POSSIBLE ROLE OF INTRANUCLEAR MEMBRANES IN NUCLEAR–CYTOPLASMIC EXCHANGE IN SPIDER CRAB OOCYTES

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It is well established that ribosomes and most of the mRNA needed to support protein synthesis during early stages of development are synthesized during oogenesis (Brown and Littna, 1964). Large quantities of rRNA and its associated proteins are synthesized by the nucleolus and transferred from nucleus to the cytoplasm (Perry, 1966). How rRNA is transferred from nucleus to cytoplasm remains obscure although several modes of transfer of material have been suggested. These include (*a*) passage through pores or annuli in the nuclear membranes (Anderson and Beams, 1956; Kessel, 1966; Stevens and Swift, 1966), (*b*) blebs or outpocketings of the outer nuclear membrane (Kessel, 1966, 1968; Scharrer and Wurzelmann, 1969 *a*), (*c*) partial disappearance of the nuclear envelope (Tashiro et al., 1968), (*d*) transport through perinuclear microtubules (Scharrer and Wurzelmann, 1969 *b*), and (*e*) nucleolar buds surrounded by pinched-off portions of nuclear envelope (Szollosi, 1965). The present study describes vesicles within the oocyte nucleus of the spider crab, *Libinia emarginata* L., and presents evidence that they play a role in nucleocytoplasmic exchange.

MATERIALS AND METHODS

Immature and mature brooding females of *Libinia emarginata* were obtained from the Marine Biological Laboratory, Woods Hole, Mass. Oocytes were collected in various stages of maturation from ovaries of mature females selected at appropriate intervals after spawning (Hinsch, 1968) or from females which had recently molted to the adult state (Hinsch, unpublished). In preparation for electron microscopy, ovaries were fixed for 90 min in Karnovsky's (1965) paraformaldehyde-glutaraldehyde mixture or in 2.5% glutaraldehyde in 0.1 M phosphate buffer. Tissues were washed in 0.1 M phosphate buffer and postfixed in buffered 1% OsO_4, pH 7.4. After dehydration through graded concentrations of ethanol, the tissues were infiltrated and embedded in Araldite. Sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate (Watson, 1958) and lead citrate (Venable and Coggeshall, 1965), and examined with a Philips 300 electron microscope.

Tissues for cytochemical study at the light microscopic level were fixed in 10% neutral formalin or Carnoy's fixative, embedded in paraffin, sectioned at 5 μ and mounted on glass slides. These sections were stained for RNA with toluidine blue, methyl green pyronin (Kurnick, 1955), or azure A, pH 4.0 (Flax and Pollister, 1949) or acridine orange. Control sections were extracted with perchloric acid or digested with ribonuclease and then stained.

OBSERVATIONS

The ovaries of newly spawned or molted females have a syncytial mass of centrally located gonial cells. Following ovulation or the molt, some of these cells begin a period of rapid growth and vitellogenesis leading to the mature oocyte.

In light microscopic studies, the cytoplasm of these oocytes is intensely basophilic. This basophilia is lost as the oocytes mature. The nucleus contains a centrally located nucleolus. This undergoes progressive vacuolization and loss of stainability with toluidine blue or azure A as the oocytes mature (Fig. 1). In sections of later stages, the nucleolus appears to be ring-shaped. Small basophilic spots are visible beneath the nuclear membrane (Fig. 1). These spots and the nucleoli fluoresce orange with acridine orange. Ribonuclease digestion reduces the stainability with toluidine blue.

Ultrastructural studies of oocytes collected during days immediately following spawning indicate that several changes occur in the nucleolus and nucleoplasm. Initially, the nucleolus appears as a solid mass of granules with very few fibrous or amorphous areas (Fig. 2). As maturation proceeds, the vacuolization seen at the light microscope level appears ultrastructurally as a progressive compartmentalization into granular and fibrous portions (Figs. 3 and 4). Very little yolk

accumulation occurs at this time. In sections of oocytes actively synthesizing yolk, the nucleolus appears ring-shaped with the central lacuna containing a homogeneous material resembling that of the surrounding nucleoplasm (Fig. 5). As these changes occur, granules resembling those in the nucleolus appear peripheral to it in the nucleoplasm. These tend to aggregate in dense clumps beneath the nuclear envelope (Fig. 5). Several small vesicles are associated with these dense clumps but do not surround them. However, somewhat later this dense material becomes more dispersed and is compartmentalized into membrane-bounded packets (Figs. 7 and 8). Material resembling that found in the packets is found in the numerous vesicles in the nucleoplasm and in the cisterna of nuclear envelope. Vesicles appear to break or bud off from the packets. Some appear to fuse with the inner nuclear membrane (Fig. 9). Blebbing of the outer nuclear membrane was also seen (Fig. 10). Granules and fibers were frequently seen in sections through nuclear pores (Fig. 9). Frequently, the packets and vesicles contain dense granules resembling the intracisternal granules found in endoplasmic reticulum of crayfish (Beams and Kessel, 1963) and *Libinia* (Hinsch and Cone, 1969) oocytes. Clumps of granular material and numerous free ribosomes appear in the cytoplasm adjacent to the nuclear envelope (Fig. 7).

