INTRANUCLEOLAR MEMBRANES AND NUCLEAR-CYTOPLASMIC EXCHANGE IN YOUNG CRAYFISH OOCYTES

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INTRODUCTION

Notwithstanding the fact that the nucleolus was one of the first cellular organelles to be described (see references 11, 22, 30, 31, 38, 42 for reviews), its significance remained largely unexplained until the studies of Caspersson and Schultz (13), Caspersson (14), and Brachet (8, 9). However, recent advances in techniques of ultrastructural cytochemistry (see references 6, 18, 40), radioautography (see references 15, 19), and biochemistry (see references 7, 10, 12, 17, 20, 29, 33, 34, 37, 39, 43) have added considerably to the knowledge of the macromolecular organization and function of the nucleolus. Nucleolar components may vary considerably in different types of cells and in the same cell under different physiological conditions (see references 5, 21, 26, 27, 31).

It is the primary purpose of the present paper to describe the presence and arrangement of a unique structural component occurring *in situ* in the nucleoli of crayfish oocytes during early stages of oocyte growth.

MATERIALS AND METHODS

The female crayfish (*Orconectes virilis*) used in this study were injected with a paraformaldehyde-

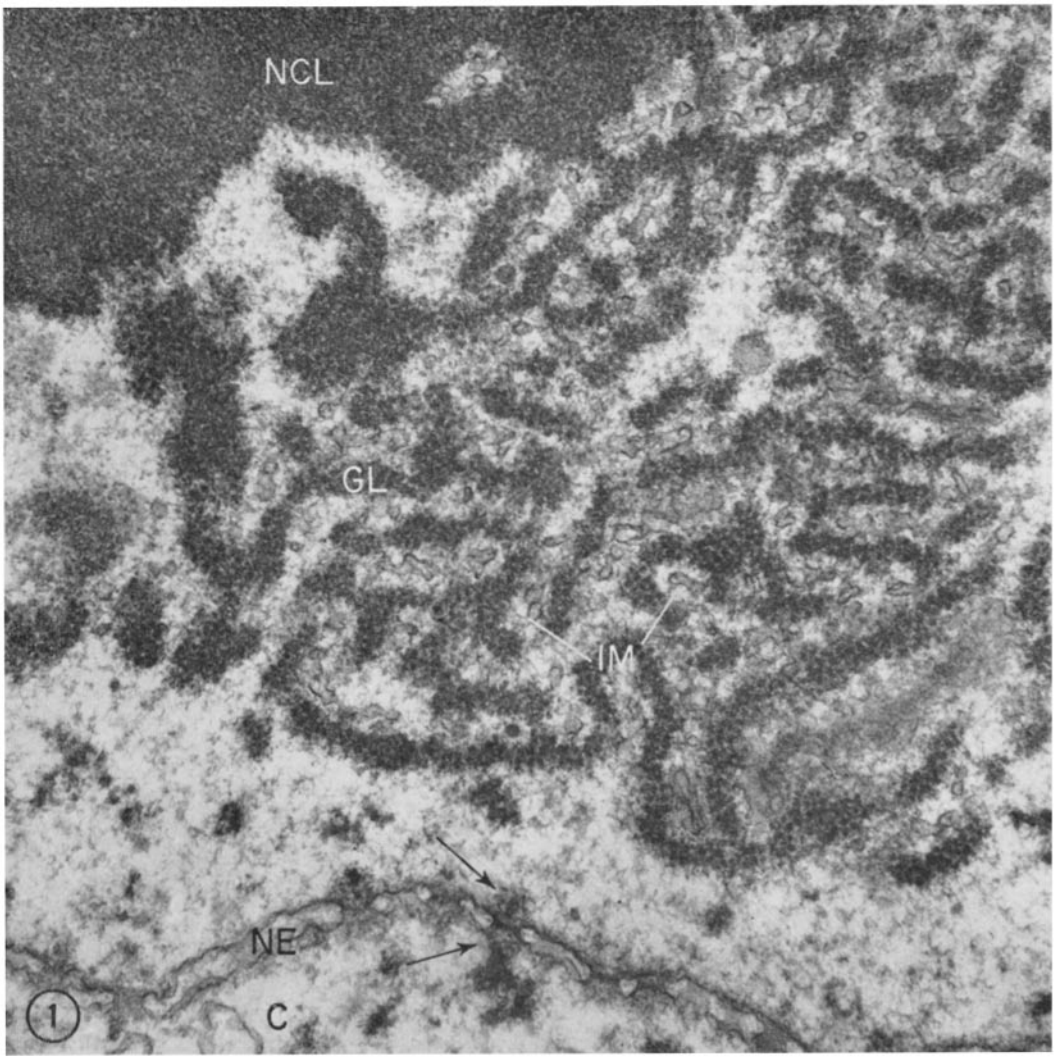


