FINE STRUCTURE OF ANNULATE LAMELLAE

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INTRODUCTION

Annulate lamellae represent a poorly understood class of cytomembranes (see references 14–18). The structural resemblance and proximity (in some cases) between the nuclear envelope and annulate lamellae have led to the concept that the annulate lamellae represent a product of the nuclear envelope although the methods propoundedly involved in the morphogenesis of annulate lamellae are indeed varied (14–17, 21, 30, 34). In almost all cases, the pores of the annulate lamellae have associated with them a dense material of variable nature. The high density associated with this region has prevented characterization of much structural detail in this area. It is the purpose of this report to describe the structural components responsible for this density in the annulate lamellae of certain germ cells.

MATERIALS AND METHODS

The annulate lamellae to be described were located in oocytes of the echinoderm, *Ophioderma panamensis*, and the amphibian, *Rana pipiens*, as well as in spermatocyte and spermatid stages of the crayfish, *Orconectes virilis*. The ophiuroids were obtained from the Pacific-Bio Marine Company (Venice, Calif.) and the Hopkins Marine Station (Pacific Grove, Calif.). The frogs and crayfish were obtained from the Iowa City region.

Individual or groups of *Ophioderma* oocytes were fixed for 2–3 hr in an ice-cold, 5% solution of glutaraldehyde in filtered sea water, or in Sörenson's phosphate buffer, to which sucrose in a concentration of 2, 5, or 7% was added. The frog oocytes were fixed for 2 hr in ice-cold, 1% osmium tetroxide in 0.1 M Sörenson's phosphate buffer. The crayfish testis was fixed for 2 hr in an ice-cold, 2% solution of glutaraldehyde in 0.05 M Sörenson's phosphate buffer, to which 0.5 mg $CaCl_2$ was added in some cases; the osmolality of this fixative approximated that reported for crayfish body fluid (33).

Following glutaraldehyde fixation, the tissues were washed with several changes of ice-cold phosphate buffer for a period of 6–12 hr. Sucrose was added to the buffer in a quantity to provide an osmolality approximating that of the particular tissue. The cells were then fixed again in an ice-cold, 1% solution of osmium tetroxide in either sea water (for *Ophioderma* oocytes) or 0.1 M Sörenson's phosphate buffer (for crayfish testis). Again, sucrose was added to the osmium tetroxide in a concentration sufficient to produce an osmolality approximating that of the tissue fluids of the particular animal. Following rapid dehydration in a series of ice-cold ethanols, the cells were embedded in Epon. Sections stained in uranyl acetate and lead citrate were studied in an RCA EMU-3G electron microscope.

OBSERVATIONS

In all three cell types, low power electron micrographs of regions of annulate lamellae illustrate a rather homogeneous region of medium density between each of the lamellae comprising a stack, with regions of increased electron opacity commonly associated with each of the pores or annuli. While ribosomes are concentrated in the cytoplasm surrounding each of the stacks and, in some instances, are attached to membranes which are continuous with the ends of the annulate lamellae, the cytoplasm immediately associated with the annulate lamellae is less particulate in appearance.

At higher magnifications, it becomes apparent that the general density associated with the annulate lamellae is primarily contributed by two

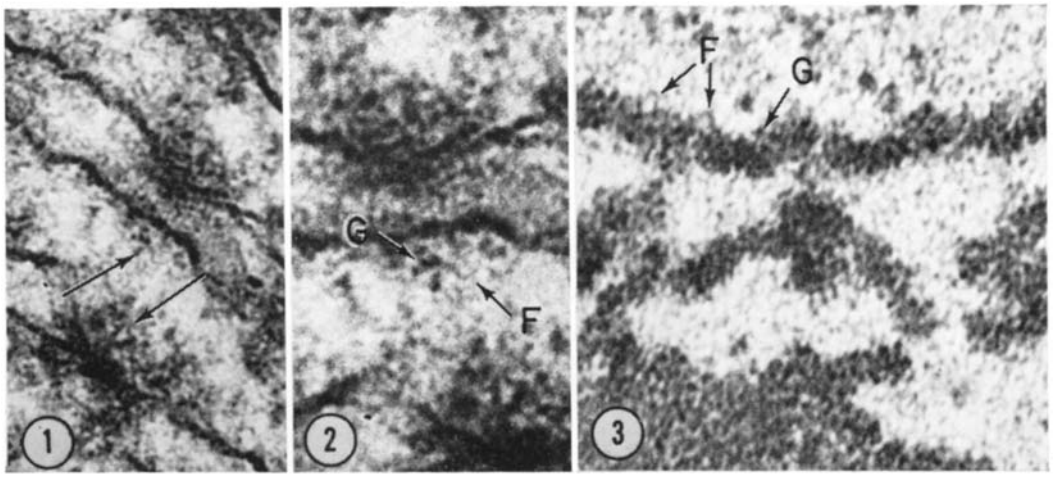

FIGURE 1 Annulate lamellae in *Ophioderma* oocyte; illustrates fibrils and granules (arrows) which appear interconnected. × 133,500.

