GIANT CENTRIOLE FORMATION IN SCIARA

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ABSTRACT

Although somatic tissues of Sciara contain 9-membered centrioles, germ line tissues develop giant centrioles with 60–90 singlet tubules disposed in an oval array. Some 9-membered centrioles still may be seen in second instar spermatogonia. Each of these centrioles is associated with a larger "daughter" or secondary centriole at right angles to it. Most centrioles of second instar spermatogonia consist of 20–50 singlet tubules arranged in an oval, sometimes associated with an even larger secondary centriole. The more recently formed centriole of a pair is distinguishable from its partner by a concentric band of electron-opaque material inside its tubules. If a pair of centrioles at right angles to each other is pictured as a "T" formed by two cylinders, the secondary centriole is always the stem of the T; the primary centriole is the top. The two centrioles are oriented at the pole of the mitotic spindle so that the tubules of the primary centriole are parallel to the spindle axis. Each daughter cell receives a pair of centrioles and, during interphase, each of these centrioles gives rise to a new daughter centriole. A Golgi area of characteristic morphology is found in association with centrioles shortly after two new ones have formed. We conclude that in Sciara a centriole may give rise to a daughter morphologically different from itself. Whether the daughter is a 9-membered or giant centriole depends on the tissue type and stage of development.

Centrioles were familiar to early cytologists as minute granules which could be identified at the focus of the astral rays after iron-hematoxylin or crystal violet staining. They classically were implicated as functioning in cell division and flagellar formation (17, 23, 28). The structure of centrioles, which are apparently present in all metazoan cells capable of division, has been clarified by the use of electron microscopy, but, beyond this, modern techniques have failed to reveal much more about these apparently indispensable cell components than already was assumed by many biologists half a century ago. The intractibility of centrioles to modern analytical techniques is due partially to their relatively small size and low number per cell that make electron microscopic studies rather arduous and cytochemical procedures extremely difficult to apply and hence contribute to the lack of success of isolation attempts necessary for biochemical analysis.

The mode of centriolar perpetuation has been particularly puzzling. Centrioles often are described as self-replicating bodies; indeed, forming centrioles generally are found only in close proximity to "mature" centrioles. But proof of any sort of genetic continuity between "mother" and "daughter" centrioles has been very elusive since, in any given species, all the centrioles look alike. No "mutant" centrioles have been described. Therefore, when variations in centriolar form were encountered during a study of spermiogenesis in the fungus gnat Sciara, it was felt that a unique opportunity was thereby provided for studying the extent to which differences in centriole morphology were propagated from parent to daughter organelle.

The somatic tissues of Sciara contain only 9-membered centrioles (26); these differ slightly from centrioles of other organisms, in that they are composed of nine doublet rather than the usual
nine triplet tubules. The spermatogonial centrioles of fourth instar larvae, however, differ markedly in size and structure from the familiar 9-membered centriole. These centrioles are composed of about 70 short, evenly spaced singlet tubules displaced in an oval which has axes of about 1.0 and 0.4 μ (26), in contrast to the 0.15 μ diameter of typical 9-triplet centrioles (10). Though their morphology is unusual, these giant centrioles exhibit the diagnostic characteristics of true centrioles: they are found in pairs at right angles to one another; they are situated at the poles of the mitotic and meiotic spindles; and they are capable of serving as basal body of a flagellum whose tubule array reflects their unusual tubule pattern (26, 27).

The present investigation was undertaken to determine the mode of origin of giant centrioles in Sciara, an organism containing mainly 9-membered centrioles, with the hope of enlarging our comprehension of the general process of centriole formation.

MATERIALS AND METHODS

The strain of Sciara coprophila used in this study is monogenic, i.e., the eggs of a given female develop into either all male or all female progeny (22). Whether the offspring are male or female depends upon whether the mother carries an X chromosome with the female-determining trait. A dominant mutation, wavy wings, which is closely linked to the sex-determining locus on the X chromosome, enables one to predict which females will bear female and which will bear male progeny (6, 7).