DISCUSSION

Nucleolar proliferation and associated amplification of genes for ribosomal RNA during oogenesis occur in amphibians (Brown and Dawid, 1968; Gall, 1968) and in echiuroid worms (Perkowska et al., 1968). This results in a large number of ribosomes in the unfertilized egg which support protein synthesis throughout early development (Brown, 1966). Changes in nucleolar structure and the accumulation of ribonucleoprotein-containing packets beneath the nuclear envelope and the large number of free ribosomes within the cytoplasm of the *Libinia* oocyte during early stages of oogenesis suggest nucleolar synthesis of these particulates. Similar packets have been reported in the oocytes of *Acheta* (Allen and Cave, 1968).

Miller (1966) described a membranous nucleolar component in the oocytes of *Triturus* and suggested that it performs a role in intranucleolar transfer of nucleoprotein precursor molecules. Kessel and Beams (1968) identified intranucleolar vesicles, but were unable to determine the fate of the membranes in the crayfish oocyte since they lose their identity as the granular lamellae of the nucleolus become shorter.

The presence of intranuclear membranes in *Libinia* suggests transport of nuclear material from

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FIGURE 1 Young oocytes at onset of vitellogenesis. Oocyte to the left has large solid nucleolus. Nucleolus of right oocyte has assumed ring-shape configuration. Basophilic clumps near nuclear membrane are indicated by arrows. Formalin-fixed, paraffin-embedded. Methyl green pyronin. × 850.

FIGURE 2 Nucleolus in an oocyte from an immature female showing almost solid nature with little amorphous area. × 7000.

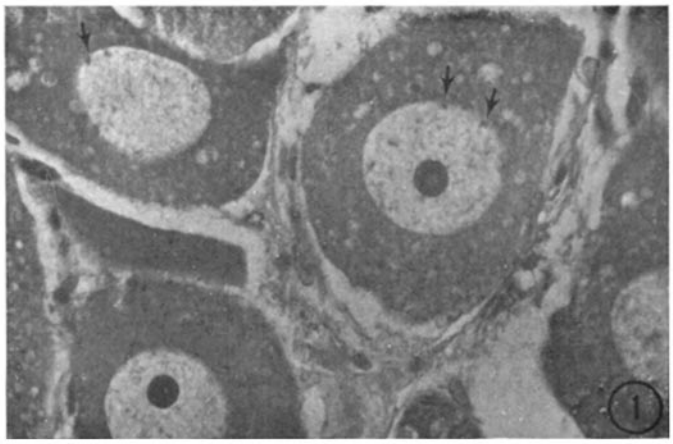
FIGURE 3 Oocyte in very early stages of vitellogenesis where the nucleolus is beginning to appear vacuolated. × 12,700.

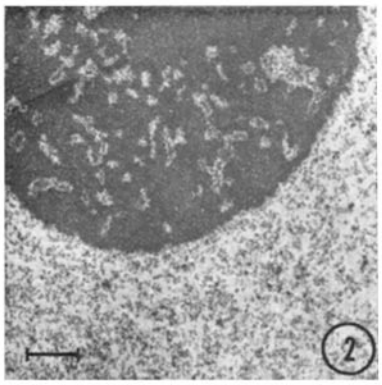
FIGURE 4 Nucleolonema of oocyte at a later stage than Fig. 3. × 12,900.

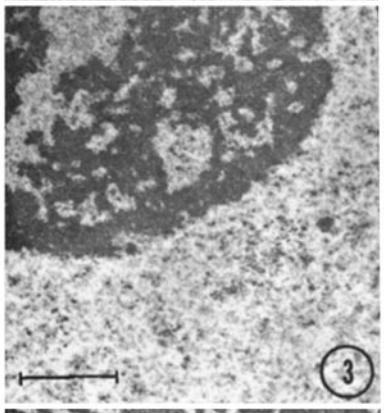
FIGURE 5 Section of nucleolus which appears ring-shaped. Central lacuna (*L*) and peripheral nucleoplasmic areas appear similar. × 19,100.

