THE SYNTHESIS OF RIBONUCLEIC ACID IN SEA URCHIN EMBRYOS

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The sea urchin egg rapidly acquires the ability to synthesize protein after fertilization. This activation is due to a changing property of the ribosomes (1). Previous work has shown unfertilized egg ribosomes are capable of directed protein synthesis in the presence of polyuridylic acid (2, 3). The purpose of this report is to present evidence suggesting the hypothesis that the activation of ribosomes is a consequence of the activation of messenger RNA synthesis. Experiments will be described showing that the sedimentation properties of ribosomes from fertilized and unfertilized eggs are virtually identical. RNA synthesis is activated following fertilization, and a portion of this RNA is found in the ribosome fraction. The bulk of the newly synthesized RNA does not possess sedimentation properties of ribosomal RNA.

METHODS

Eggs of Strongylocentrosus purpuratus were obtained, washed, fertilized, and stripped of fertilization membranes by standard procedures (4). Ribosomes and mitochondrial free supernatants containing ribosomes were prepared by the method of Hultin (1). RNA was prepared by the phenol method (5). It was essential to

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carry out the initial extraction with hot phenol and sodium dodecyl sulfate, and to carry out the preparation with alacrity until the first alcohol precipitation. C-14 uridine (28mC/mM) was counted on a low background Geiger counter after addition of carrier RNA and trichloroacetic acid precipitation. H-3 uridine (2.77C/mM) was counted by liquid scintillation counting in Bray's solution (6) with an efficiency of 8%. All counting was carried out with a maximum error of 3%. Quenching was monitored with internal standards. Sucrose density gradient sedimentation of RNA was carried out according to Hiatt (7).

RESULTS

The sedimentation properties of ribosomes from unfertilized and fertilized eggs are virtually identical. Ribosomes from both classes of eggs showed a similar magnesium dependence, forming two smaller subunits in 10^{-4}M magnesium. Figure 1 presents results obtained from sedimenting a mixture of radioactive ribosomes from fertilized eggs and non-radioactive ribosomes from unfertilized eggs. The peaks of radioactivity and optical density sediment identically. There is a suggestion of heavy labelled ribosomes at the leading edge which may correspond to the polysome fraction found in reticulocytes (8). The appearance of this small heavy fraction is reproducible, and it is more evident in nuclear ribosomal preparations. If the mitochondrial free supernatants are not treated with desoxycholate, a small radioactive microsomal pellet is found at the bottom of the centrifuge tube.

If unfertilized eggs are exposed to large quantities of radioactive uridine for long periods of time, no incorporation into RNA can be detected. In contrast, fertilized eggs demonstrate a low level of incorporation; the rate of incorporation

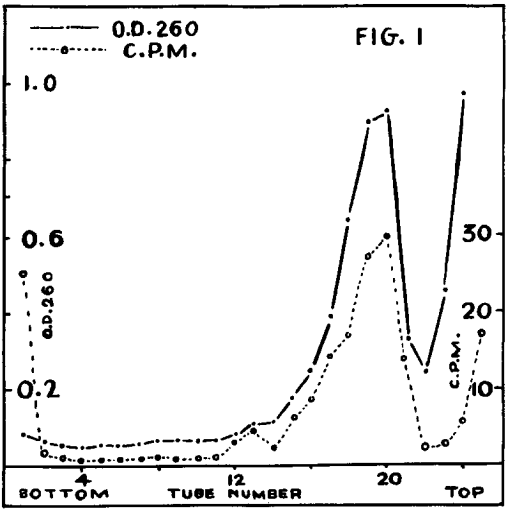

Figure 1. 0.6 ml. of packed cells were suspended in 10 ml. of sea water 100 minutes after fertilization and exposed to 16 μC of C-14 algal hydrolysate (800μC/mg.) for 45 seconds. Mitochondrial free supernatant (0.2 ml.) from these cells was mixed with 0.8 cc. of an identical preparation from unfertilized cells and centrifuged for two hours through a 15-30% linear sucrose gradient (made in 0.007M magnesium acetate, 0.04M Tris, pH 7.6) at 24,500 R.P.M.

increases during early cleavage. The sedimentation properties of the newly synthesized RNA were examined in a sucrose density gradient. The RNA synthesized during cleavage after a short exposure to uridine is heterogeneous; regions of radioactivity do not correspond to ribosomal RNA. There is also a large amount of radioactive small molecular weight RNA associated with the "soluble" RNA component (Figure 2a). This light fraction contains small amounts of radioactive DNA. The other regions of radioactivity are completely acid and alcohol precipitable, ribonuclease labile, and desoxyribonuclease insensitive. Continued development of eggs after exposure to isotope leads to increased labelling in all regions of the sedimentation diagram, but no definite peak of radioactivity is found in the region of heavy ribosomal RNA (Figure 2b). Exposure of late blastula and early gastrula to radioactive uridine leads to a labelling of ribosomal

