INTERCELLULAR MIGRATION OF CENTRIOLES IN THE GERMARIUM OF DROSOPHILA MELANOGASTER

An Electron Microscopic Study

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ABSTRACT

A cluster of centrioles has been found in the early *Drosophila* oocyte. Since the oocyte is connected to 15 nurse cells by a system of intercellular bridges or ring canals, the possibility that the cluster of centrioles arose in the germarium from an intercellular migration of centrioles from the nurse cells to the oocyte was analyzed in serial sections for the electron microscope. Initially, all of the 16 cells of the future egg chambers possess centrioles, which are located in a juxtanuclear position. At the time the 16 cell cluster becomes arranged in a lens-shaped layer laterally across the germarium, the centrioles lose their juxtanuclear position and move towards the oocyte. By the time the 16 cell cluster of cells is surrounded by follicle cells (Stage 1), between 14 and 17 centrioles are found in the oocyte. Later, these centrioles become located between the oocyte nucleus and the follicle cell border and become aggregated into a cluster less than 1.5 μ in its largest dimension. The fate of these centrioles in the oocyte is not known. The fine structure of the germarium and the early oocyte is also described.

INTRODUCTION

Animal cells in interphase typically have two centrioles in a juxtanuclear position. This number may vary with the cell type and the stage of the generation cycle of the cell. One or both of the mature centrioles in a cell may be associated with a daughter centriole in the process of generation. Modifications of this basic pattern have been found in a number of instances which are related to the formation of cilia or flagella. For example, a modification of the typical duplicative pattern occurs during spermatogenesis in viviparid snails (Gall, 1961). In the development of the atypical multiflagellate sperm the two typical centrioles be-

come surrounded by a cluster of procentrioles which elongate and become the basal bodies of the multiflagellate sperm. Similarly, large clusters of duplicated centrioles have been reported in ciliated mammalian bipolar sensing cells (Dirksen and Crocker, 1966; Heist and Mulvaney, 1968). In these cells, centrioles are seen in their usual juxtanuclear position and also throughout the length of dendritic processes which extend approximately 40 to 80 μ from the cell body. Observations of centrioles in various positions between the nuclei of the cell body and the plasma membrane of the dendritic process suggest an intracellular migration

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of the centrioles from the cell body to the tip of the process prior to ciliogenesis.

The clustering of centrioles in the atypical sperm of viviparid snails and the extensive intracellular movement of centrioles in bipolar sensing cells serve as examples of exceptions to the typical centriole number and intracellular location in animal cells. The developing oocyte of *Drosophila melanogaster* provides another exception. A cluster of six centrioles has been found in Stage 3 oocytes (Mahowald, 1962). This cluster could have arisen either by duplication of the two preexisting oocyte centrioles or as the result of an *inter*cellular migration of centrioles to the oocyte from the 15 nurse cells joined to the oocyte by intercellular bridges or ring canals. A three-dimensional reconstruction of the 16-cell clusters of cystocytes by means of serial sections was employed as an approach to answering the question of the manner of origin of this centriole cluster in the *Drosophila* oocyte.

MATERIALS AND METHODS

The ovaries of *Drosophila melanogaster*, Oregon R strain, were removed from 3-day-old flies, and the ovarioles were teased apart in cold 1 or 2% glutaraldehyde buffered with 0.1 M phosphate at pH 7.4. The ovarioles were fixed for 30 min at 4°C, washed overnight in 0.2 M sucrose in phosphate buffer, then postfixed for 2 hr at 4°C in either 1% osmium tetroxide in 0.5 M phosphate with 0.08 M NaCl or 1% osmium tetroxide buffered with Veronal acetate and with Zetterqvist's salts. Tissues were dehydrated in ethanol and embedded in Araldite according to Luft (1961). Serial sections of selected germaria were cut on an LKB Ultrotome III (LKB Instruments, Inc., Rockville, Md.) picked up on 0.2% Formvar film-loops, and mounted on 0.2 × 1.0 mm slit grids. Sections were stained with 7.5% uranyl magnesium acetate and with lead citrate (Frasca and Parks, 1965). A thin carbon coat was evaporated on the grids for stability. Each serial section was photographed at ×4000 with an RCA EMU-4 electron microscope.

Since serial sections permit three-dimensional reconstruction of biological structures, this procedure was used to reconstruct the 16-cell clusters in the anterior portion of the ovariole and to determine centriole position within the cells during the various stages of germarial development. From the work of Brown and King (1964), it is known that the 16 cells are interconnected in a regular and predictable pattern (cf. Figs. 6 and 7) so that the centrioles can be localized within a specific cell of the cluster. This procedure makes it possible to determine the sequence of movement of centrioles within the 16-cell clusters during oogenesis.

OBSERVATIONS

Structure of the Germarium

Each ovary of *Drosophila melanogaster* is composed of approximately 20 ovarioles held together by a peritoneal sheath. Each ovariole consists of an anterior germarium and a posterior vitellarium which is composed of successive egg chambers (Koch, Smith, and King, 1967). The most posterior 16 cell cluster of the germarium is designated as Stage 1. The vitellarium contains oocytes in various stages of development from Stage 2 to Stage 14 which is the mature egg (King, Rubinson, and Smith, 1956). King and his colleagues have studied the process in *Drosophila* in great detail, and more recently have analyzed serial sections of the germarium to study the origin of the oocyte in the anterior portion of the ovariole (Koch and King, 1966; Koch, Smith, and King, 1967). However, they have not reported any observations on the distribution of centrioles in the germarium. In our study we have restricted our analysis of serial sections to the location of centrioles within the 16-cell clusters of the germarium and to the proximal postgermarial chambers.

The germarium can be divided morphologically into three regions (Koch and King, 1966) (Fig. 1). The anteriormost region contains the stem cell or cells which divide to form another stem cell and a cystoblast. The cystoblast undergoes four successive divisions to form 16 cystocytes interconnected by ring canals, which presumably result from incomplete cytokinesis (Figs. 1, 2) (Meyer, 1961). The ring canals occur in a regular and predictable pattern such that two cells have four ring canals, two cells have three canals, four cells have two canals, and eight cells have one canal (Brown and King, 1964). The two cells with four ring canals begin meiosis as judged by the presence of synaptonemal complexes in their nuclei, and have been termed pro-oocytes (Koch, Smith, and King, 1967). The second germarial region, that of pronounced mesodermal cell invasion, may in turn be divided into an anterior (2 *a*) and a posterior (2 *b*) portion (Fig. 1) on the basis of the extent of mesodermal cell invasion and the position of the 16 cell cluster within the germarium. In the anterior region (Fig. 1), the clusters are surrounded by only thin strands of cytoplasm of prefollicular cells, and two or more clusters may be positioned across the width of the germarium. In the posterior region (Figs. 1, 3), follicle cell invasion is more extensive
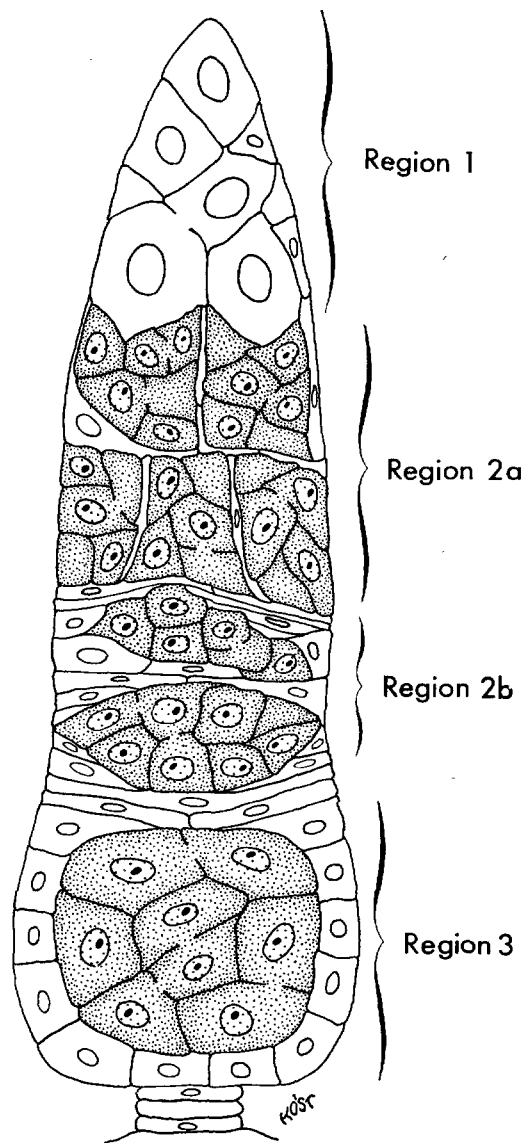
Region 1

