*DEMONSTRATION OF POLYRIBOSOMES AFTER FERTILIZATION OF THE SEA URCHIN EGG**

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Fertilization of sea urchin eggs initiates an increased amino acid incorporation into proteins.[1, 2] This stimulation of protein synthesis has been attributed to activation of ribosomes.[3] Nemer[4, 5] and Wilt and Hultin[6] found that the synthetic messenger RNA polyuridylic acid stimulates homogenates from unfertilized eggs to synthesize polyphenylalanine. After fertilization, messenger RNA may combine with ribosomes to form polyribosomes.[5, 7] Monroy and Tyler[7] recently reported the presence of polyribosomes in fertilized sea urchin eggs.

This paper reports the presence of active polyribosomes, some of which are bound to membranes, in fertilized sea urchin eggs prior to the first mitotic division. The relative proportion of membrane-bound polyribosomes, suggested to be the major site of protein synthesis,[8] and of free polyribosomes is also presented.

Materials and Methods.—Eggs of *Lytechinus variegatus* were washed three times and resuspended in sterile sea water at 22–24°C and fertilized. Thirty minutes after fertilization either a carbon-14-labeled hydrolyzate of algal proteins, or a mixture of nine uniformly labeled amino acids (L-leucine, L-isoleucine, L-valine, L-glutamic acid, L-phenylalanine, L-serine, L-tryptophane, L-aspartic acid, and L-alanine) was added to equal samples of fertilized eggs and unfertilized eggs. Five minutes later the cells were washed three times in a cold isotonic NaCl:KCl solution,[9] washed and resuspended in "homogenizing medium," and then gently homogenized by three strokes of a Teflon pestle in a Duall tissue homogenizer. The homogenizing medium contained 0.01 *M* $MgCl_2$, 0.24 *M* KCl, and 0.01 *M* Tris-HCl at pH 7.6, and contained 1 mg/ml of washed bentonite, unless the supernatant was to be treated with ribonuclease. After centrifugation at 10,000 × *g* at 5°C, the supernatant was collected, treated at 4°C for 20 min with 5 μg/ml of ribonuclease[10] or 0.3 per cent deoxycholate[11] (depending upon the experiment), and layered on sucrose gradients.

One ml of the 10,000 × *g* supernatant was layered on top of each sucrose gradient made up with a bentonite-treated homogenizing medium. The sucrose gradients were spun for 2 hr at 25,000 rpm on the SW 25 rotor of the Spinco Model L centrifuge at 0–4°C. Fractions were collected for optical density reading (260 mμ), and were then precipitated with 5 per cent trichloroacetic acid in the presence of added carrier albumin (250 μg/ml). Each precipitate was collected on a Whatman GF/C filter, and was washed three times with 5 per cent trichloroacetic acid and three times with ethanol. The filters were dried, and the radioactivity was determined in a Nuclear-Chicago gas flow counter.

Results.—Early experiments[12] using a linear 15–30 per cent sucrose gradient showed that protein synthesis occurs on polyribosomes in fertilized eggs. However, ribonuclease treatment of the supernatant from fertilized eggs shifted more radioactivity to the ribosome fractions than could be accounted for by loss from the