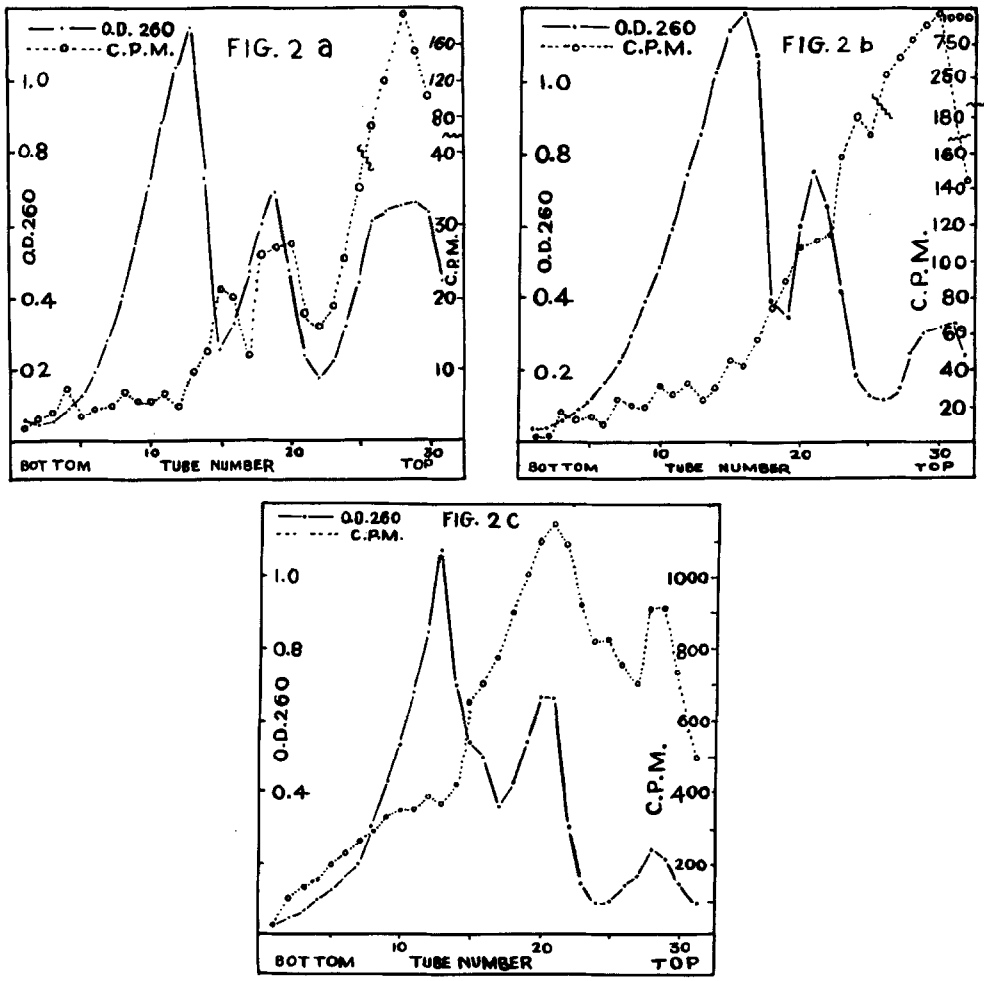

Figure 2. Sucrose density gradient analysis of labelled RNA from fertilized sea urchin eggs. Preparations were derived from:
a) 1 ml. of packed cells in 10 ml. of sea water 35 minutes after fertilization exposed to 20 μC of C-14 uridine for 40 minutes.
b) 1 ml. of packed cells in 10 ml. of sea water 45 minutes after fertilization exposed to 100 μC of H-3 uridine for 30 minutes followed by washing and development for 3 hours.
c) 1 ml. of packed cells in 10 ml. of sea water 12 hours after fertilization exposed to 50 μC of H-3 uridine for 5 hours.

RNA (Figure 2c). The synthesis of these classes of RNA during cleavage may represent synthesis of slowly accumulating ribosomal RNA precursors, or messenger RNA, or both. Because of the large internal acid soluble pool, kinetic studies are not decisive in resolving this issue. Labelled RNA extracted from purified

nuclear ribosomes shows a sedimentation heterogeneity similar to that observed in Figure 2b, except the "soluble" RNA peak is missing. If the labelled RNA is a ribosomal precursor, it sediments in association with fully formed ribosomes. These experiments and other supporting evidence will be published subsequently, in extenso.

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