Region 2a

Region 2b

Region 3

FIGURE 1 Drawing of the morphological regions of the germarium of *Drosophila melanogaster*. The large clear cells at the tip of the germarium are the stem cell and cystocytes of Region 1, whereas the smaller clear cells represent the prefollicular and follicular cells.

and the cluster is arranged laterally across the entire width of the germarium in the shape of a convex lens. In the third germarial region (Fig. 1), the cells are now arranged in a cluster surrounded by a cuboidal layer of follicle cells.

Although some features of the fine structure of the germarium have been described (Koch and

King, 1966; Koch, Smith, and King, 1967; King, Aggarwal, and Aggarwal, 1968), a number of further ultrastructural characteristics of both the mesodermal and germinal tissue of the germarium should be noted. The mesodermal or prefollicular cells are found along the periphery of the germarium in Region 1, although thin projections of these cells extend between some of the cystoblasts. The cytoplasm of these cells is sparse (Fig. 2), but this appearance is probably due to fixation damage. The germarium is bordered by a homogeneous tunica propria, and external to this border are bands of musculature which circumvent the germarium. Usually only the prefollicular cells come into direct contact with the tunica propria, but in places the cystocytes contact it directly (Fig. 2). In Region 2 (Fig. 3), the prefollicular cells have penetrated between the clusters of cystocytes and have become arranged as thin bands of cytoplasm separating the clusters from each other. These long processes never penetrate within a cluster. Microtubules are commonly found running the length of these processes. In addition to the usual organelles characteristic of active cells (mitochondria, plentiful ribosomes, and a prominent nucleolus), a multivesicular body (Figs. 3, 4) is present. Germinal cells occasionally have similar vesicles (Fig. 3, below long arrow) but these vesicles always contain dense inclusions or myeloid material, both of which are absent in the multivesicular bodies of prefollicular and follicular cells.

The germinal cells or cystocytes of Regions 1 and 2 possess a very dense cytoplasm (Figs. 2–4), lack appreciable amounts of endoplasmic reticulum (ER) although ribosomes are plentiful, and possess many Golgi regions. The presence of densely-staining fibrous material on the outer nuclear membrane (Figs. 2, 3) distinguishes these cells from the nongerm line cells in *Drosophila*. These fibrous bodies frequently are bordered on the cytoplasmic surface by ER which bears ribosomes only on the membrane facing the cytoplasmic matrix (Figs. 2, 3). Presumably these structures are the source of the fibrous bodies surrounded by ER which are frequently found in cystocytes (Fig. 3, long arrow). These fibrous structures in cystocytes are probably the same type of material which results from the fragmentation of polar granules in the embryo.[1] They are not

