recipient eggs, and none of these eggs developed normally. These results indicate a real difference in the "developmental capacity" of germ cell and somatic cell nuclei. Many of the germ cell nuclei are shown to be developmentally totipotent, while by the same test the somatic nuclei are shown to be severely restricted in capacity to promote development.

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*EXPERIMENTALLY INDUCED ACTIVATION OF THE RIBOSOMES OF THE UNFERTILIZED SEA URCHIN EGG**

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It has been suggested that the activation of protein synthesis which follows fertilization in the sea urchin egg depends primarily on the establishment of an interaction between ribosomes and messenger RNA (mRNA). In the unfertilized egg such an interaction is apparently prevented by some structural condition of the ribosomes that is changed upon fertilization.[1–4] The alternative possibility, the absence of mRNA in the unfertilized egg, is disproved by experiments showing (*a*) that actinomycin does not prevent protein synthesis until the blastula stage, under conditions in which RNA synthesis is severely inhibited;[5] (*b*) that enucleated egg fragments which have been activated parthenogenetically incorporate amino acids into proteins just as actively as normal fertilized eggs;[6, 7] (*c*) that RNA extracted

from unfertilized eggs can act *in vitro* as a template for protein synthesis.[2] In these last-mentioned experiments it was shown that while ribosomes from rat liver or sea urchin embryos were stimulated *in vitro* by RNA extracted from unfertilized eggs, neither this RNA nor those prepared from sea urchin embryos or from rat liver nuclei had any effect on the ribosomes of unfertilized eggs (see also refs. 8 and 9).

On the other hand, the latter were effectively stimulated by poly U.[9, 10] This result is not contradictory to the previous ones because the binding affinity of poly U is much greater than that of natural messengers.[11]

In fractionation experiments aimed at localizing the mRNA store in the unfertilized egg, the active component was found to be associated with the ribosomal pellet.[2] The results presented in this note permit an interpretation of this finding.

The purpose of these experiments was to attempt to release the inhibition of the ribosomes of the unfertilized egg, thus gaining an insight into the conditions that are responsible for it. It had been found earlier that in the sea urchin egg, fertilization is immediately followed by the transient activation of proteases,[12] but the significance of this finding had remained obscure. The results to be described show that trypsin is effective in removing *in vitro* the inhibition of the ribosomes of the unfertilized egg, and suggest that the activation of the proteases may indeed be instrumental in activating the ribosomes and hence in activating protein synthesis at fertilization.

Methods.—Materials: Eggs and embryos of *Paracentrotus lividus* have been used in these experiments. The procedure for the collection of the eggs, fertilization, and development has been described earlier.[13]

Preparation of ribosomes: The jelly coat was removed from unfertilized eggs by acid (pH 5) sea water.[13] Eggs and embryos were washed repeatedly in filtered sea water, and the embryos once in Ca^{++} and Mg^{++} free sea water, and then gently homogenized in 4 vol of medium A of Robinson and Novelli[14] with the addition of 5×10^{-3} *M* mercaptoethanol. The homogenate was centrifuged in the cold at 10,000 *g* for 10 min. The ribosomal pellet was obtained from the supernatant centrifuged at 105,000 *g* for 60 min. The pellet from unfertilized eggs was colorless and translucent and had an RNA-protein ratio of 0.60–0.74. The pellet from blastulae or gastrulae was always heavily colored and the RNA-protein ratio was 0.40–0.60. These ratios were not significantly changed when the pellets were prepared from postmitochondrial supernatants treated with DOC (0.7%), thus suggesting that the membranes of the endoplasmic reticulum were not responsible for the low values of the RNA-protein ratios. Purification of the ribosomes could be obtained if the 105,000 *g* pellets, with or without previous treatment with DOC, were run through a sucrose density gradient; the material of the ribosomal (monosomal) peak then had an RNA-protein ratio of 1.0–1.4. An opaque sediment was always present at the bottom of the tubes; its RNA-protein ratio was 0.1–0.2. The nature of this material is not known. In the present experiments nonpurified ribosomal pellets were used. For the assay of amino acid incorporation, the pellet was resuspended in 0.05 *M* Tris buffer pH 7.6 containing 2.5×10^{-2} *M* KCl and 4×10^{-3} Mg acetate. The conditions of incubation are given in the table legends.

Trypsin treatment: The ribosomes to be treated with trypsin were suspended in 1 ml of the buffer described containing 100–200 μg of crystalline trypsin and incubated for 30 min at 30°C. The incubation mixture was then layered on top of 0.15 *M* sucrose (made up in the buffer) in the 12-ml tubes of the 40 rotor of the model L Spinco ultracentrifuge and centrifuged at 105,000 *g* for 60 min. The same procedure was used for the treatment of ribosomes with chymotrypsin and ribonuclease.