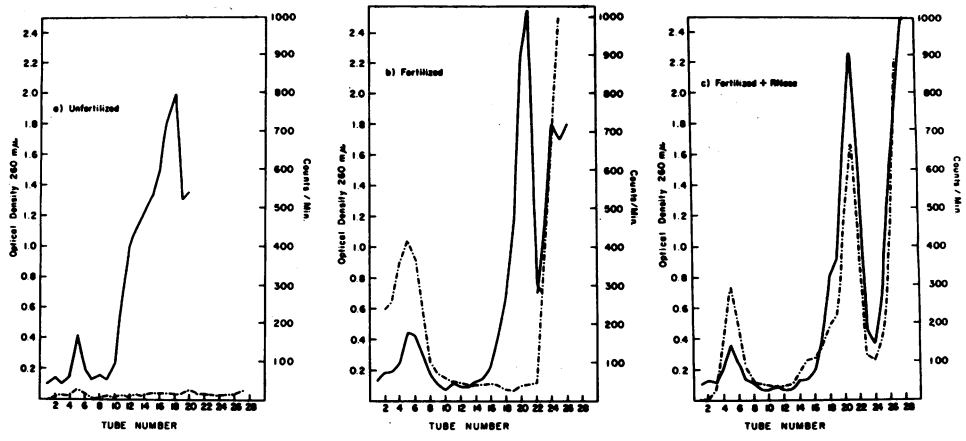

FIG. 1.—Demonstration of heavy polyribosomes. Five ml of 50% sucrose was placed beneath the linear 15–30% sucrose gradients upon which the supernatants were layered. The bottom fractions from the sucrose gradient tubes are indicated at the left of all figures. Optical density at 260 mμ is indicated by a solid line (——) and counts/min by a dashed line (– · – · –). Twenty-eight fractions were collected. Preparation of experimental material is described in the text.

polyribosome region. This suggested that the additional radioactivity recovered in the ribosome region after ribonuclease treatment might be due to very large polyribosomes in the nonribonuclease-treated sample which sedimented to the bottom of the sucrose gradient tube, and were not collected in the early fractions. To recover these, a 5-ml cushion of 50 per cent sucrose was placed beneath the linear 15–30 per cent sucrose gradient.[8]

There was considerable optical density at the 30 and 50 per cent sucrose interface (tubes 4–6) of all three samples shown in Figure 1. The extract from unfertilized eggs (Fig. 1*a*) had little radioactivity at the interface, or elsewhere along the gradient. The optical density peak at the interface (Fig. 1*a*) was removed by deoxycholate treatment without resulting in a demonstrable polyribosome region (unpublished experiments). Fertilized eggs (Fig. 1*b*) have significant radioactivity at the interface, most of which was detected in the ribosome region after ribonuclease treatment (Fig. 1*c*). The incomplete removal of the radioactivity by ribonuclease (Fig. 1*c*) suggests that, in addition to free polyribosomes (tubes 1–10), polyribosomes bound to membranes may also occur at the interface region.

As an aid in estimating the relative proportions of free and membrane-bound polyribosomes, supernatants from fertilized eggs were treated with ribonuclease, and ribonuclease plus deoxycholate. It has been reported[18] that ribonuclease degrades membrane-bound polyribosomes without causing their release from membranes. One would, therefore, expect the degraded membrane-bound polyribosomes in a sample treated with ribonuclease to be released from membranes by subsequent deoxycholate treatment.

Figure 2*a* (tubes 1–6) shows a significant amount of radioactivity in the 60 per cent sucrose cushion and at the 30 and 60 per cent sucrose interface. Treatment of the supernatant with ribonuclease (Fig. 2*b*) moves most of the radioactivity to the ribosome region (tubes 19–26). Further treatment of the ribonuclease-treated sample with 0.3 per cent deoxycholate (Fig. 2*c*) eliminated essentially all of the

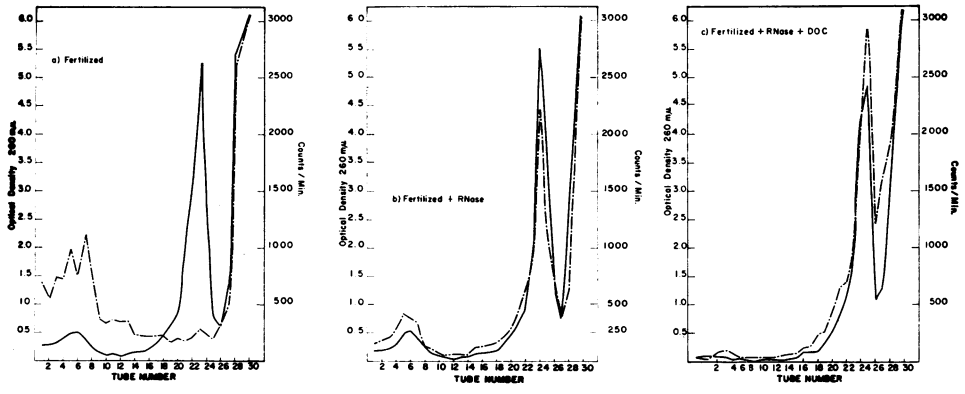

FIG. 2.—Sequential effect of ribonuclease and deoxycholate upon polyribosomes. Five ml of 60% sucrose was placed beneath the linear 15–30% sucrose gradient upon which the supernatants were layered. After treatment with ribonuclease, one half of the sample was layered on the sucrose gradient (Fig. 3*b*), and the other half was treated for 1 min with 0.3% deoxycholate prior to layering on the gradient (Fig. 3*c*). Thirty-two fractions were collected. Optical density is indicated by a solid line (——) and counts/min by a dashed line (– · – ·).

radioactivity and optical density from the 60 per cent sucrose cushion and at the interface.