[1] Mahowald, A. P. 1970. Polar granules of *Drosophila*. III. The Continuity of polar granules during the life cycle of *Drosophila*. Manuscript in preparation.
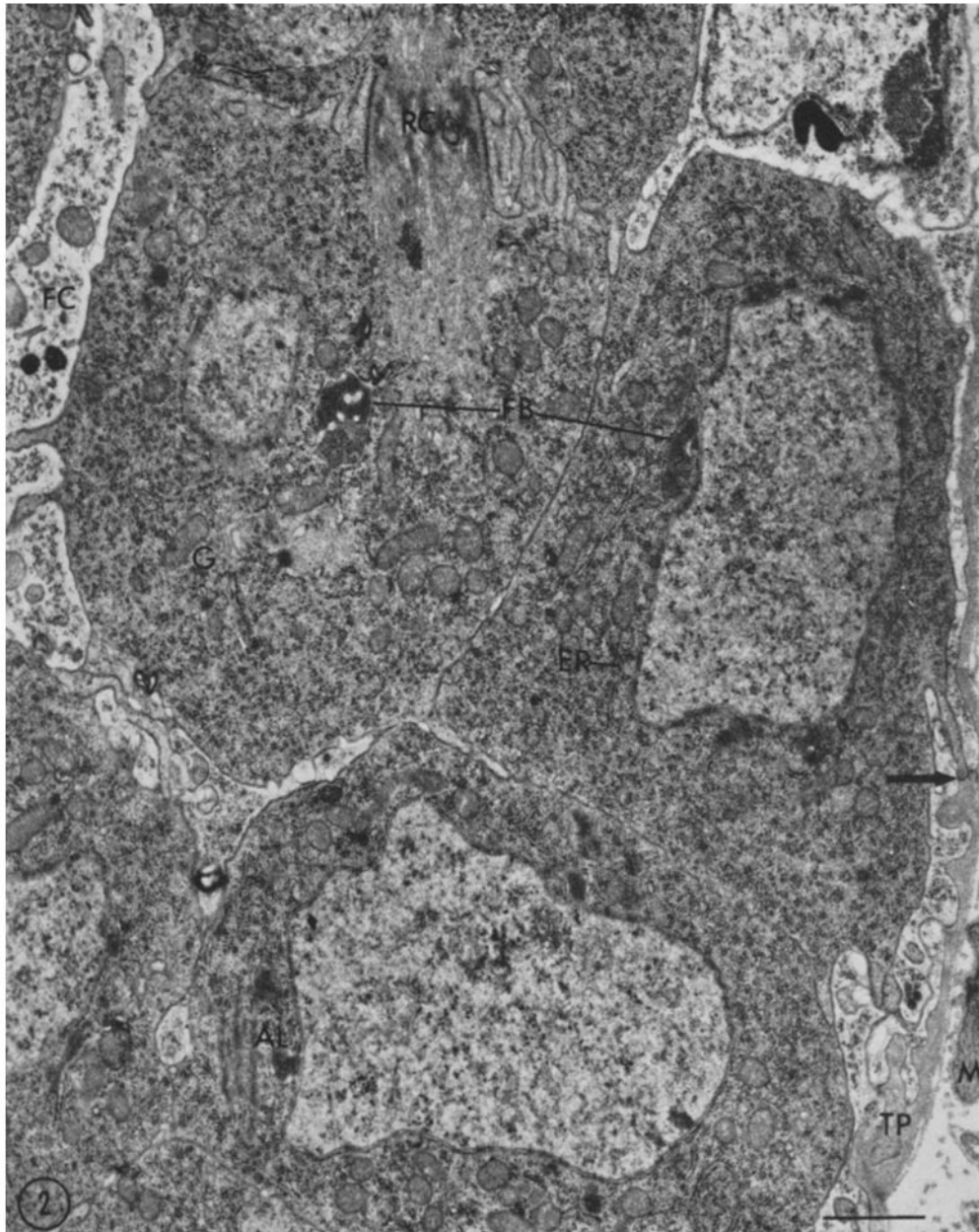
FIGURE 2   Electron micrograph of a longitudinal section of Region 1 of the germarium. The large dense cells are the cystocytes during the time of the four divisions which produce the cluster of 16 cells. Two cystocytes are joined by a newly formed ring canal (RC) which is still filled with microtubules resulting from the mitotic spindle. Fibrous bodies (FB) are attached to the outer nuclear membrane of cystocytes and to the endoplasmic reticulum (ER). Annulate lamellae (AL) are common in these cells. Golgi complexes (G) and mitochondria are typical. Mesodermal or prefollicular cells (FC) are found along the periphery of the germarium, although processes extend into the anterior region separating the different clusters of cystocytes from one another. The germarium is bordered by a homogeneous tunica propria (TP), and external to this border are bands of musculature (M) which run around the germarium. The arrow indicates a place of contact of the cystocytes with the tunica propria. The magnification line is 1 μ unless noted differently. $\times$ 13,500.
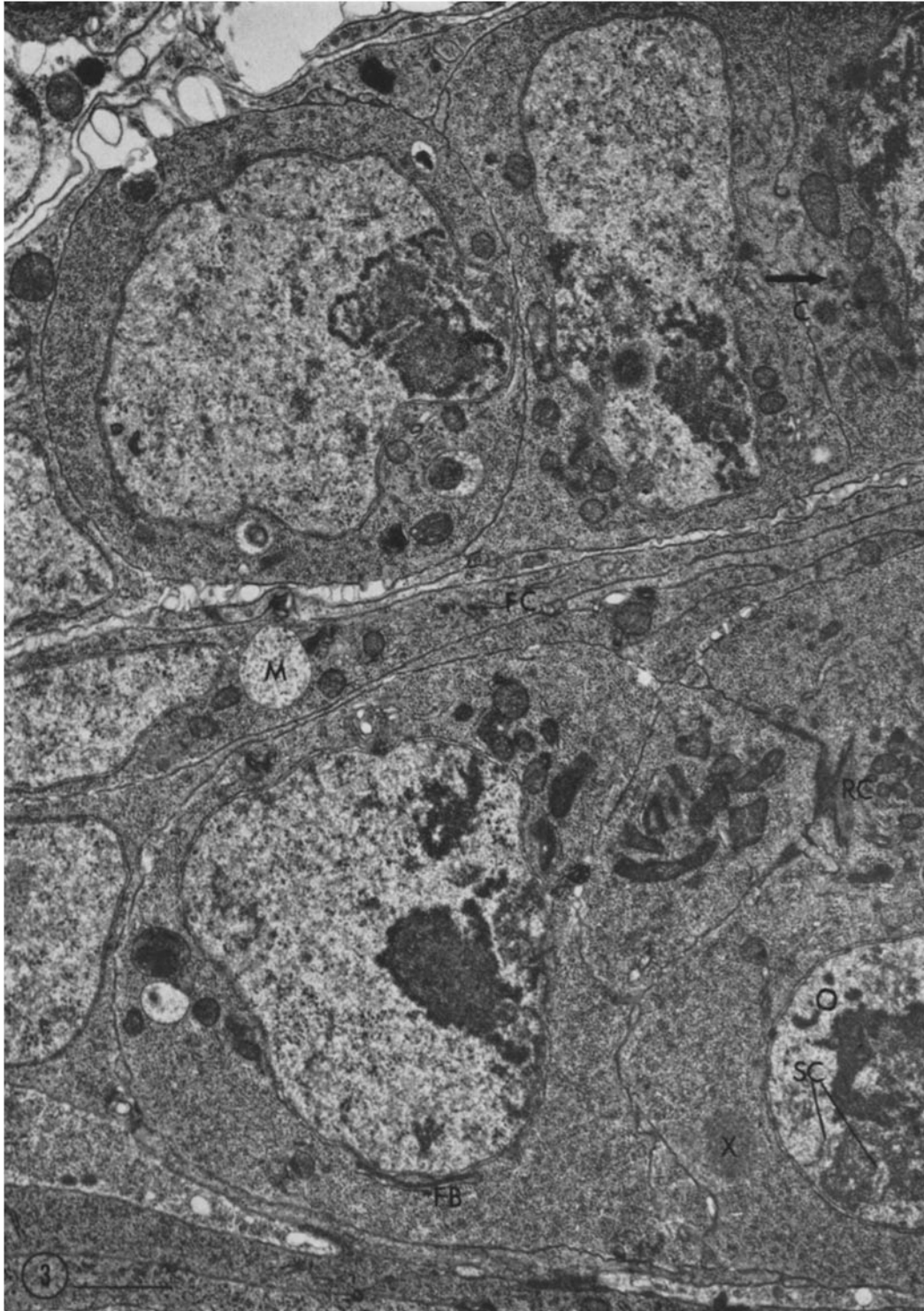
FIGURE 3   Electron micrographs from the serial sections of two 16-cell clusters (Figs. 7 a, 7 b) from Region 2 b of the germarium. The younger of the two clusters is at the top of the figures. In one cell of this cluster a transverse section of a centriole (C) is found adjacent to a ring canal, and a second centriole (arrow) is seen in oblique section adjacent to the first centriole. The oocyte for this cluster is out of the plane of section. In the older cluster at the bottom of the figures the oocyte (O) is present. Two oblique sections of ring canals (RC) are present above the oocyte nucleus. Mitochondria are clustered in
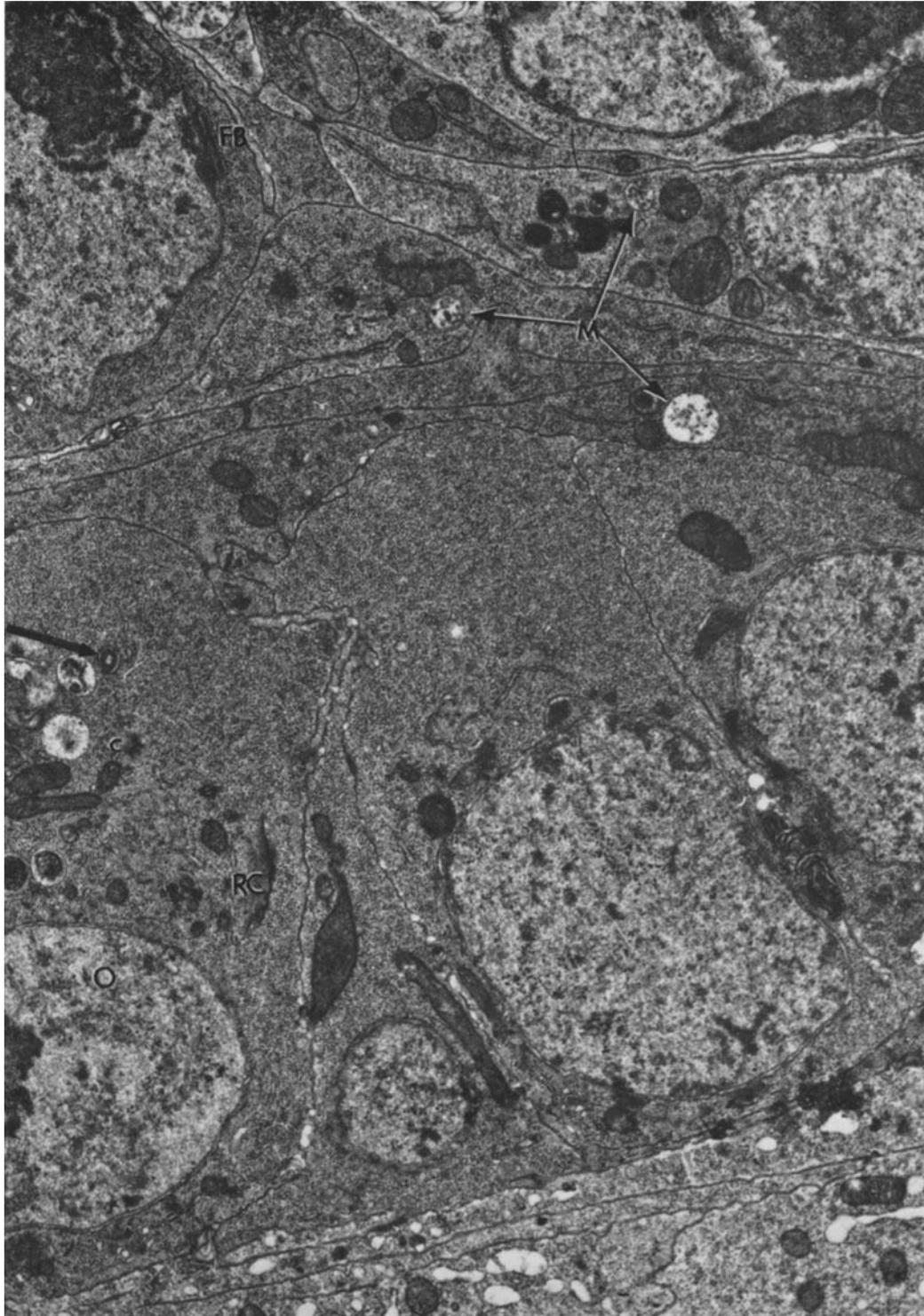
this region, and in serial sections 15 centrioles were found in this area (one centriole [c] is shown in oblique section). Long bands of fibrous material (FB) are present on the nuclear membrane of the cystocytes, and frequently these bands possess ER along their cytoplasmic border. A ribosome-free area is present in the oocyte (X). The prefollicular cells (FC) are arranged as thin bands of cytoplasm separating the clusters from each other. Multivesicular bodies (M) are common in these cells. Synaptonemal complex (SC). × 14,500.