Preincubation: Preincubation of ribosomes of unfertilized eggs was carried out by resuspending them in the supernatant of the 105,000 *g* centrifugation (postribosomal supernatant), fortified by the ingredients of the incubation mixture for the assay of amino acid incorporation, with the omission of radioactive amino acids, poly U, and RNA. In the case of ribosomes of embryos,

when preincubation was to precede trypsin treatment, the postmitochondrial supernatant was used, fortified as described. Following preincubation, ribosomes were collected by centrifugation at 105,000 *g* for 60 min.

RNA and rat liver cell sap: RNA was extracted and purified from eggs and embryos as previously described.[2] For the preparation of the cell sap, rat livers were handled as described.[15] Homogenates were prepared in medium A and centrifuged at 15,000 *g* for 15 min: the supernatant was then centrifuged at 105,000 *g* for 120 min. The upper third of the final supernatant was discarded and the remaining clear layer was collected and stored at −20°C in 4.0-ml portions and used when required.

Chemicals: ATP and GTP were from Sigma; PEP and PEP-kinase from Boehringer; crystalline ribonuclease (RNase), crystalline trypsin from Worthington; crystalline chymotrypsin from Armour; poly U from Miles Chemical Company; C^{14}-phenylalanine and C^{14} algal protein hydrolysate from the Radiochemical Centre, Amersham, England.

Analytical and counting procedure: Proteins were estimated by the method of Lowry *et al.*,[16] RNA by the orcinol reaction. Samples for counting were dried on the bottom of the vials to which 2 ml of 0.3% PPO and 0.03% POPOP mixture in toluene were added and counted in an EKCO scintillation counter.

Results.—The data presented in Table 1 show that following treatment with trypsin, ribosomes of unfertilized eggs (*a*) can be stimulated by RNA extracted

TABLE 1

EFFECT OF TREATMENT WITH TRYPSIN ON THE ABILITY OF RIBOSOMES FROM UNFERTILIZED EGGS TO INCORPORATE AMINO ACIDS INTO PROTEINS

Expt. no.	Amino acid(s)	Addition	Cpm/mg Ribosomal RNA Control	Trypsin-treated
1	Phenylalanine	None	0	122
	"	Poly U	270	340
2	Phenylalanine	None	0	72
	"	Poly U	360	2200
	AH	None	25	260
	"	RNA blastula	0	475
3	AA	None	18	448
	"	RNA unfert.	9	644

Incubation procedure: The incubation mixture contained in 1.0 ml 25 μM Tris buffer pH 7.6; 4 μM Mg acetate; 50 μM KCl; 20 μM mercaptoethanol; 4 μM ATP; 0.5 μM GTP; 10 μM PEP; 10 μg PEP-kinase; about 4 mg (estimated as proteins) of rat liver cell sap. Additions: poly U, 1 mg; total RNA from blastula (RNA blastula), 1.3 mg; total RNA from unfertilized eggs (RNA unfert.), 1.23 mg. Radioactive amino acids: C^{14}-phenylalanine (7.8 μC/μM), 1 μC; C^{14} algal protein hydrolysate (AH), 1 μC; 1 μC total of a mixture of C^{14}-phenylalanine, lysine, valine, glycine, and glutamic acid (AA). In expt. 1, 0.193 mg, in expt. 2, 0.483 mg, and in expt. 3, 0.327 mg of ribosomes were used. After 30 min at 30°C, the reaction was terminated by addition of 100 μM of phenylalanine or amino acid mixture and 0.4 ml of 50% TCA. The precipitate was extracted with cold and hot (90°C) 5% TCA, hot alcohol-ether, and ether; it was then dissolved in ammonia, dried in the vials, and counted. Trypsin treatment was with 100 μg/ml for 30 min. A sample of the ribosomal preparation was precipitated with 5% TCA and analyzed for RNA content, and this was used as a reference.

either from sea urchin embryos or from unfertilized eggs; (*b*) show a markedly increased response to poly U; (*c*) become capable of carrying out *in vitro* incorporation of amino acids into proteins in the absence of any exogenous RNA or poly U. Results indicated in (*a*) and (*b*) show that trypsin has exposed previously unavailable sites for attachment of RNA and of poly U.

Chymotrypsin, acting under the same experimental conditions, fails to cause any stimulation. Since trypsin is often contaminated with RNase, some experiments were also carried out in which ribosomes were treated with RNase at the concentration of 0.25 μg/ml followed by washing as in the trypsin experiments. This treatment abolished the ability of ribosomes to be stimulated by poly U.