Larvae were selected from matings of known female producers or male producers. Larval gonads (testes or ovaries, depending on the culture) were fixed for 1–4 hr in 2.5% glutaraldehyde buffered in 0.05 M Sorenson’s phosphate buffer (pH 7), rinsed several times in 0.1 M phosphate buffer (pH 7.6), and postfixed for 1–2 hr in 1% OsO₄ in 0.1 M phosphate buffer (pH 7.6). Tissues were dissected in cold (0–4°C) fixative, and subsequent fixation was carried out in the cold. Tissues were dehydrated in cold ethanol and embedded in Epon 812 according to Luft (21). Sections

Figrues 1–4 Transversely or nearly transversely sectioned 9-membered centrioles adjacent to longitudinally cut giant centrioles in second instar Sciara spermatogonia. The doublet nature of the centriolar elements can be most clearly discerned in Fig. 1. × 105,000.
RESULTS

Nine-Membered Centrioles in Sciara

Somatic tissues of *Sciara* embryos and larvae appear to possess only 9-membered centrioles. Although most of the centrioles of male second instar germ line tissue are giant centrioles, we always find some 9-membered centrioles in these early testis cells. In order to positively identify a centriole as the 9-membered type, the centriole must appear in cross-section or near cross-section. We have observed cross-sectional profiles of 15 9-membered centrioles in spermatogonia of second instar larvae. In the most favorably transected centriole (Fig. 1), the tubular components on one side of the centriole appear as doublets; the tubules on the opposite side are sectioned obliquely. Therefore the nine doublets of the centriole probably are not exactly parallel to the long axis of the centriole, but are slightly tipped as is apparently the case in 9-triplet centrioles (1, 10). In all cases where a cross-sectional profile is seen, the section longitudinally transects another centriole lying adjacent to the 9-membered centriole (Figs. 1–4). The distance between the two sides of the longitudinally sectioned centriole is considerably greater than the diameter of the adjacent 9-membered centriole. The longitudinally cut centriole is, therefore, almost certainly larger than its partner and thus probably is composed of more than nine tubules. 9-membered centrioles also are found at right angles to larger centrioles at the pole of the mitotic spindle in second instar spermatogonial divisions (Fig. 5).

Giant Centrioles of the Testis

Most of the centrioles of second instar larval spermatogonia are giant centrioles similar to but smaller than those found in fourth instar larvae at the onset of spermatogenesis. Giant centrioles of second instar testis are composed of only 20–50...
tubules, in contrast to the 70 or more singlet tubules making up the centrioles of larval testis just prior to pupation. These smaller giant centrioles contain occasional doublet tubules similar to those comprising 9-membered centrioles of Sciara (Figs. 6 and 7), whereas the centrioles of fourth instar larvae contain only singlets.

Two distinct types of smaller giant centrioles are found in second instar larvae. In one type, a thin concentric band of electron opaque material lies inside the centriole tubules and is separated from them by a distance of about 400 A (Figs. 9, 10 and 11). Viewed in cross-section these centrioles display oval (Figs. 9 and 10) or rectangular profiles. This type of centriole is easily distinguishable from the other type which lacks this band of electron-opaque material (Fig. 8). When a centriole lacking a dense line is seen in cross-section, it is always adjacent to a longitudinally sectioned centriole in which the dense line is present (Figs. 6, 7, and 8). We shall refer to centrioles without a dense line as primary centrioles and those with a dense line as secondary centrioles, for reasons which are given in the discussion of centriole formation later in the text. Primary centrioles seen in cross-section usually are flattened slightly on the side which borders the adjacent longitudinally sectioned secondary centriole (Figs. 6 and 8). When a secondary centriole is sectioned transversely, its primary centriole partner is not in the plane of section and is not seen.

In a three-dimensional reconstruction of the spatial relationship which the primary and secondary centriole must have to each other (Fig. 12, configuration I), it is clear why a section which cuts the secondary centriole in cross-section will not include the adjacent primary centriole. From Fig. 12 it also is apparent that it is possible to transect the primary centriole longitudinally without cutting through the adjacent secondary centriole. However, it is not possible to cut the secondary centriole longitudinally without cutting through the primary centriole. Indeed, we have observed that whenever a longitudinally sectioned centriole does not lie adjacent to another centriole it is a primary centriole, i.e., does not have the band of dense material (Figs. 13 and 14). From the above considerations, especially the fact that each of 54 different transversely or nearly transversely sectioned primary centrioles we have observed was found to lie adjacent to a longitudinally cut secondary centriole, we conclude that centrioles always occur in pairs in which one member of the pair is a primary and one a secondary centriole, and that they always bear the same geometric relationship to one another.