present in nongerminal cells. The amount of this material found on the nuclear membrane is approximately equivalent for all of the cystocytes in Region 1, but in Region 2 b less material is found along the nuclear envelope of the two pro-oocyte nuclei. In Region 3, the nuclear envelope of the true oocyte has completely lost this material. The nurse cells retain it throughout oogenesis. Its possible fate is presented elsewhere.[1]

A ribosome-free area is present in the oocyte (Fig. 3). This is the same structure described previously in a Stage 3 oocyte (Mahowald, 1962, Fig. 12). In the serial sections of the germarium it has only been found in the oocyte, but in a postgermarial stage it has been found in a nurse cell. Its significance is unknown.

The formation of ring canals has not been studied in detail in *Drosophila* ovaries. However, in one of the germaria studied, two of the cystocytes have just completed mitosis, and cytokinesis has become arrested around the spindle remnant (Fig. 2). The rim of the canal has already accumulated the electron-opaque material that is characteristic of these canals (Meyer, 1961). The amorphous material that borders the inside rim of the canal (Figs. 3–5) has not yet formed. The ring canals increase in size according to the age of the 16 cell cluster bearing them. In Region 2 a, all the ring canals are less than 1 μ in diameter, except for the canal which joins the two pro-oocytes which is more than 1 μ. In Regions 2 b and 3, the canal between the two pro-oocytes is approximately 2 μ in diameter, while the other canals vary from below 1 μ to about 1.5 μ in diameter. Since mitochondria,

Golgi complex, ER, and centrioles (see below) can be found within the canals as early as Region 2 a, it is clear that the nutrient stream between the future nurse cells and the oocyte begins in this region of the germarium. Microtubules are found in all cystocytes, but they do not show any clear orientation with respect to the ring canals or other cellular structures.

After the completion of the fourth and last cystocyte division and another period of DNA synthesis (Chandley, 1966), the two cells with four ring canals form synaptonemal complexes. While the pro-oocytes are in Region 2, the synaptonemal complexes are attached to the inner nuclear membranes of these cells. These two pro-oocytes are also distinguishable from the 14 prospective nurse cells by their slightly larger size and by the fact that their outer nuclear membrane possesses less fibrous material than is found on the prospective nurse cells. These differences between the pro-oocytes and the nurse cells increase as the cluster progresses in Region 2. All of the cystocytes have prominent nucleoli during the time that they are in the germarium (Figs. 3–5).

The oocyte proceeds through a regular sequence of changes in position within the 16-cell cluster as it passes through Region 2 into Region 3. In Region 2 a the pro-oocytes are located at random within the 16 cell cluster (Koch and King, 1966), and the clusters themselves are irregularly shaped and do not stretch completely across the germarium (Fig. 1). In Region 2 b the 16 cell cluster becomes arranged as a lens-shaped layer of cells across the germarium (Figs. 1, 3), and the pro-

FIGURE 4   Longitudinal section of Stage 1 oocyte (Fig. 7 c) showing special mitochondrial cluster in the region of the entry of the four ring canals, two of which are just out of the plane of section (arrows). Golgi complex (G) and ER are present in the mitochondrial cluster. In this oocyte, 14 of the 17 centrioles found were located within a 3 μ region at the base of the cell (C) adjacent to the follicle cell border. The other three centrioles were scattered, two of them being located adjacent to the nuclear envelope and one adjacent to a ring canal. The nucleolus (Nu) is still present in the oocyte nucleus. Fibrous bodies (FB) are found along the nuclear envelopes of nurse cells, but not on the envelope of the oocyte or follicle cell nuclei. Portions of four nurse cells (NC) can be seen bordering the oocyte on the left, and five follicle cells (FC) on the right. × 15,000.