FIGURE 2 Enlargement illustrating three granules (*G*) which appear attached at surface to thin fibril (*F*). × 208,000.

FIGURE 3 Periphery of a nucleolus in *Ophioderma* oocyte illustrating dense granules (*G*) which are embedded in a matrix and fibrils (*F*). × 72,500.

structural components. One of the structural components is a small, dense granule (Figs. 1, 2, 4–6, 9, 10). These granules vary somewhat in size, the smallest being ~40 A in diameter and the largest ones measuring 70–90A in diameter. In general, however, the granules have diameters of about 50–60 A. It is not known whether the size variations of the granules represent stages in the growth or dissolution of the granule; nor is the three-dimensional shape of the granule known. The dense granules are widely dispersed in the interlamellar cytoplasm (Figs. 4, 9, 10). However, in the ophiuroid oocyte, the concentration of these granules is greater in the pore region than elsewhere (Figs. 5, 6). At the highest magnifications, granules of similar size and density also appear to reside with the membranous cisternae of the annulate lamellae and, in some instances, to comprise the membranes themselves (Fig. 6). This appearance, to some extent, may be due to the plane of section with respect to the membranes and annular material of the lamellae.

The second structural component associated with the annulate lamellae is a very thin fibril (Figs. 2, 9, 10). These fibrils may be associated with the pore region, but also form a filamentous meshwork between the lamellae. The width of the fibrils is more difficult to determine in thin sections since these fibrils are so small and lack contrast. In general, however, they range from those barely resolvable to those approximately 30 A in width.

The fibrils and granules demonstrate a close spatial relationship in all regions of the annulate lamellae. Thus, in both the pore region and interlamellar regions, the fibrils often appear to interconnect the dense granules to form a network (Figs. 5, 6, 9, 10). In other areas, the small granules appear attached by their surface to the fibrillar component (Figs. 1, 2).

Ruthmann (32) found that annulate lamellae in crayfish spermatocytes show a basophilic stain, but that no ribosomes were associated with the lamellae. In the frog oocyte as well, ribosomes do not appear to be directly associated with the membranes of the annulate lamellae, but these lamellae also show a basophilic stain.[1] This fact has led to earlier suggestions that, if annulate lamellae have RNA associated with them, the RNA is in nonparticulate form. Merriam (21) has suggested that

[1] Kessel, R. G. Unpublished observations.