FIGURE 1 Early stage in formation of branching and anastomosing lamellae (*GL*) from the periphery of a dense nucleolus (*NCL*). Membranous vesicles and lamellae (*IM*) are surrounded by the granular lamellae. Nuclear envelope (*NE*), cytoplasm (*C*), and a region of nuclear-cytoplasmic exchange (arrows) are indicated. × 50,000.

glutaraldehyde fixative as described by Karnovksy (23). The animals were subsequently dissected, and portions of the ovary were transferred to fresh fixative where they remained at room temperature for periods of 3–5 hr. Following several rinses in ice-cold 0.1 M phosphate buffer (pH 7.4) for a period of 3–12 hr, the oocytes were dehydrated rapidly in a series of ethanols, treated with propylene oxide, and embedded in Epon 812 (28). Sections obtained with a Sorvall MT-2 ultramicrotome were mounted on copper grids and stained with uranyl acetate (44) and lead citrate (36). The sections were subsequently studied in an RCA EMU-3G electron microscope.

RESULTS

Crayfish oocytes have been extensively studied at the electron microscope level by Beams and Kessel (3, 4), especially with regard to the process of cytoplasmic differentiation and vitellogenesis. These studies have provided evidence that intra-

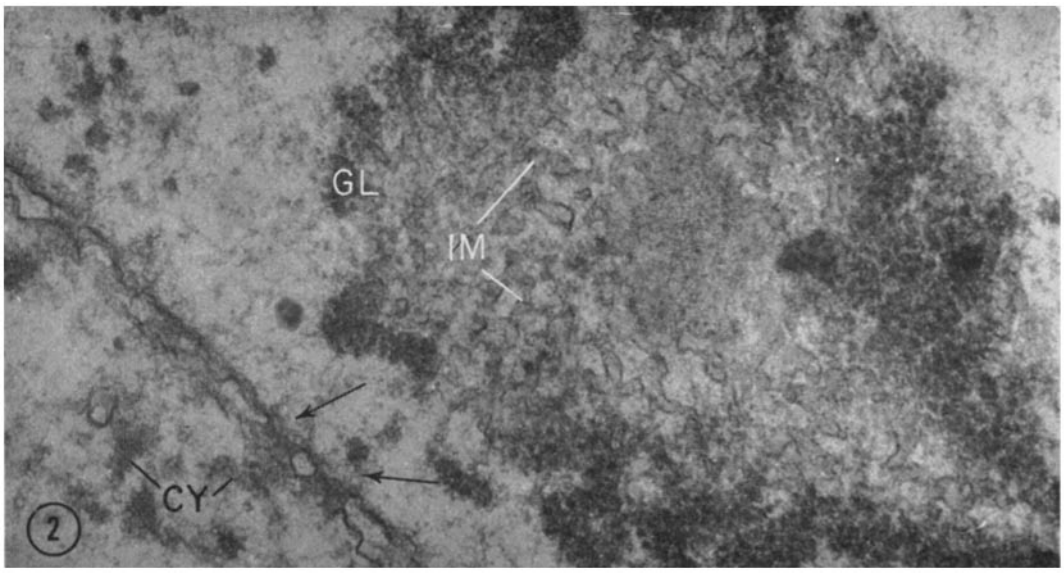


FIGURE 2 This nucleolus illustrates the branching and anastomosing system of intranucleolar membranes (*IM*). Granular lamellae of nucleolus indicated at (*GL*). Cytoplasmic nuages (*CY*) and nuclear pores (arrows) are identified. × 72,500.

ooplasmic synthesis of yolk proteins is a prominent feature of crayfish oogenesis.