FIGURE 5   A cross-section through the supranuclear region of Stage 1 oocyte showing the four ring canals (1–4) which join the oocyte in this region. Because the germarium containing this oocyte was not studied in complete serial sections, it is not known which canal originated at the first division, or the second and so on. Two other ring canals (RC) are found in this picture and in one a centriole (C) is located within the canal. The nuclei present in the figure are from adjacent nurse cells. × 16,000.
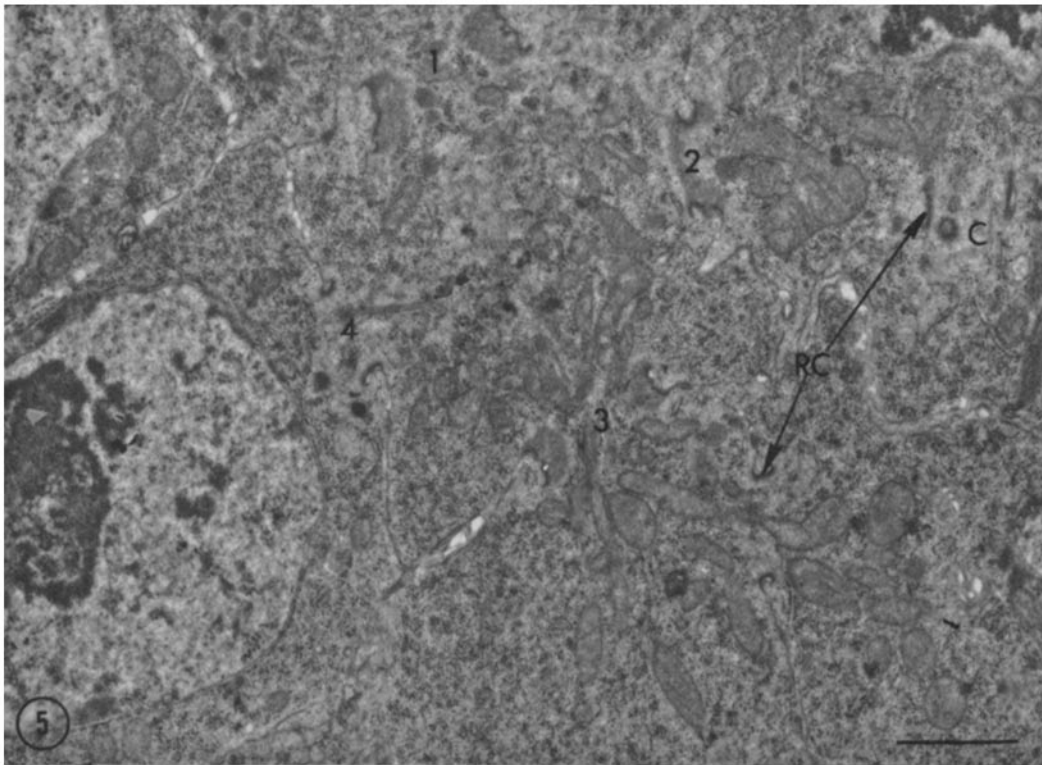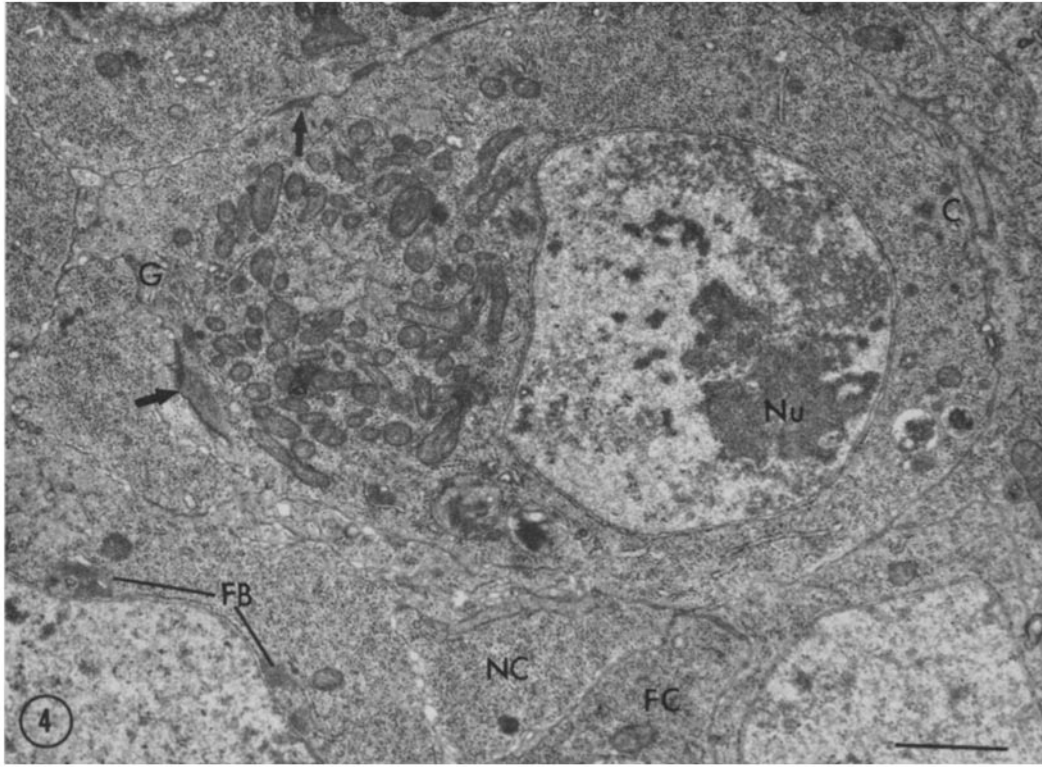
oocytes are always situated in the center of the "lens." Presumably this is due to the fact that seven other cystocytes are attached to each pro-oocyte, and the pro-oocytes to one another. When the cluster becomes lens-shaped, the best steric arrangement is to have the two central cells of the complex located in the center of the lens. As the 16 cell cluster moves into Region 3 of the germarium, it becomes transformed into a ball of cells (Fig. 1). Since the pro-oocytes are always the most posterior cells of this ball of cells, the change in organization is probably accomplished by the lens-shaped layer folding downward in the middle, moving the pro-oocytes from a central location in the layer to a posterior position in the ball of cells. The ovariole musculature seems capable of producing this type of cellular rearrangement. The germarium has rings of circular muscle fibers (Koch and King, 1966; cf. Fig. 2) which upon contraction would produce a downward pressure, much like squeezing a tube of paste. This would result in the type of folding postulated above.

In Region 3 or the Stage 1 oocyte, certain clear changes appear. In addition to the centriolar movement (see below), the oocyte loses all the fibrous material attached to the nuclear membrane while the number 2 cell (the former pro-oocyte) retains some of the fibrous material on the nuclear membrane. The synaptonemal complexes of the oocyte are no longer attached to the nuclear membrane and are not so abundant in thin sections as in Region 2, and the complexes in the former pro-oocyte have disappeared almost completely. Moreover, the oocyte has now a large cytoplasmic region which is filled with mitochondria and other cytoplasmic organelles (Fig. 4). In a cross-section through this region (Fig. 5) it is possible to show the presence of the four ring canals and the great accumulation of mitochondria in this region. This accumulation of mitochondria is obviously due to the nutrient stream from the nurse cells. At this time the microtubules do not show any preferential arrangement in relation to the nutrient stream, although some type of organized arrangement would be expected if they had a role to play in this flow of material.

In the postgermarial clusters, the changes noted in the Stage 1 oocyte continue. The synaptonemal complexes gradually disappear while the chromosomes become compacted into the karyosome (Smith and King, 1968). The nucleolus of the oocyte disappears and a round granular structure remains (Mahowald and Tiefert, 1970) which may

be identical to the endobody described in oocyte nuclei of insects by Bier, Kunz, and Ribbert (1967). The cytoplasm in the oocyte continues to increase in amount. The former pro-oocyte redifferentiates into a nurse cell, although it is still distinguishable from the other 14 nurse cells because of the smaller amount of fibrous material on its nuclear membrane.

Distribution of Centrioles

11 16-cell clusters from three germaria were studied in serial sections. In addition, three oocytes from postgermarial chambers were sectioned and studied. The results of observations on eight 16-cell clusters from two germaria are summarized diagrammatically in Figs. 6 and 7. The four clusters represented in Fig. 6 and the last cluster in Fig. 7 are from one germarium. The first three clusters in Fig. 7 are from a second germarium.