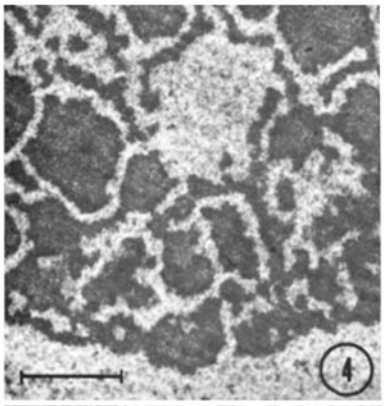
FIGURE 6 Nucleus of early vitellogenic oocyte. Numerous granules are apparent throughout nucleoplasm. Dense aggregations (*A*) form in peripheral regions. Several membrane-bounded vesicles (arrows) associate with but do not surround these aggregations. Nuclear envelope is infrequently interrupted by pores at this stage. *C*, cytoplasm. × 17,400.

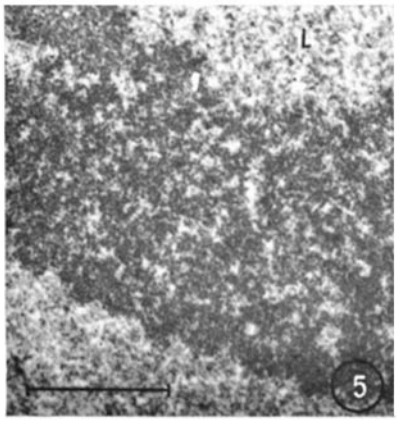
FIGURE 7 Packets (*p*) of ribonucleoprotein appear in nucleoplasm while condensations of material accumulate in cytoplasmic vesicles adjacent to the nuclear envelope (arrows). Cytoplasmic vesicles adjacent to nucleus are free of ribosomes whereas the more distant endoplasmic reticulum has several. × 18,000.







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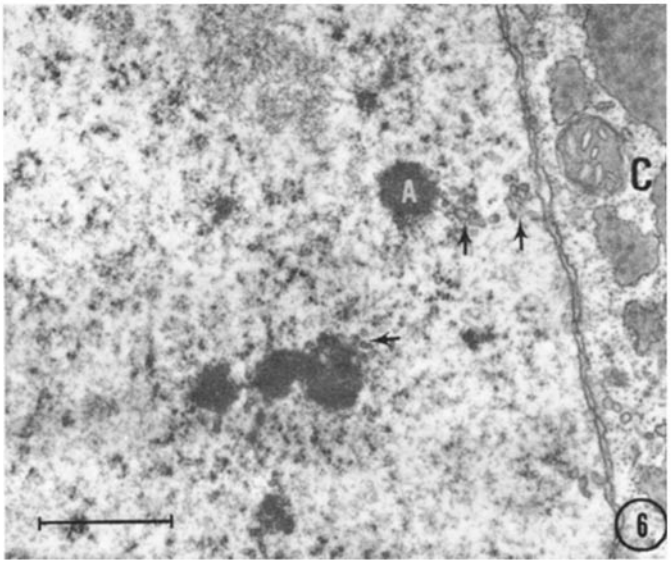
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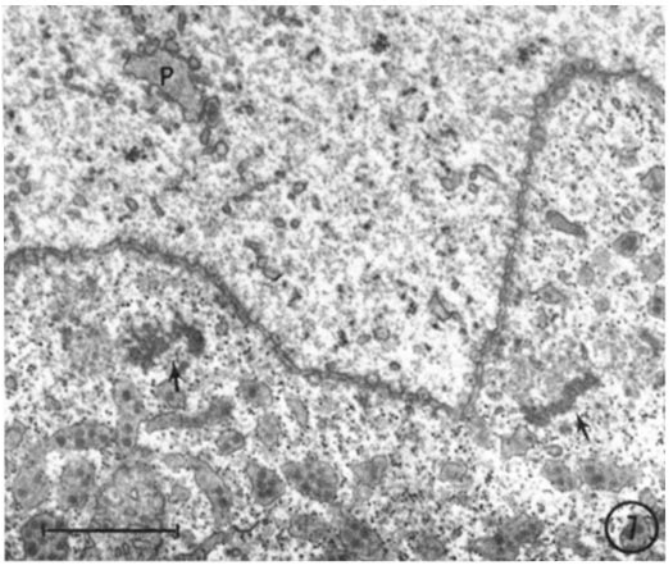
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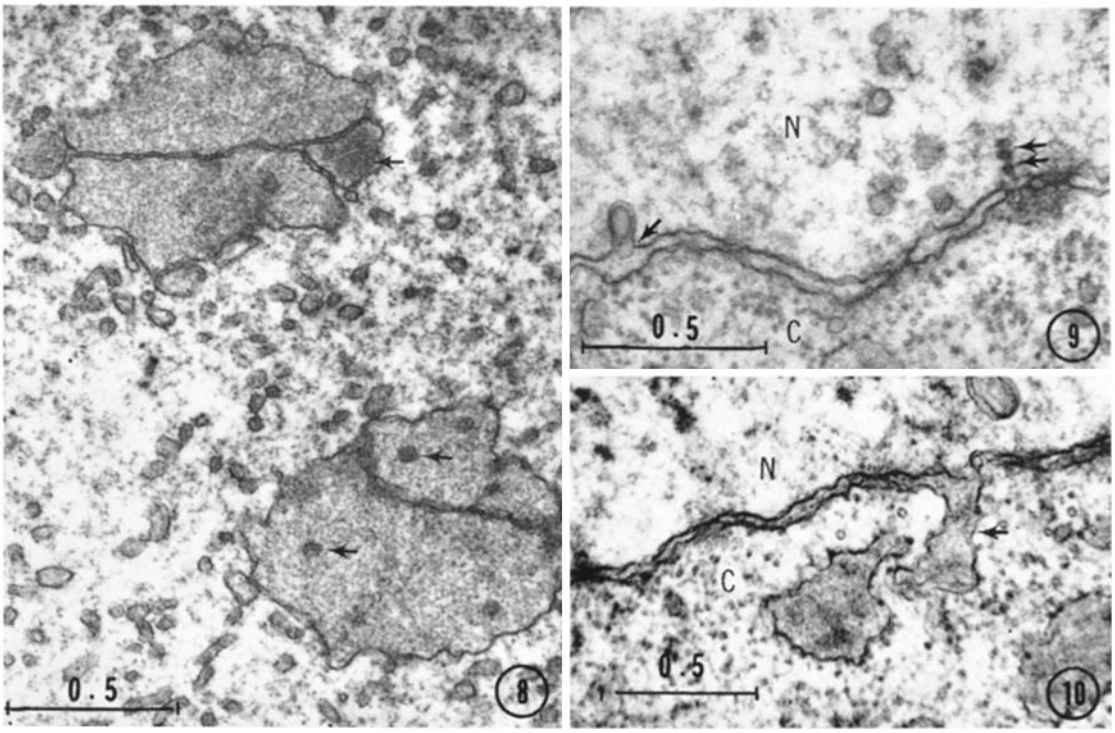