The observations on nucleolar fine structure, which will be described, were made on crayfish oocytes ranging from 150 to 250 μ in diameter. In oocytes of such size, a number of nucleoli, each several microns in diameter, is located in the peripheral region of the nucleoplasm. Initially, the nucleoli are extremely dense, and little internal detail is apparent with the electron microscope. Subsequently, a portion of the periphery of each nucleolus (Fig. 1) becomes associated with a branching and anastomosing system of coarse strands. This network first becomes apparent on that side of the nucleolus adjacent to the nuclear envelope and consists of dense granules embedded in an amorphous matrix. Thin filaments are frequently observed to be associated with these coarse strands of granules. It appears that RNA is present in two distinct morphological forms, granular and fibrillar, in nucleoli of many cells, while the matrix in which the RNA is embedded is primarily protein since it is reported to be easily removed with pepsin digestion (see reference 6).

As soon as the nucleoli begin to "spin out" the network of coarse strands of granules, a membranous component becomes evident in the nucleoli (Fig. 1). These membranes occur in the form of numerous vesicles and short lamellae which are enclosed by the nucleolar network (Fig. 1). The membranes are never observed at the free margin of the nucleolus, however, nor are they visible in any other portion of the nucleoplasm. This condition suggests either that the membranes are preformed and present within the compact nucleolus and only become visible as a loosening of nucleolar organization occurs, or, conversely, that the membranes are synthesized under the influence of the nucleolus and, therefore, become apparent concomitant with the formation of the coarse strands or lamellae from the periphery of the nucleolus. In some sections, a branching and anastomosing configuration of the intranucleolar membranes is apparent (Fig. 2).

It appears that many of the dense nucleoli in each oocyte nucleus undergo a partial or complete transformation into a network of coarse lamellae. After this is accomplished, the coarse strands comprising the network become organized into stacked parallel rows (Fig. 3). However, occasional connections are encountered between the stacked lamellae either at their ends or along their length (Fig. 3). As the ordered configuration of nucleolar lamellae is established, the membranes also be-

come oriented in rows within the nucleolus (Figs. 3, 4). Thus, the membranes, still consisting of vesicles and lamellae of different lengths, are arranged in alternate rows between the granular lamellae. In some cases, thin filaments extend between the membranous lamellae and the coarse granular lamellae (Fig. 4). This highly structured arrangement apparent in the nucleoli has been observed in nearly all the nucleoli in a single oocyte as well as in a number of different oocytes, so the condition would appear to represent a consistent and characteristic behavior of the nucleoli in this oocyte.

Following the establishment of the nucleolar organization just described, the nucleoli decrease in size. As this occurs, the intranucleolar membranes decrease in number. The fate of the membranes is unknown, but they appear to lose their identity as the granular lamellae become shorter. In no case were membranes observed in other regions of the nucleoplasm. The decrease in nucleolar size appears to occur at least in part by a progressive fragmentation of small pieces from the ends of the granular lamellae (Figs. 3, 4). Thus, small isolated fragments similar to the granular nucleolar lamellae appear at various distances from the nucleoli. Many of these isolated nucleolar fragments are located in proximity to pores in the nuclear envelope (Figs. 2, 3). Indeed, frequent examples of nuclear-cytoplasmic exchanges in such oocytes can be demonstrated (Figs. 5–8). Dense, amorphous masses of material are present in both the nucleoplasm and cytoplasm, and they are connected by similar strands of material extending through the nuclear pores (Figs. 5–8). The nature of the material present within the nuclear pores and which is apparently in transit from the nucleus to the cytoplasm is difficult to determine. In certain instances, extremely small granules as well as thin filaments, similar to those recently described as associated with the pores of annulate lamellae (25), are apparent, and they appear to be embedded in an amorphous matrix (Figs. 5–8). Thus, the material in transit through the nuclear pores appears somewhat different structurally from the nucleolar ribonucleoprotein material. A similar configurational change in ribonucleoprotein during its exit from the nucleus has been noted in other cells (see references 24, 41).