In the anterior part of the second germarial region (2 a), the 16-cell clusters have already formed, synaptonemal complexes are forming in the two pro-oocytes, and the cell clusters are separated from adjacent ones by thin cytoplasmic strands of prefollicular cells. Prior to the intercellular movement of centrioles, a change in their intracellular location can be observed. In the younger cluster of cystocytes of Region 2 a (Fig. 6 a) the centrioles are, for the most part, in a juxta-nuclear position, while in the next cluster of the same germarium (Fig. 6 b) nearly all the centrioles are located adjacent to the cell membrane, usually in the vicinity of the ring canals. At this same time most centrioles are no longer arranged at right angles to one another, although two centrioles are frequently found together.

The major centriolar movement occurs in the posterior portion of the second germarial region. The age difference between successive 16-cell clusters within one germarium is approximately 12 to 24 hr (Chandley, 1966), so that a random sample of germaria would yield clusters of different ages. If the extent of centriolar migration is used as an index of the age of the cluster within Region 2 b, a sequence of centriolar movements is apparent. In the youngest clusters found in this region (Figs. 7 a, 6 c) there is a clear indication of movement toward one of the two pro-oocytes (cell number 1), and in an older cluster of this region (Fig. 7 b) the accumulation of centrioles in the oocyte appears to be nearly complete. 15 centrioles are found in the cytoplasmic region where the four ring canals join the oocyte (Fig. 3). This localization of centrioles
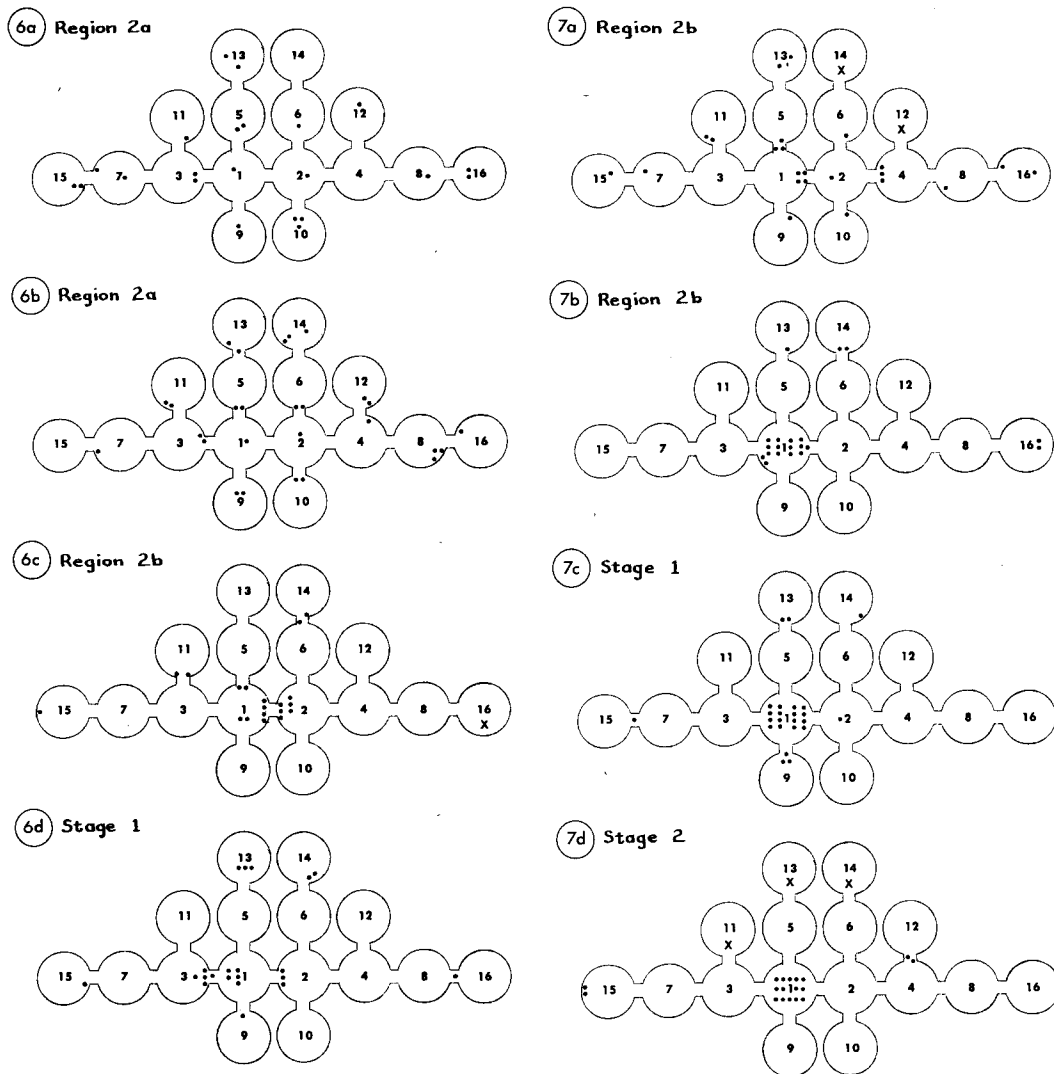
FIGURES 6 and 7 Diagrammatic representations of the 16-cell clusters, after Brown and King (1964). Cells 1 and 2 are the pro-oocytes. After centrioles have begun to move intercellularly, cell 1 is recognizable as the oocyte. The small dots represent centrioles which were clearly identified as centrioles, either because the section was in a transverse direction or because the centriole was found in three adjacent sections and could be clearly identified as a centriole. Procentrioles and doubtfully identified centrioles are not included in these illustrations. If the centrioles were juxtanuclear

in location, the dots are placed adjacent to the number of the cell, whereas if they were adjacent to a ring canal they are drawn next to the circle or in the canal depending upon the location. In the oocytes of the cluster in Fig. 7 d all of the centrioles were aggregated together (Fig. 9), and in Fig. 7 c 14 of the 17 centrioles were located between the oocyte membrane and the follicular cell border (Figs. 4, 8). Cells marked with X were missed in the serial sections. The clusters of Figs. 6 and 7 d are from one germarium, and the clusters of Figs. 7 a, 7 b, and 7 c are from a second germarium.

was also found in one of the Stage 1 (Region 3) oocytes of one germarium (Fig. 6 d), while in the second germarium studied (Fig. 7 c) 14 of the 17 centrioles of the Stage 1 oocyte were localized in

the posterior region of the oocyte, between the nucleus and the follicular cell border (Figs. 4, 8), in a disk-shaped area 2.5 μ in width.