In transverse sections of secondary centrioles, all the tubules are occasionally seen in cross-section; more often, however, the tubules of one side are seen in cross-section while the tubules of the opposite side are sectioned obliquely (Figs. 15 and 16). In longitudinal section the sides of secondary centrioles are seen to diverge (Fig. 17). Therefore, secondary centrioles generally take the form of a bilaterally flattened, truncated cone. The form of primary centrioles is less regular, but their sides are also often divergent.

In sections which cut a primary centriole transversely, so that it appears as an ellipse, and which cut the adjacent secondary centriole longitudinally, so that it appears as two parallel rods, the length of the longer axis of the ellipse is often less than the distance between the parallel sides of the adjacent secondary centriole (Figs. 18-23). In some cases it appears that, even if the primary centriole were flattened longitudinally, the greater axis would not be so long as the distance between the two sides of the adjacent secondary centriole. Therefore one must conclude that the secondary centriole is larger and presumably consists of a greater number of tubules than the adjacent primary centriole. Thus the change from 9-membered centrioles in testes of young larvae to giant centrioles in testes of late fourth instar larvae occurs by a series of increases in number of tubules in each newly formed centriole. In late fourth instar larvae the number of component tubules in centrioles varies. In fact, during spermiogenesis the giant centrioles, which still range in size, serve as basal bodies to giant flagella in which the number of tubules also varies from about 60 to 90 (26, 27).

Fibrous material in varying amounts often is found inside giant centrioles (Figs. 13 and 17). The chemical nature of this material is not known. Ribosomes, endoplasmic reticulum, microtubules, and mitochondria also occur in giant centrioles (Figs. 11 and 14).

Behavior of Giant Centrioles during Mitosis

The nuclear membrane remains almost entirely intact throughout the spermatogonial divisions in Sciara. To our knowledge, no other metazoa have been found in which the nuclear membrane does not break down during cell division although this
Figures 6 and 7  Most of the tubules which comprise the small giant centrioles of second instar spermatogonia are singlets; however, a few doublet tubules (arrows) can be discerned. Fig. 6, × 115,000; Fig. 7, × 122,000.

Figure 8  Transversely sectioned primary centriole lying adjacent to longitudinally cut secondary centriole. Second instar spermatogonium. × 160,000.
FIGURES 9-11 Transversely cut secondary centrioles in second instar spermatogonia. Secondary centrioles can be identified by the thin concentric band of electron-opaque material which lies inside the centriole tubules and is separated from them by a distance of about 400 Å (arrows). mi, mitochondrion. Fig. 9, X 114,000. Fig. 10, X 96,000. Fig. 11, X 90,000.

phenomenon is common among the protista (14, 15, 32).

In prophase, microtubules extend from two pairs of centrioles on either side of the nucleus into the nucleoplasm (Fig. 24). These microtubules are never actually continuous with the tubules of the centrioles. Microtubules directed towards the two pairs of centrioles can be seen in the cytoplasm of early prophase cells before tubules are discernible in the nucleoplasm. This suggests that spindle tubules are cytoplasmic in origin.

During prophase, the tubules of the primary
I and II are three-dimensional diagrammatic representations of the two possible configurations of one primary ($1^\circ$) and one secondary ($2^\circ$) centriole when one is adjacent and perpendicular to the other. The two-dimensional profiles diagrammed in $a$, $b$, $c$, and $d$ are obtained by representative planes of section through centrioles disposed as in configuration I. The images diagrammed in $e$, $f$, $g$, and $h$ would be obtained by various planes of section through centrioles in configuration II, but none of these images are ever obtained. We, therefore, conclude that the actual relationship of secondary to primary centrioles is always as diagrammed in configuration I. When sectioned, a secondary centriole without any primary partner could give an image such as $g$, and a lone primary centriole could give an image such as $f$, and these are never obtained; therefore we believe that spermatogonial centrioles of second instar *Sciara* larvae always occur in pairs.