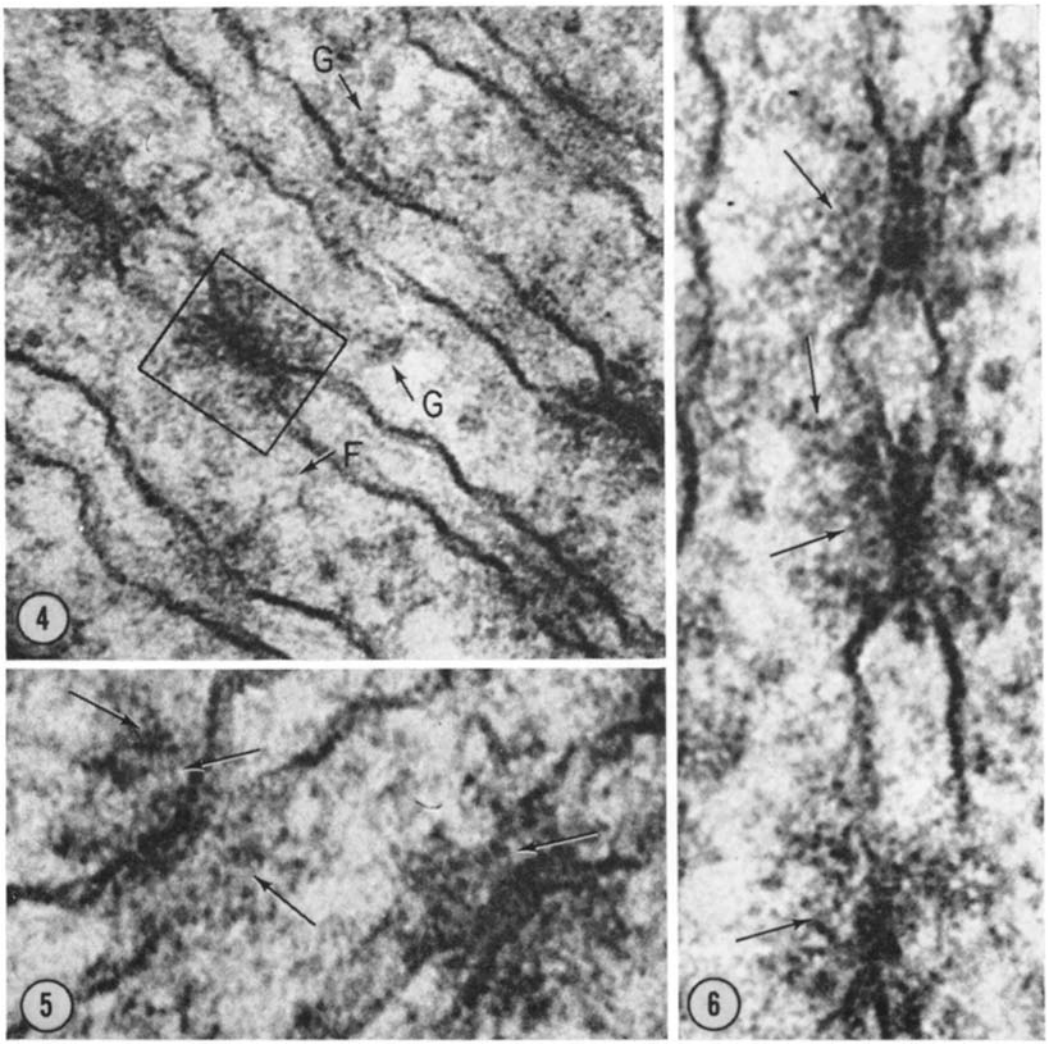

FIGURE 4 Annulate lamellae in *Ophioderma* oocyte. One pore region is outlined. Interlamellar granules (*G*) and fibrils (*F*). × 133, 500.

FIGURES 5 and 6 Enlargements of several pore regions. In all cases, numerous, dense granules as well as fibrils are concentrated in the pore region. The arrows direct attention to those areas in which the network formed by the interconnection of the granules and fibrils is particularly evident. × 208,000.

in the case of the *Dendraster* egg the annulate lamellae may synthesize 150 A particles.

In view of the aforementioned comments, the nucleolus of the *Ophioderma* oocyte was examined for structural components which might resemble those associated with the annulate lamellae in the same cell. In this oocyte, large nucleoli situated in proximity to the nuclear envelope appear to "spin" out coarse strands (nucleolonema) from their surfaces which then undergo fragmentation. The coarse strands emanating from the surface of these nucleoli consist of a number of dense granules which range from 60 A to 130 A and are embedded in a less dense matrix (Fig. 3). Fine fibrils approximately 30 A in width are also associated with the nucleolus (Figs. 3, 7). In many instances the small granules and filaments associated with the nucleolus appear interconnected, forming granule–fila-