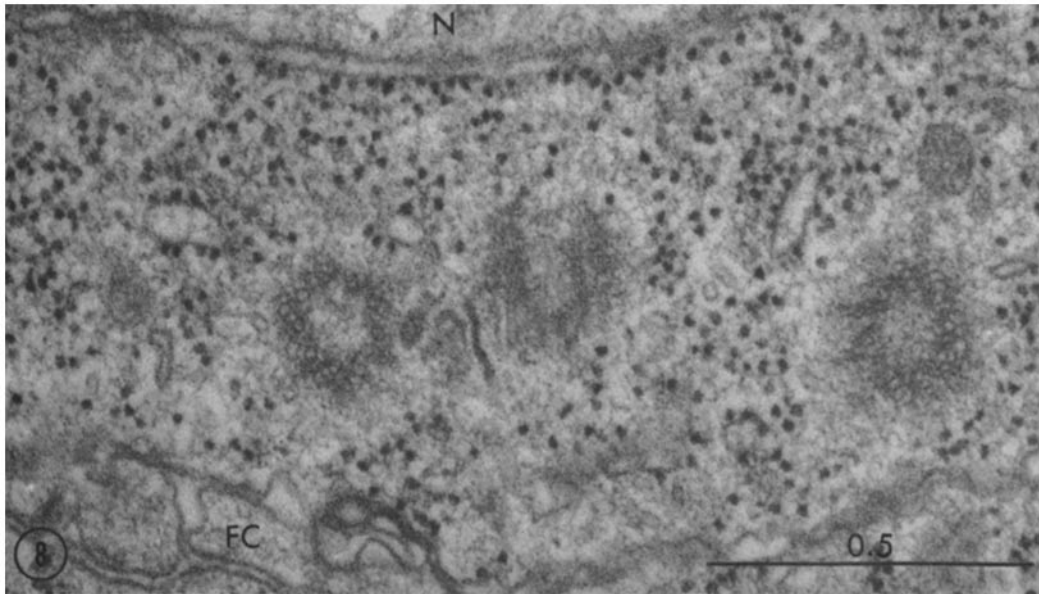In the postgermarial oocytes studied all the

FIGURE 8   High magnification micrograph of three of the centrioles in the oocyte of a Stage 1 cluster of cells (Fig. 7 c). The centrioles are located between the oocyte nucleus (N) and the follicle cells (FC). At this stage, the centrioles have not yet come into contact with each other. × 87,000.

oocyte's centrioles become localized within the same region as in late Stage 1 oocytes, and in its dimensions the cluster of centrioles becomes more compact. In the Stage 2 oocyte studied the centriolar complex was 1.3 μ in width, and in the Stage 4 studied the complex was only 1.2 μ in width. In these later stages of centriolar aggregation, individual centrioles become continuous with neighboring centrioles (Fig. 10), but in none of the instances examined were all of the centrioles fused in this manner (Fig. 9). The centrioles still retain the typical ribosome-free area surrounding them (Figs. 8–10). During this sequence of movement and aggregation of centrioles, microtubules were not found associated with either individual or aggregated centrioles.

The fate of this centriolar complex has not been determined. Because of the area of the cluster (less than 2 μ across), it would be tedious to locate centrioles in serial sections in older stages of the oocyte.

The structure of the centriole does not appear to change during these migrations. The centriole in Drosophila is typically composed of nine sets of triplet tubules (Fig. 8). There is always a single central tubule (Mahowald, 1963), 180 A in diameter. Adjacent to many of the centrioles, both before

migration and after the centrioles have clustered in the oocyte, procentrioles (Gall, 1961) are frequently found. It is doubtful that more centrioles are being formed, because the total number of centrioles found in the 16 cell cluster decreases during the course of the migration. In the oldest chamber examined (Fig. 7 d) 18 centrioles were found, while prior to the migration (Fig. 6 b) 27 centrioles and during the migration 25 centrioles were found (Fig. 7 a). It is possible that the procentrioles were formed prior to the cellular changes which allowed centrioles to move from their juxtanuclear position, and that further development was arrested.

DISCUSSION

The centriolar migration, accumulation, and localization in the oocyte takes place during the 3 day period in which the 16 cell cluster is passing through the germarium and the following early chambers of the vitellarium. Distinct features of this centriolar movement can be recognized. In the early 16-cell clusters of the anterior portion of germarial Region 2, two centrioles are found in the typical juxtanuclear position in each cell, but in the older clusters of this region the centrioles have lost their juxtanuclear location and are now found
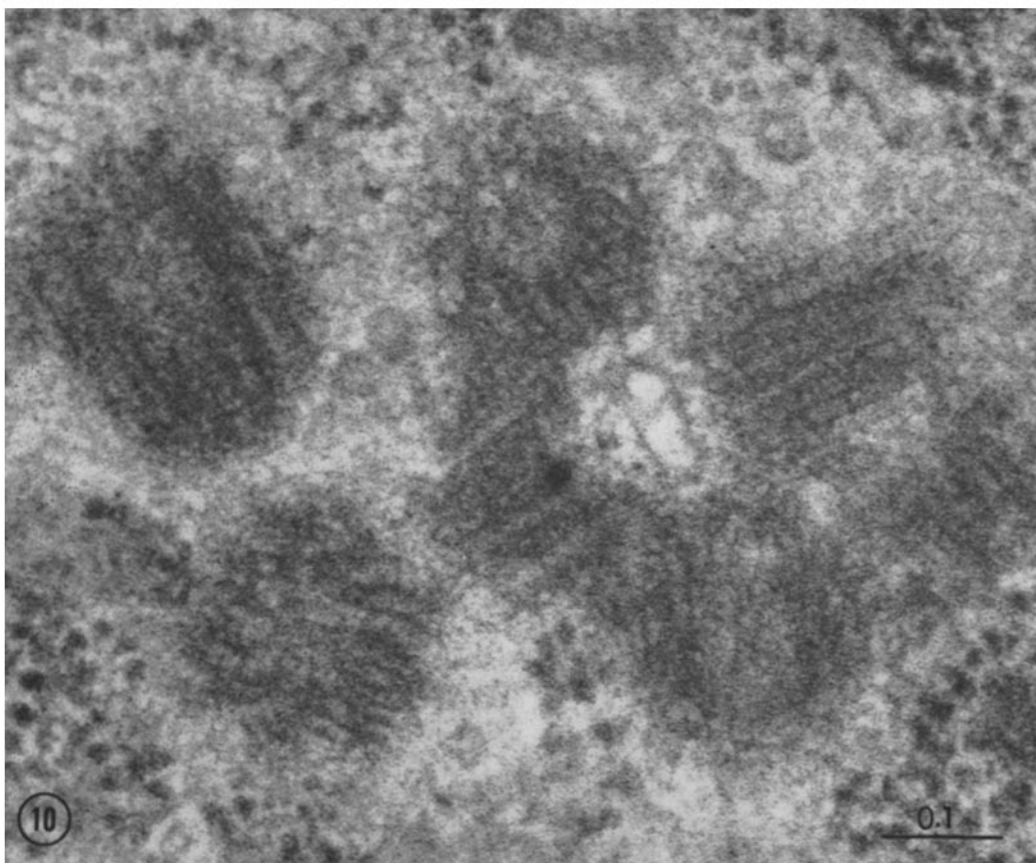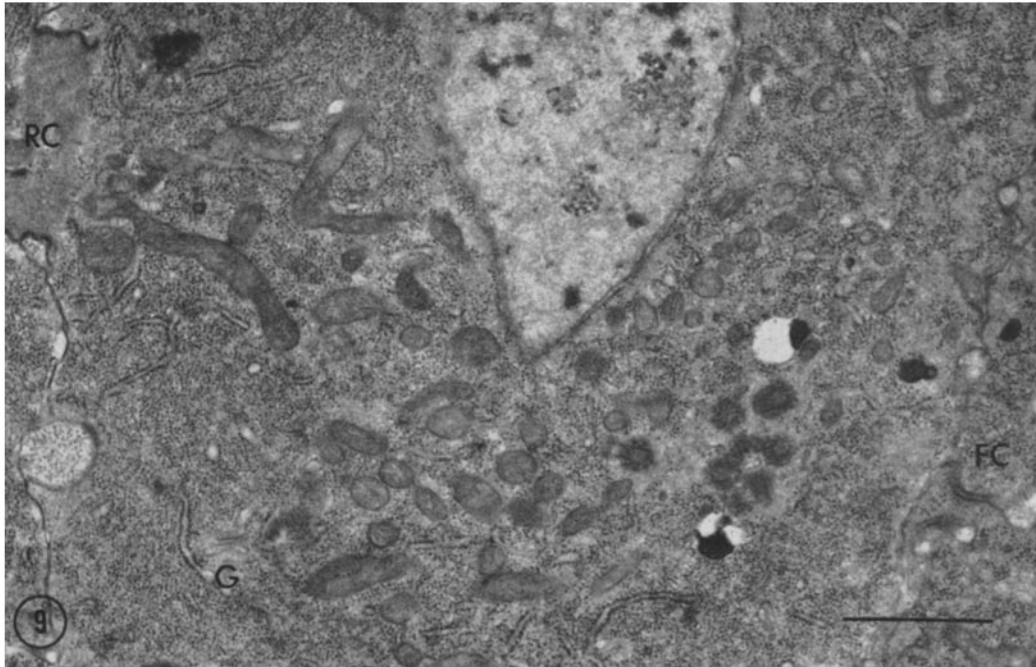
FIGURE 9   Low magnification of the oocyte in a Stage 2 chamber. The centrioles are located between the nucleus and the follicle cell (FC) border. Individual centrioles have begun to "fuse." Golgi complex (G) and ring canal (RC). × 20,000.