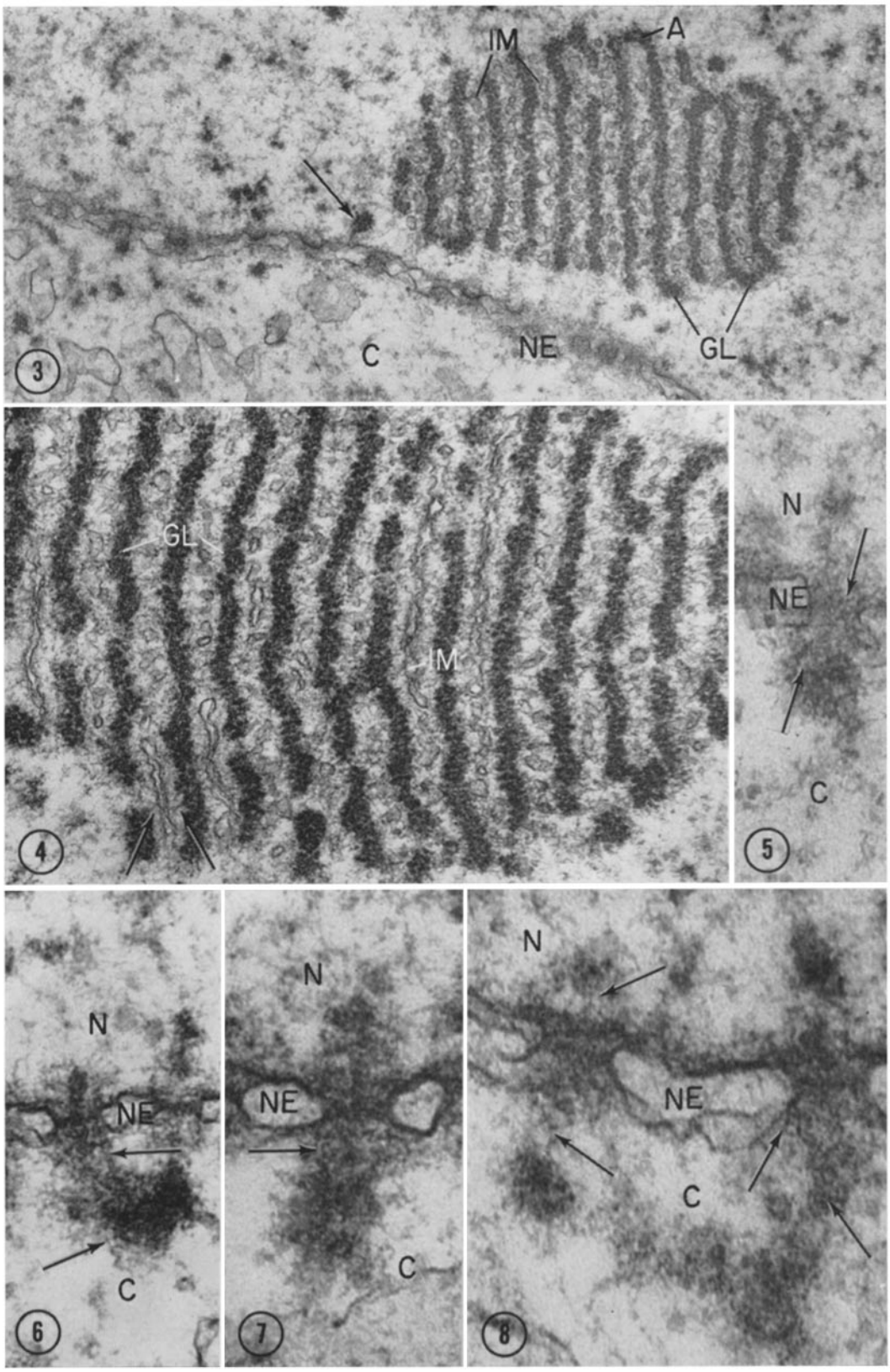
DISCUSSION

As far as can be determined, an extensive system of intranucleolar membranes *in situ* has not previously been described. However, in studies on isolated nucleoli of *Triturus* oocytes, Miller (29) has recently obtained information which appears to be directly related to the observations recorded in the present study. Under certain experimental conditions for isolating *Triturus* oocyte nucleoli, Miller (29) described a situation in which a low-contrast spherical object was found attached to many of the expanded fibrous nucleolar cores. Miller (29) further indicated that "although no direct evidence is available, this component appears to be membranous in nature," since the "membranous" nucleolar component was described as having phase-contrast and electron-transmission characteristics similar to those of known membranous structures when treated in a similar manner. Miller (29) then suggested that "the membranous nucleolar component may play a role in an intranucleolar transfer or transformation of the ribosomal RNA precursor molecules following their transcription on the DNA in the nucleolar cores."

FIGURE 3 This nucleolus illustrates the alternate stacked arrangement of the coarse granular lamellae (*GL*) of different lengths and intranucleolar membranes (*IM*). Small nucleolar masses (arrows) are located between the nucleolus and nuclear envelope (*NE*). Continuity between adjacent granular lamellae in the nucleolus is evident at (*A*). Cytoplasm, *C*. × 34,000.

FIGURE 4 Enlargement of the stacked nucleolus illustrating the alignment of vesicles and lamellae (*IM*) between granular lamellae (*GL*). In region indicated by arrows, thin filaments appear to interconnect the membranous and granular lamellae. × 50,000.

FIGURES 5–8 All figures illustrate one or two pore regions in the nuclear envelope (*NE*). Dense material is present in each of the pores and is continuous with material in the nucleoplasm (*N*) and cytoplasm (*C*). A tubular structure in a nuclear pore is indicated in Fig. 6 (arrow). Small granules and filaments are evident in the dense masses in all figures (arrows). Figs. 5 and 6, × 97,500; Figs. 7 and 8, × 140,000.


IM