The most interesting result of these experiments is that trypsin-treated ribosomes have acquired the ability to carry out incorporation in the absence of exogenous

RNA or poly U. This, together with the previously reported observation that in preparations of unfertilized eggs the RNA fraction most active as a template is the one extracted from the ribosomal pellet,[2] suggests the presence of ribosomes carrying an attached mRNA chain, the complex, however, being rendered inactive by a protein coat.

The experiments show further that the activity of the trypsin-treated ribosomes is strongly increased by poly U.

We have found that if trypsin treatment is followed by preincubation of ribosomes in their own postribosomal supernatant, the endogenous incorporating activity is considerably increased. The endogenous activity now almost equals that obtained in the presence of poly U; indeed, addition of poly U has very little, if any, effect. Hence, as a result of preincubation, the ribosomes have become fully active and unable to accept any further poly U (Table 2).

It should be noted that for preincubation to be effective it must be applied *after* trypsin treatment (Table 2). This suggests the complete removal (proteolytic or otherwise) of the inhibitory protein coat made possible only after the preliminary action of trypsin.

Similar experiments were carried out on ribosomes prepared from embryos. Such

TABLE 2

EFFECT OF TRYPSIN TREATMENT OF RIBOSOMES OF UNFERTILIZED EGGS FOLLOWED BY PREINCUBATION IN THEIR OWN POSTRIBOSOMAL SUPERNATANT ON THE ENDOGENOUS AND POLY U-STIMULATED INCORPORATION OF C^{14}- PHENYLALANINE INTO PROTEINS

Trypsin treatment	Pre-incubation	Expt. 1		Expt. 2	
		No poly U	Poly U	No poly U	Poly U
−	−	...	...	0	240
−	+	...	...	0	217
+	−	149	520	...	...
+	+	645	585	451	485

Incubation procedure as indicated in Table 1. Trypsin treatment was with 200 μg/ml for 30 min at 30°C. Preincubation was carried out for 30 min. Expt. 1 contained 0.444 mg, and expt. 2, 0.394 mg of ribosomes. Activities are expressed in cpm/mg ribosomal RNA.

ribosomes show very little response to poly U. If preincubated for $^1/_2$ hr, they lose most of their endogenous incorporating activity (stripped ribosomes) and become susceptible to stimulation by either poly U or sea urchin RNA (Table 3). Trypsin treatment of unstripped and stripped ribosomes decreases their response to poly U (Table 3). Experiments are in progress to elucidate further the differences between ribosomes of unfertilized and fertilized eggs.

It is conceivable that in the course of later embryogenesis, ribosomes or ribosomal aggregates with their attached mRNA may be kept in a nonfunctional condition by

TABLE 3

EFFECT OF PREINCUBATION AND TRYPSIN TREATMENT ON THE INCORPORATION OF C^{14}-PHENYLALANINE INTO PROTEINS BY RIBOSOMES OF BLASTULAE AND ON THEIR RESPONSE TO POLY U

Trypsin treatment	Pre-incubation	Expt. 1		Expt. 2	
		No poly U	Poly U	No poly U	Poly U
−	−	600	680	228	396
−	+	51	285	42	423
+	−	160	201	69	334
+	+	181	164	133	78

Incubation procedure, trypsin treatment, and preincubation as in Tables 1 and 2. Expt. 1 contained 1.24, and expt. 2, 1.16 mg of ribosomes in controls and preincubated samples and 0.47 and 0.40 mg of ribosomes, respectively, in trypsin-treated samples.

a protein envelope, activation resulting from the removal of the envelope at the required time through the operation of proteases.

That this may be so is borne out by the observation[17] that the nonfunctioning ribosomal aggregates in the skin of the chick embryo become functional at the time of keratinization. It would be interesting to find out whether or not the nonfunctioning ribosomal aggregates can be activated by proteases.

Conclusion.—Our interpretation of the results presented in this note is that the mRNA that is synthesized during oögenesis becomes attached to ribosomes. The mRNA-ribosome complex is, however, made inactive by a protein coat, the activity being released by the removal of the coat. We further suggest that the protease(s) that are activated upon fertilization may be instrumental in this process.

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FORMATION OF HUMAN LACTATE DEHYDROGENASE ISOZYME PATTERNS IN VITRO

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Biochemical,[1] immunologic,[2] and genetic[3] evidence, as well as recombination experiments,[4] indicates that each of the five commonly encountered lactate dehydrogenase (LDH) isozymes is a tetramer composed of varying proportions of A and B subunits: LDH 5 = AAAA, LDH 4 = AAAB, LDH 3 = AABB, LDH 2 = ABBB,