FIGURE 10   High magnification of the centriole cluster in Fig. 9, showing the continuity between adjacent centrioles. × 160,000.

adjacent to the plasma membrane, usually adjacent to a ring canal. Subsequently, the centrioles accumulate in one cell so that this characteristic distinguishes the future oocyte from the companion pro-oocyte prior to other distinguishing characteristics. And then finally, within the oocyte the centrioles aggregate into a centriolar complex which is located away from the point of entry into the oocyte.

A morphological analysis of serial sections does not give much information on the kinds of forces that could be responsible for this movement and aggregation of centrioles. But from this study we can distinguish certain features. Since centrioles are ordinarily located adjacent to the nucleus in a centrosome region, the first change appears to be a loss of the control for this location. Much of the accumulation of the centrioles in the future oocyte could be explained as a passive movement in the nutrient stream towards the oocyte. At the time of centriole movement (Region 2 *b*) the pro-oocytes are located in the center of a lens-shaped cluster of cells. The cause of the nutrient stream probably is the contraction of the circular musculature surrounding the germarium, and presumably the direction of movement is toward the center of the germarium (the location of the pro-oocytes) and in a posterior direction since the clusters themselves ultimately move in a posterior direction. Although the volumes of individual cells of the cluster have not been calculated, it is clear, both from the size of the pro-oocytes in individual sections and from the number of thin sections which contain these two cells, that these cells are the largest ones of the 16 cell cluster. Presumably, this is due to a movement of cytoplasm into these cells from the remaining cells of the cluster. Thus the centrioles could be moved passively by this nutrient stream to the pro-oocytes. Since the difference between the two pro-oocytes is not evident at the time the centrioles become accumulated in one of the cells, it is possible that some more specific cause accounts for this accumulation in one pro-oocyte. The nature of this force is unknown.

The localization of centrioles within the oocyte between the nucleus and follicular cell border appears to be unrelated to the nutrient stream. The mechanism producing this localization is unknown. A reasonable hypothesis for the aggregation of centrioles (as distinguished from their specific localization adjacent to the follicle cells) is that the ability of centrioles to be associated in

double becomes reestablished. This "attractive force" arranging centrioles together might be capable of gathering and retaining all of the oocyte centrioles in one location. Not all of the nurse cell centrioles become located within the oocyte. The reason for this lack of uniformity is not known.

This is the first description of an *inter*cellular movement of centrioles, so far as is known. Many centrioles are found in a variety of cell types prior to ciliogenesis (Heist and Mulvaney, 1968) and to flagellogenesis (Gall, 1961), but in these instances the multiple centrioles arise by duplication of the centrioles present within the cell and they become the basal bodies of cilia or flagella.

Possible Functions of Centriolar Migration

King and his colleagues have made extensive studies of the germarium of *Drosophila* ovaries in order to discover the mechanisms active in selecting one of the 16 cells, which result from the four cystocyte divisions, to become the oocyte (King, 1964; Brown and King, 1964; Koch and King, 1966; Koch, Smith, and King, 1967). Since both cells with four ring canals begin meiosis, those authors have postulated that the presence of these canals is critical in the initial differentiation of two cells of the 16 cell cluster into pro-oocytes. The subsequent selection mechanism which provides that one pro-oocyte becomes the true oocyte is the amount of contact present between the oocyte plasmalemma and the follicle cells. Koch and King (1968) have made three-dimensional models of a Stage 1 chamber and have found that the oocyte has seven times more area of contact with follicle cells than does the pro-oocyte which re-differentiates to become one of the nurse cells. Thus they postulate that the plasmalemma interaction between the oocyte and follicle cells is responsible for determining which of the two pro-oocytes becomes the true oocyte.

In our further analysis of the germarium with serial sections for the electron microscope, two further differences have been noted which occur at the same time that one pro-oocyte is selected for oocyte development. The first observation is that as soon as the centrioles begin to move intercellularly, the future oocyte already is distinguishable. In the earliest stages found one of the two pro-oocytes has more centrioles, and the centrioles present in the other pro-oocyte are located principally in the large ring canal joining the two cells. It is not known whether at this time there is a

difference in the amount of contact between the oocyte and the follicle cells, but it is clear that these two cells are already different from one another at this stage. The second observation concerns the presence of fibrous bodies located on the nuclear envelope. These structures have the same ultrastructure as the fibrous bodies which appear on the nuclear envelope at the time of the fragmentation of the polar granules in the embryo[1] (Mahowald, 1968). During oogenesis these fibrous bodies are found on the nuclear envelopes of the 15 nurse cells but are absent from the oocyte throughout oogenesis. In the germarium they are present on all 16 cystocytes until Stage 1 when the oocyte has lost these structures. However, earlier in Region 2 b the amount of this material on the two pro-oocytes is distinctly less than on the 14 nurse cells. At Stage 1, the number 2 cell, which becomes a nurse cell after having lost synaptonemal complexes, has a diminished amount of this material as compared to the other nurse cells, and the oocyte has none. Thus the loss of this distinctive material may also have a role to play in cellular determination. Probably the most satisfactory manner of considering the mechanism of oocyte determination is to view it as involving all three of these observations: centriolar accumulation, loss of fibrous material from the nuclear envelope, and finally contact with the follicular epithelium. Which of these factors is the principal one in determining the oocyte and which are secondary results of this determination is difficult to determine. The selec-

tion mechanism could be the location of the cell in the lens-shaped cluster in Region 2 b which makes the cell most able to expand as the nutrient stream begins. The accumulation of centrioles would occur because the nutrient stream would cause cellular material to accumulate in that cell, and the greater size of the cell would produce increased contact with the prefollicular epithelium. Thus, a series of events may be initiated which stabilize the oocyte in its future development and which result at the same time in the redifferentiation of the second pro-oocyte into a nurse cell.

The fate of the centriole cluster in the oocyte after Stage 4 is unknown. Since the number of centrioles found in Region 2 a prior to migration is double that found in the cluster, it is possible that centrioles are breaking down during these stages of oogenesis. Since the maturation divisions of the mature egg lack centrioles (Huettner, 1933), probably all the centrioles disappear as recognizable and functional structures. There is some evidence in echinoderm eggs that centrioles may appear in the activated egg (Dirksen, 1961), but it is unknown whether this is also true of insect eggs, and specifically the *Drosophila* egg.

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