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**Figure 12**

Centriole Number

A dividing cell clearly possesses four centrioles, two at each pole of the spindle (Fig. 27). Each daughter cell then receives two centrioles which must then give rise to two more centrioles before the next division. In spermatogonial interphase two pairs of centrioles are sometimes found in close proximity to each other (Figs. 28 and 29), and at other times at some distance apart (Fig. 17). Although a cell frequently is found to contain four centrioles, we have never observed more than four centrioles in one cell. In six of the eight cases where we observed four centrioles in close proximity, all four centrioles lacked a concentric band of dense material, as do primary centrioles. In the other two instances, and in all cases where the two pairs were some distance apart (Fig. 17), each pair had the usual appearance of one primary and one secondary centriole. When in close proximity to one another, the four centrioles are found in one of two specific spatial relationships. In the first array (Fig. 28), the four centrioles are in a row and the tubules of the two secondary centrioles are aligned. Although the secondary centriole may not contain the concentric dense band, it can be identified by its relationship to the adjacent centriole (see Fig. 12). In the other array (Fig. 29), the two secondary centrioles lie side by side and the tubules of the two primary centrioles aligned. When the four centrioles occur in close proximity, a Golgi area of characteristic morphology often is associated with them (Figs. 28 and 29). This type of
Golgi zone rarely is observed except in association with four centrioles in close proximity to each other.

**Centrioles of Second Instar Larval Oogonia**

We have examined second instar larval oogonia less extensively than spermatogonia; however, the centrioles appear to be the same in both types of germ cells. As in spermatogonia most of the centrioles are smaller giant centrioles. In a few cases we have observed four giant centrioles in the same cell. They always occur in the relationships described in the preceding paragraph. On one occasion we observed a 9-membered centriole in an oogonium.

**Centrioles of Fourth Instar Larval Oocytes**

By the late fourth instar (stage c, d, and e) (12) all the oocyte nuclei contain the chromosomal cores indicative of meiotic prophase (9, 24). Each primary oocyte is connected to a polytene nurse cell, also of germ origin (7), by a cytoplasmic bridge (Fig. 30). We have not observed many centrioles in these large cells; however, all the centrioles which have been seen in cross-section are either smaller giant centrioles or 9-membered centrioles. They occur both in polytene nurse cells (Fig. 30), where they sometimes are mishapen and thus probably degenerating (Fig. 31), and in primary oocytes, where in two cases we have observed four giant centrioles in the same oocyte (Fig. 32).

**DISCUSSION**

**Centriole Formation**

When found in pairs, centrioles of a wide range of organisms are arranged so that the long axis of
When viewed in transverse section the tubules of one side of secondary centrioles are seen in cross-section while the tubules of the opposite side are sectioned obliquely. In Fig. 15, arrows indicate doublets. Second instar spermatogonia. Fig. 15, $\times 102,000$; Fig. 16, $\times 111,000$.

one is perpendicular to the long axis of the other (10, 11, 20, 30, 34). Since centrioles are known to form at right angles to pre-existing centrioles (4, 8, 13, 25), the perpendicular position of two mature centrioles may be a consequence of their disposition during replication, although it also may have some other significance. In *Sciara*, the morphological dissimilarity of the members of each pair enables us to demonstrate that pairs of centrioles are disposed in a specific configuration: if the two centrioles are pictured as a T formed of two cylinders, the primary centriole is always the top of the T and the secondary centriole intersects it in the middle. Therefore, in any section which transects both centrioles of a pair, one can distinguish between the primary and secondary centrioles by their spatial relationships. (See legend of Fig. 12 for more detailed explanation.)

Since the giant centrioles of spermatogonia of second instar larvae are comprised of fewer tubules than those of spermatogonia of fourth instar larvae, it is logical to assume that centrioles of increasing size are formed at each successive interphase. (There are approximately three spermatogonial cell generations between second and fourth instar.) Since we frequently observe primary centrioles adjacent and at right angles to larger secondary centrioles (Figs. 18–23), we conclude that primary centrioles represent the original, or mother, centrioles and that secondary centrioles are newly formed daughter centrioles.