In an attempt to distinguish free polyribosomes from those bound to membranes, we used a 20–40 per cent sucrose gradient with a 1-ml cushion of 60 per cent sucrose at the bottom of the tube. In the sample from fertilized eggs (Fig. 3*b*) both the polyribosome region (about tubes 8–12) and the interface between the 40 and 60 per cent sucrose solutions (tubes 1 and 2) had considerable radioactivity. Treatment with ribonuclease (Fig. 3*c*) removed most of the radioactivity in the polyribosome fraction, and less at the interface. Figure 3*a* shows that 0.3 per cent deoxycholate significantly reduced the radioactivity at the 40 and 60 per cent (tubes 1 and 2) sucrose interface and accentuated the polyribosome region (tubes 8–12). In analogy to the reticulocyte system,[10] the distribution of polyribosomes, indicated in Figures 3*a* and *b*, may indicate that few protein types are being synthesized during this phase of development in the sea urchin egg.

Discussion.—Sea urchin eggs undergo an increase in detectable polyribosomes and protein synthesis after fertilization. The messenger RNA and ribosomes required for the formation of polyribosomes are probably present in the mature, unfertilized egg because protein synthesis occurs in parthenogenetically activated enucleate egg fragments,[14, 15] and incorporation of RNA precursors into RNA is insignificant until the blastula, or later developmental stages.[16–20] Though the messenger RNA and ribosomes are present prior to fertilization, we have not detected polyribosomes until after fertilization.

The studies reported in this paper show that a small proportion (about 10%) of the active polyribosomes are bound to membranes in the fertilized sea urchin egg. Hultin[3] has suggested that membranes are not required for *in vitro* protein synthesis by supernatants prepared from sea urchin eggs. However, the significance of membrane-bound polyribosomes may be that they perform a different role in the cell, or synthesize proteins at a different rate[13] and of a qualitatively different nature than the free polyribosomes.

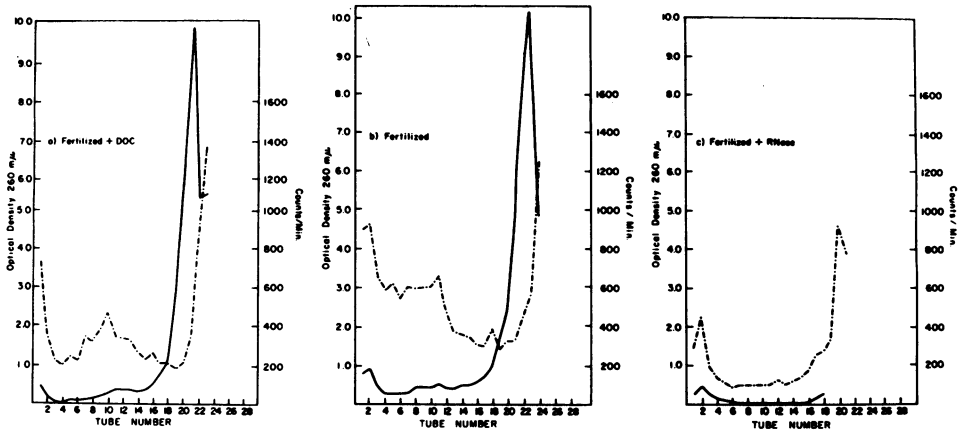

FIG. 3.—Release of polyribosomes by deoxycholate. One ml of 60% sucrose was placed beneath the linear 20–40% sucrose gradient upon which the supernatants were layered. In (*c*) the optical density fractions 18 through 25 were lost. Twenty-five fractions were collected. A solid line (——) indicates optical density and a dashed line (– · – ·) counts/min.

Summary.—Polyribosomes were detected prior to the first mitotic division in fertilized eggs of the sea urchin *Lytechinus variegatus*. The radioactivity of labeled amino acids incorporated into nascent proteins was shifted by treatment with ribonuclease from the polyribosome region to that of the ribosome, indicating the presence of messenger RNA. Some of the polyribosomes were bound to membranes.

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