FIGURE 8 Two membrane-bounded packets of ribonucleoprotein and associated vesicles in nucleoplasm. Contents of vesicles resembles that in the packets. Granular aggregates of material frequently form in the packets (arrows). × 45,100.

FIGURE 9 Fusion of a nuclear vesicle with inner nuclear membrane (arrow) and granules above nuclear pore (double arrow). Cisterna of nuclear envelope is filled with material. *N*, nucleus, *C*, cytoplasm. × 48,400.

FIGURE 10 Blebbing (arrow) of outer nuclear membrane. *N*, nucleus, *C*, cytoplasm. × 40,200.

the nucleus to the cytoplasm by a mechanism in addition to the passage of fibrous or granular material through the nuclear pores. This mechanism includes the fusion of nuclear vesicles with the inner nuclear membrane; second, the passage of the material in the vesicles into the cisterna between layers of the nuclear envelope; and finally, blebbing of the outer nuclear envelope to form endoplasmic reticulum with associated nucleolar material (e.g., rRNA).

The origin of these intranuclear membranes is of interest. The vesicular membranes may arise by proliferation of the nuclear envelope or they may arise *de novo*. The vesicles do not assume the typical structure of annulate lamellae so frequently seen in the cytoplasm of developing oocytes (Kessel, 1968). In fact, annulate lamellae do not appear in the cytoplasm of *Libinia* oocytes during vitellogenesis (Hinsch and Cone, 1969). However, Dallai (1967) reported nuclear vesicles in the epithelium of the testes in *Collembola*. He felt that they formed from the inner nuclear membrane and were related to the endoplasmic reticulum producing activity of the outer nuclear membrane.

This study is Contribution No. 132 from the Institute of Molecular Evolution. The investigation was supported in part by the NIH grant 5T1 HD26-06 (Fertilization and Gamete Physiology Training Program, Marine Biological Laboratory, Woods Hole, Mass.), by NASA grant NaG-689, NSF grant No. GB 3899 and a grant from the Florida Division of the American Cancer Society to Dr. C. B. Metz.

The author's work was done under tenure of a NIH Special Fellowship.

Received for publication 17 February 1970, and in revised form 4 June 1970.

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