The correspondence of primary centrioles to mothers and secondaries to daughters is borne out further by the finding that the giant centrioles which lie next to transversely sectioned 9-membered centrioles are secondary centrioles, i.e., they display the band of dense material, (Figs. 1–4). Since no 9-membered centrioles are observed in fourth instar spermatogonia, spermatocytes, spermatids, or sperm (26, 27), it is logical to assume that the giant centriole is the newly formed member of the pair.

One wonders whether mother and daughter centrioles of other organisms are also orientated in a specific way, but since all mature 9-membered centrioles look alike, it is difficult to demonstrate this relationship. When centrioles are forming, however, they are shorter and the tubules are less clearly defined than those of mature centrioles.

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FIGURE 17 Two pairs of centrioles in an interphase spermatogonium. All four centrioles have been sectioned longitudinally. Note that the primary and secondary centrioles bear a specific spatial relationship to each other (configuration d of Fig. 14). m, fibrous material. × 34,000.
Figures 18–23  The longer axis of the primary centriole is greater than the distance between the two sides of the adjacent longitudinally cut secondary centriole. Arrows, dense band characteristic of secondary centrioles. Second instar spermatogonia. Fig. 18, × 63,000. Fig. 19, × 77,000. Fig. 20, × 50,000. Fig. 21, × 64,000. Fig. 22, × 100,000. Fig. 23, × 134,000.
FIGURE 24  Prophase spermatagonium. Microtubules extend from a pair of centrioles on either side of the nucleus into the nucleoplasm. $P_1$ and $P_2$, primary centrioles. $S_1$, secondary centriole. Arrows, nuclear membrane. Second instar. $\times$ 36,000.
FIGURE 25  Same cell as Fig. 24. Section parallel to that in Fig. 24. The transversely sectioned secondary centriole, $S_2$, identifies $P_2$ in Fig. 24 as a primary centriole. $P_1$, primary centriole. $S_1$, secondary centriole. $\times 25,000$.

Thus a distinction can be made between the forming centriole, or procentriole, and the adjacent perpendicularly disposed mother centriole. We have examined published micrographs of forming centrioles lying adjacent to mature centrioles (8, 11, 25), and we note that mother and daughter centrioles are disposed in relationships which we have termed A and D in Fig. 12 if, in relationship I, the mother centriole were in the position of the primary and the daughter in the position of the secondary. In the atypical primary spermatocytes of the snail Viviparus, Gall describes the formation of many centrioles at right angles to one mother centriole (13). A number of his published micrographs show cross-sections of a mother centriole adjacent and at right angles to several procentrioles, but none show cross-sections of even one procentriole lying adjacent to a longitudinally cut mature centriole. The mother centriole of other organisms is, therefore, analogous to the primary centriole of Sciara testis, and the daughter centriole corresponds to the secondary. We conclude that centrioles probably always arise in the configuration I of Fig. 12 where $I^o$ is the mother centriole.

Evidence for the proliferation of centrioles by means of an autonomous self-replication process consists, for the most part, of the observation that centrioles arise in the vicinity of pre-existing centrioles (35). Recent cytochemical and biochemical evidence for the presence of DNA in basal bodies of Tetrahymena (2, 16, 29) is not yet conclusive. Although light microscopic observations led some early cytologists to the belief that centrioles might divide by a process similar to fission, it appears in electron micrographs that this may not be the case; new centrioles arise at right angles to, and often at some distance from, pre-existing centrioles. It is difficult to imagine a process of self-replication which occurs in such a manner. In any case, self-replication is perhaps not an apt description of what we observe in Sciara. Here giant centrioles may arise next to 9-membered centrioles, and
daughters of giant centrioles are not exact replicas of their mothers. The factor which determines whether the daughter of a 9-membered centriole will be another 9-membered centriole or a giant centriole appears to depend on the cell type and stage of development of the tissue. The term self-replication implies that a genetic system is involved. We cannot exclude, on theoretical grounds, the possibility that centrioles of unlike morphology are genetically identical, i.e., contain an element which generates centrioles of varying appearance but replicates itself precisely. But our data are also consistent with the idea that a mother centriole may serve as a focal point for the assembly of centriolar proteins, coded for by nuclear genes, and also may place constrictions on the orientation of the forming organelle. Although the evidence is not yet conclusive, we feel that the latter interpretation is more likely.