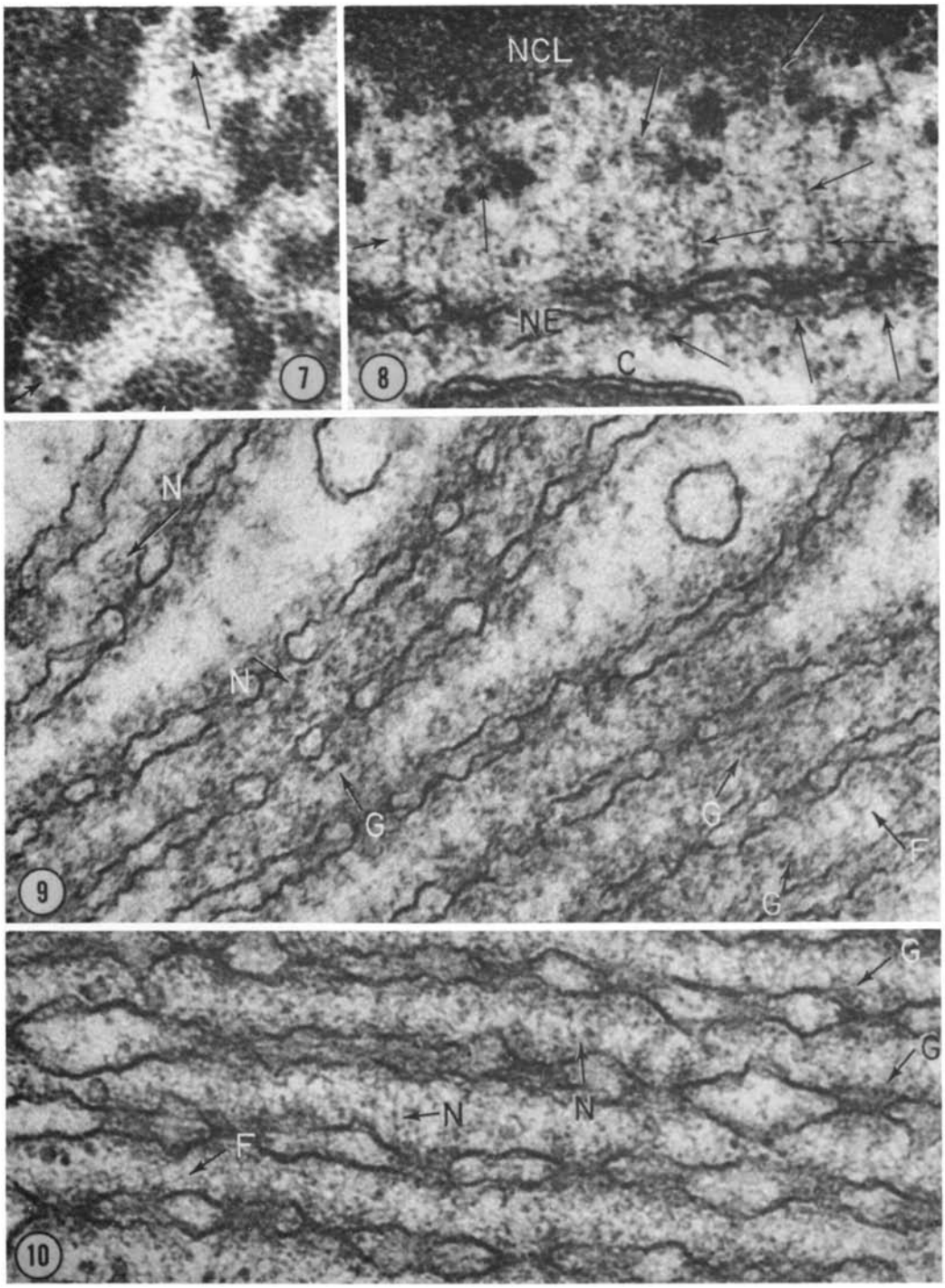

FIGURE 7 Portion of nucleolus in *Ophioderma* oocyte. Arrows indicate regions where small granules appear interconnected by thin filaments. × 72,500.

FIGURE 8 *Ophioderma* oocyte. Portion of nucleolus (*NCL*) present in nucleus. Cytoplasm, *C*, nuclear envelope, *NE*. Note wide distribution of granule–filament complexes (arrows). × 87,500.

FIGURE 9 Annulate lamellae in crayfish spermatocyte. Fibrils (*F*), granules (*G*), and regions of their interconnection (*N*) are illustrated. × 97,800.

FIGURE 10 Annulate lamellae in *Rana pipiens* oocyte (~300 μ diameter). Granules (*G*) and fibrils (*F*) are indicated. Connections between granules and fibrils are pointed out at (*N*). × 87,500.

ment complexes similar in arrangement to those associated with the annulate lamellae (compare Figs. 7 and 8 to Figs. 5 and 6). Such granule–filament complexes not only comprise the nucleolus and nucleolar fragments but also are present in the nucleoplasm between the nucleolus and nuclear envelope (Fig. 8). Further, the structures are present on both sides of the nuclear envelope where they appear to be associated with the nuclear pores (Fig. 8). Thus, the fibrils associated with the nucleoli and nucleolar fragments in *Ophioderma* oocytes have a size range comparable to that of the fibrils associated with the annulate lamellae. The smallest granules constituting the nucleolonema or its fragments are similar in size to the largest granules associated with the annulate lamellae.