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Thus, the membranes described in the crayfish oocyte nucleoli constitute direct evidence to support the suggestion by Miller (29) that membranes may constitute a structural component of the nucleolus. It is obvious that the membranous component of crayfish oocyte nucleoli is extensively developed and especially prominent, a feature which may indicate that the function performed by such a system is especially emphasized in this particular cell. If this is the case, the nucleoli in crayfish oocytes would appear to constitute a useful system on which biochemical studies might be designed to determine with more precision the functional significance of intranucleolar membranes. Davies and Small (16) have recently described a membranous component associated with chromosomes in certain cells.

Prominent masses of electron-opaque material, strongly staining for acidic protein, have been described in the cytoplasm close to the nuclear envelope by a number of investigators (1, 2, 24, 32, 35, 41). They were originally referred to as "nuages" in spider oocytes by André and Rouiller (2). These nuages contain processes which extend into the pores of the nuclear envelope and, in some cases, are also in contact with particles in the nucleoplasm (see references 1, 24, 41). Swift (41) has suggested that elongate processes which extend through the annuli of the nuclear envelope in *Chironomus* salivary gland cells represent deformed nucleoprotein particles in the act of passing into the cytoplasm. Thus, the nuage material would then represent a configurational change from a particulate component within the nucleus to coalesced and amorphous masses outside. Swift (41) further noted that the nuage material was often shaped like tornadoes, with narrow basal processes near the nuclear envelope, but there were fewer and larger cloudlike masses farther from the nuclear margin, a condition which suggested to him that the nuage structure had been formed by a coalescence of material from many annuli and that the direction of movement was from the nucleus to the cytoplasm (see also reference 24). It seems likely that the nuage material described in a wide variety of cells, especially in young oocytes including those of the crayfish, is involved in the transfer of RNA from chromosome to cytoplasm, but the exact functional significance remains obscure (see references 24, 41).

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