DECOUPLING OF PROTEIN AND RNA SYNTHESIS DURING DEUTERIUM PARTHENOGENESIS IN SEA URCHIN EGGS*

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The synthesis of protein in eggs is very strongly enhanced at the time of fertilization. There is, indeed, some doubt as to whether mature, unfertilized eggs of the sea urchin synthesize protein at all, since slight contamination by oocytes and follicle cells could account for the small measured incorporation of amino acids. The increase in rate shortly after fertilization is six to twenty-fold in Arbacia punctulata, and most of the label incorporated during early cleavages is found in the mitotic apparatus and in nuclei (Gross and Cousineau, 1963a). RNA synthesis in the unfertilized egg is likewise marginal, but here the increase in rate at fertilization is small (Nemer, 1963). Polyribonucleotide made for some hours after fertilization is probably entirely messenger and transfer RNA (Nemer, 1963, Wilt, 1963).

The usual interpretation is that activation of protein synthesis after fertilization reflects the synthesis of new messengers, but some lines of evidence in addition to the incorporation rates suggest an alternative. Experiments on enucleate eggs and homogenates (Tyler, 1963), and on eggs treated with Actinomycin D (Gross and Cousineau, 1963b,c) may indicate the

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presence in unfertilized eggs of preformed, inactive messenger RNAs, which become competent to function as templates only after fertilization.

One strategy by which these alternatives can be examined is to attempt an effective decoupling of protein and RNA synthesis. Experiments of this type with Actinomycin will be reported elsewhere. We describe here another technique, which uses two of the known effects of heavy water on Arbacia eggs, i.e., parthenogenetic activation of cell division and suppression of nucleic acid synthesis.

Deuterium Parthenogenesis

D_2O is an unusually effective antimitotic agent. In the fertilized egg, mitosis is stopped immediately and at any stage by making the medium 75+% D_2O (Gross and Spindel, 1960, Marsland and Zimmerman, 1963). This effect is reversible, as is the blockade of DNA synthesis (Gross and Harding, 1961) and the inhibition of RNA turnover (Cousineau, 1963). It is therefore surprising that D_2O can be employed as a parthenogenetic agent. When unfertilized eggs are immersed in 85% D_2O, the cytoplasm becomes packed, after an hour, with cytasters. When the eggs are washed free of deuterium, they become activated, i.e., "fertilization" membranes are raised, and the cells divide. The cleavages begin in half the normal first cleavage time, and continue for about eight hours. The furrows segment the eggs randomly,but the mitoses are mostly normal. These "embryos" eventually disintegrate without visible differentiation.

Autoradiographic experiments on fertilized eggs show that deuterium-cytasters are very heavily labeled when formed in the presence of a labeled amino acid in the medium (Cousineau, 1963). Also, there is evidence that a certain amount of new protein has to be made in order for division to take place (Hultin, 1961). These facts suggested that the formation of cytasters in the unfertilized egg, during a parthenogenetic pretreatment in heavy water, might represent appreciable synthesis of new protein.

Protein Synthesis During D_2O Treatment

Several pulse and continuous incorporation experiments were done with C^{14}-amino acids. In all, the unfertilized eggs incorporated exogenous precursor into protein during D_2O storage. The rate reached a peak just before the full development of cytasters, i.e., in about an hour at 21.5°C. Incorporation was two to five times as rapid as in unfertilized control eggs stored in normal sea water. To the extent that the control incorporation represents the activity of residual oocytes and other contaminant cells (one per 10^3 eggs), the deuterated cells may be described as having had their protein-synthetic machinery switched on.

When the deuterium is removed by washing, the rate of protein synthesis is further increased, exceeding, for a time, that in the normally fertilized controls. Parthenogenetic eggs have made, after two hours in D_2O and 30 minutes in normal sea water, as much new protein as is made by fertilized control eggs in their first full hour of development. It is significant that the parthenogenetic eggs show multiple and irregular cleavages 30-40 minutes after removal of the D_2O, whereas the controls take about an hour to reach 50% first cleavage.

Results of one experiment are plotted semilogarithmically in figure 1. This form of presentation permits compression of the counting data within a reasonable scale, and emphasizes the changes taking place in the first two hours. Experimental details are given in the legend. In this, as in the other experiments, the high rate of protein synthesis evoked by D_2O treatment is maintained for many hours, a favorable feature not observed in other methods for the production of artificial parthenogenesis.

Synthesis of RNA

Incorporation of labeled precursors into RNA of unfertilized eggs is very slight. Autoradiograms of sectioned unfertilized eggs exposed to tritiated precursors show no intracellular activity, although fertilized eggs, with only slightly more bulk counts per sample, do show some.

