eggs of Paracentrotus. RNA added after 8 minutes of preincubation. Incorporation without added RNA was of 700 c.p.m.; this value was subtracted from the counts of the RNA-containing samples. Test system: S-30 fraction of rat liver.

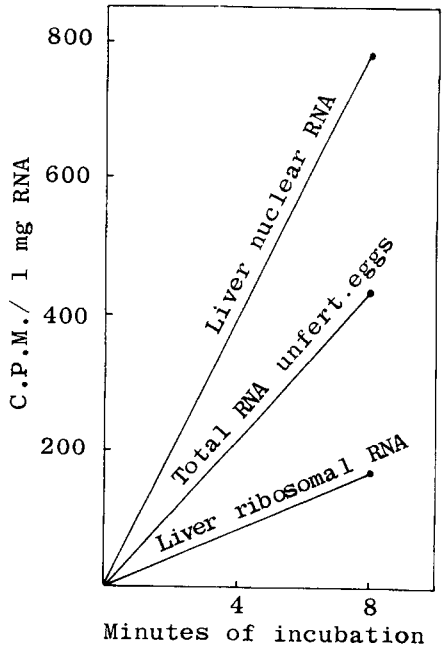

Fig.2 - Incorporation into proteins of a mixture of 0.71 μC L-alanine + 1.42 μC L-phenylalanine + 0.71 μC L-lysine + 0.14 μC L-valine stimulated by total RNA from unfertilized Paracentrotus eggs and by nuclear and ribosomal rat liver RNA. RNA added at the beginning of incubation. Test system: S-30 fraction of rat liver.

containing ribosomes and RNA from unfertilized eggs was negligible thus confirming the results of Wilt and Hultin (1962) and of Brachet et al. (1963). Control experiments with poly-U gave stimulation comparable to those obtained by Nemer (1962), Tyler (1962) and Wilt and Hultin (1962). With a ^{14}C algal protein hydrolysate a slight incorporation could be observed. On the contrary a system containing

ribosomes of blastulae not only had a high level of basal incorporation but this was further stimulated by eggs or blastula total RNA.

Discussion.

These results strongly support the view that the unfertilized egg contains a store of mRNA. Now there are two alternatives: (1) either mRNA is present in the egg in a condition that prevents its utilization by the ribosomes and which is changed by the extraction and purification procedure; or (2) the ribosomes of the unfertilized egg have some structural defect that prevents their combining with an otherwise normal mRNA. The latter alternative, originally suggested by Hultin (1961), is supported by the low level of stimulation of the ribosomes from unfertilized eggs by RNA preparations that were active with ribosomes of rat liver and of blastula. Of special interest is the fact that the most active RNA fraction from unfertilized eggs is the one extracted from the microsomal pellet. Of course, this is no evidence that the active fraction is bound to the ribosomes; this question is currently being examined. However, preliminary experiments in which RNA extracted from the pellet was fractionated on a sucrose gradient showed that the activity was largely confined to the 28 S fraction.

References.

1. Barondes, S.H., Dingman, C.W., and Sporn, M.B., Nature, 196, 145 (1962).
2. Brachet, J., Decroly, M., Ficq, A., and Quertier, J., Biochim.Biophys.Acta, 72, 660 (1963).
3. Cammarano, P., Giudice, G., and Novelli, G.D., Biochem.Biophys.Res.Comm., 12, 498 (1963).
4. Denny, P.C., and Tyler, A., Biochem.Biophys.Res.Comm., 14, 245 (1964).
5. Gross, P.R., and Cousineau, G.H., Biochem.Biophys. Res.Comm., 10, 321 (1963).
6. Gross, P.R., Spindel, W., and Cousineau, G.H., Biochem. Biophys.Res.Comm., 13, 405 (1963).
7. Hiatt, H.H., J.Mol.Biol., 5, 217 (1962).

8. Hultin, T., Bxptl.Cell Res., 25, 405 (1961).

9. Lowry, O.H., Rosenborough, N.J., Farr, L.A., and Randall, R.J., J.Biol.Chem., 193, 265 (1951).
10. Monroy, A., and Tyler, A., Arch.Biochem.Biophys., 103, 431 (1963).
11. Nemer, M., Biochem.Biophys.Res.Comm., 8, 511 (1962).
12. Robinson, C.L., and Novelli, G.D., Arch.Biochem. Biophys., 96, 459 (1962).
13. Tyler, A., Proc.Conf. Immuno Reproduction. The Popul. Council, (1962) p.13.
14. Tyler, A., Amer.Zool., 3, 109 (1963).
15. Wilt, F.H., and Hultin, T., Biochem.Biophys.Res.Comm., 9, 313 (1962).