DISCUSSION

By far the largest amount of information available at present suggests that the annulate lamellae are derived exclusively from the nuclear envelope. Should this situation continue to be the rule, then it would appear reasonable to regard the annulate lamellae as nuclear adjuncts formed as an elaboration of the nuclear envelope. Since the annulate lamellae migrate into other regions of the cytoplasm, perhaps carrying with them nuclear-derived information, this migration would provide a means for establishing nuclear control over remote regions of the cytoplasm. Such a function, which has been discussed previously (24, 35), would emphasize the notion that the annulate lamellae may represent, in some unknown manner, the agent or messenger of the nucleus. It should be emphasized, however, that direct experimental evidence to support such an hypothesis is lacking.

Only a few studies have demonstrated any specific association between formed product and the pores of annulate lamellae. Granular aggregates measuring 30–45 mμ in diameter illustrate a specific alignment with respect to the pores in stacks of annulate lamellae in tunicate oocytes (17). These annulate lamellae also have ribosomes attached to their surface and are continuous at their ends with rough-surfaced vesicles (17). With respect to perinuclear stacks of annulate lamellae in carp oocytes, a granular product forms a layer equidistant between each lamellae comprising the stack.[1] Furthermore, small granules and filaments appear to be associated with intranuclear annulate lamellae in the tunicate oocyte nucleus (reference 17, Fig. 13).

In a number of cell types, it has been described that ribosomes are not attached to membranes of annulate lamellae (2, 5, 6, 12, 14, 20, 30, 32). However, in several other instances, ribosomes have been described as being directly attached to the membranes of annulate lamellae (11, 16–18). While Ruthmann (32) reported that RNA-containing granules could not be detected in annulate lamellar systems in crayfish spermatocytes, the lamellae do show an intensely basophilic stain; that author indicated that it is "probable that the annulate lamellae are somehow connected with protein synthesis." Merriam (21) has pointed out that annulate lamellae in sand dollar oocytes have a high intrinsic density which does not disappear when the section is bleached with hydrogen peroxide, and hence, they react to the treatment in the same way as the 150 A particles of the heavy bodies. Several studies (1, 11, 28) on various sea urchin oocytes have shown that annulate lamellae may be associated with masses of ribonucleoprotein; the latter probably are derived from the nucleolar material of the nucleus (1). Examples of annulate lamellae that are continuous at their ends with granular endoplasmic reticulum are numerous (4, 6–10, 13, 15, 17, 18, 28, 30–32). These facts, when considered together, imply that the annulate lamellae have some type of role in protein synthesis or perhaps some sort of transcriber function (35).

It is of interest to compare the filaments and granules associated with annulate lamellae to the nuclear and cytoplasmic materials of similar size range. In this connection, the filaments associated with the annulate lamellae are of a size range comparable to that of DNA filaments ($\sim$ 30 A) described in *Triturus* oocyte nucleoli (23) and in the DNA-containing axes of the lateral loops of the lampbrush chromosomes of the same cell (22). They are only slightly smaller than "small" RNP particles and RNA-containing fibrils illustrated and described in certain nucleoli (3). A number of studies have demonstrated the presence of DNA fibrils occurring in electron-lucent regions within the matrix of mitochondria (19, 25–27). When these fibrils appear in a dispersed state, they are of the order of 20–50 A in width. There are, however, no reports of DNA associated with annulate lamellae.

In a study on the identification of glycogen in

amphibian embryos, Perry (29) described glycogen particles ~325 A in diameter which in turn are composed of 40 A grains. However, glycogen does not appear to be directly associated with the annulate lamellae in the three cell types described here.

Additional studies are necessary before any correlation can be made between structures associated with the annulate lamellae and those in the nucleus. However, the possible presence of an RNP particle associated with annulate lamellae that is smaller than the particles associated with the nucleolus and ergastoplasm could account for the reports of basophilia in annulate lamellae in certain cells even though no cytoplasmic ribosomes appear to be associated with these lamellae.

The membranes and pores in annulate lamellae may provide only the framework within which the biochemical machinery necessary for the functional activity of this cytoplasmic organelle is organized. The fibril and small granule herein described may represent only a portion of the biochemical component. The ribosome which has been described as associated with the membranes of annulate lamellae in some cells may represent still another component. It would, in fact, be of considerable interest to know whether, in such cases, the annulate lamellae exert any influence over the formation or activity of the ergastoplasm with which they are often continuous. It is not known at what time the small granules and fibrils become associated with the annulate lamellae during their morphogenesis. However, these two structural components have been observed to be associated with forming stacks of annulate lamellae in *Rana pipiens* oocytes.

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