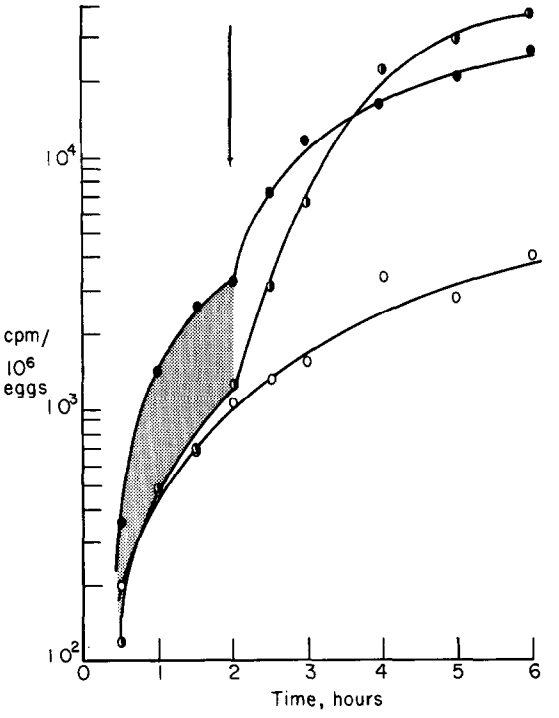

Figure 1. A suspension of Arbacia eggs was divided into three parts. The eggs were centrifuged and resuspended as follows: "D" in 85% D_2O sea water, "HU" and "HF" in normal sea water. Cell density was 2 X 10^4/ml. Each suspension contained in the medium 0.5 µc/ml. C^{14}-L-valine, at a concentration of 10 µg/ml. Samples were removed at intervals and pipetted into cold TCA (final conc., 5%) containing 0.5 mg/ml. unlabeled valine. At two hours after t=0, the suspensions were centrifuged and all cells resuspended in normal sea water containing the same amount of C^{14}-valine as initially. Sampling was continued for another 4 hours. The samples were washed in cold TCA, extracted with boiling TCA, washed again with cold TCA and water, dissolved in 1N NaOH, reprecipitated with acid, collected and washed on Millipore filters. These were dried and mounted on planchets, then counted to at least 4000 counts in a gas-flow counter. At two hours, just after resuspension, the "HF" eggs were fertilized. O——O, unfertilized control; "HU". ●——●, control fertilized at 2 hours; "HF". ●——●, eggs in deuterium for first two hours, in H_2O sea water thereafter; "D". Shaded area is the zone of decoupling between protein and RNA synthesis.

In order to study RNA synthesis during the deuterium activation of protein synthesis, experiments like the one just described were performed, but with C^{14}-uridine as precursor. Egg suspensions (experimentals in D_2O, controls in normal sea water) were sampled at intervals and the incorporation stopped with TCA containing excess unlabeled uridine and cytidine. Counts in the washed cells, dried on Millipore filters, were recorded. The filters

(which had been tacked lightly to planchets) were then incubated in individual dishes with ribonuclease in Tris-EDTA buffer at pH 7.2. Papers were mounted on a chimney-filter and the contents of the dishes washed through. After more TCA and water washes, the papers were counted once again. The radioactivity in RNA was obtained by difference. Separate experiments showed that 90% of the RNA'ase-insensitive activity is removable by DNA'ase. The results are summarized in Table I.

Table I

Minutes after immersion in D- or H- sea water	Net cpm in RNA for 2 X 10^4 eggs (unfertilized)	
	H_2O	D_2O
35	5	7
60	10	6
120	32	6

Suspensions contained 2 X 10^4 eggs/ml. At t=0, uridine-2-C^{14} was added to a final concentration of 3.3 µg/ml., 0.4 µc/ml. Treatment of samples described in the text. Counts listed are corrected for background (3 cpm) and averaged from several runs to 100 total counts. Efficiency was about 10% for this method.

Incorporation into the RNA of control eggs was slightly higher than normally observed under these conditions: a clean suspension of fertilized eggs may incorporate 2 - 3 times as much label in one hour. Since these experiments were done at the end of the season, the animals may already have shed gametes, making the oocyte contamination larger than usual. But regardless of the amount of control incorporation, these cells did not make protein at more than the baseline rate for unfertilized eggs, while D_2O-treated eggs, which did, incorporated less uridine into RNA than the controls. With such low counts, there is no point in quantitative argument. For the purpose of these experiments, it is sufficient to observe that eggs whose protein synthesis had been activated, and which were making labeled cytasters, showed no increment in RNA synthesis over controls.

Discussion

The simplest interpretation is that activation of protein synthesis can occur in this system without concomitant messenger RNA synthesis. On one general hypothesis, the post-fertilization burst of protein synthesis depends upon the synthesis of new non-4S RNA. If this were so, and if templating by mRNA is obligatory, then the templates for the proteins made during storage in D_2O must already be present in the unfertilized egg. The alternative, an unknown activating effect of D_2O on some undetected RNA, newly synthesized, is made unlikely by the absence of significant isotope effects on the physical properties of purified nucleic acids (Crespi and Katz, 1962, Mahler, et al., 1963). There is, however, one caution to be observed: it is never certain that a failure of incorporation means a failure of synthesis. This is not to imply that in the experiments described uridine did not enter the deuterated eggs. It enters at the same rate as in controls. The implication is rather that the rate of synthesis of RNA might well be high in the unfertilized egg (and in D_2O), and still be non-demonstrable because of a closed pool of immediate precursors to RNA. The fact that there is some labeling of RNA, especially after fertilization, and that this labeling can be reduced, with Actinomycin, for example (Gross and Cousineau, 1963b), mitigates this argument to some degree.

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