A pair of centrioles, one primary and one secondary, is found at the pole of the telophase spindle in spermatogonial divisions in Sciaræ so that each daughter cell must receive two centrioles. At prophase one pair of centrioles is situated on each side of the nucleus; therefore, as in other known cases (1), a new pair of centrioles must be formed during interphase. It is probable that the secondary centriole becomes a primary shortly before the new centrioles are formed since the two newly formed centrioles presumably will be secondaries, and the 1:1 ratio of primaries to secondaries always is maintained. Shortly after the new centrioles are formed we might expect to find four centrioles together. Indeed we do find four centrioles together in interphase spermatogonia, and in most cases all four have the appearance of primary centrioles. This implies that the secondary centriole becomes a primary centriole shortly before centriole formation. Then a new centriole resembling a primary forms in the position characteristic of the secondary centriole in relation to each of the original centrioles. At some time before or during the formation process, the two original centrioles move apart so that they no longer are perpendicular to each
FIGURE 27 Late anaphase. The tubules of the primary centriole ($P_1$) are directed in the direction of the opposite pole. Primary centriole $P_2$ is tipped slightly, but its tubules point in the general direction of the opposite pole. Second instar spermatogonia. $\times \ 20,000$.  

DAVID M. PHILLIPS Giant Centriole Formation in Sciara
In interphase spermatozoa two pairs of centrioles sometimes are found in close proximity to each other. Usually all four centrioles have the morphology of primary centrioles. A characteristic type of Golgi region (g) is associated with the four centrioles. Second instar. Fig. 28, X 46,000. Fig. 29, X 55,000.

Other but each is perpendicular to a newly formed secondary centriole. The two new centrioles then develop a dense line. Before the two pairs move apart to opposite sides of the nucleus, we see four centrioles together, two primaries and two secondaries.

When four centrioles were found together, they almost always were seen in one of two spatial arrays. In one array, the tubules of the two primary centrioles are aligned, and in the other the tubules of the two secondary centrioles are aligned. These relationships may be a function of the mode of centriole formation, or they may be a result of a tubule-aligning property of centriole tubules.
FIGURE 30 In the ovaries of fourth instar larvae each primary oocyte (lower cell) is connected by a cytoplasmic bridge (b) to a nurse cell (upper cell). Four longitudinally cut giant centrioles can be seen in the nurse cell (arrow). × 11,000.
Figure 31  Misshapen centrioles, perhaps in the course of degeneration, in a nurse cell. Fourth instar. X 80,000.

Figure 32  Four giant centrioles in a primary oocyte. Arrow, chromosomal core. Fourth instar. X 37,000.
toplasmic microtubules appear to be aligned by the tubules of giant centrioles at the onset of flagellar formation in *Sciara* spermiogenesis and develop into a giant flagellum which reflects the tubule pattern of the centriole (26). Centrioles also seem to provide a point of focus for spindle fibers. The lining up of centrioles in *Sciara* may be but another manifestation of this centriole property.

**Centriole Orientation**

During germ cell development of many animals, centrioles may become very long (18, 31, 33) although an increase in width or number of tubules such as occurs in *Sciara* has to our knowledge never been described. Their increased length has facilitated light microscopic observations of centriole orientation during cell division. The two centrioles often are disposed in a "V" with the apex of the V directed towards the opposite pole (3, 11, 19). Costello (5) concluded, from studies of the orientation of centrioles during maturation division in flatworm eggs, that the orientation of centrioles may determine the type of spiral cleavage, i.e., dexiotropic or leiotropic. The fact that centrioles are oriented precisely in cell division suggests that their orientation is important. During gonial divisions in *Sciara* each pair of centrioles is oriented so that the tubules of one centriole point toward the opposite pole while the tubules of the other centriole are perpendicular to the long axis of the spindle. Furthermore, it is consistently the primary centriole which is directed towards the opposite pole; this suggests that the primary and secondary centriole are not only morphologically but also functionally dissimilar.

**Centrioles in Oocytes**

Although it seems that 9-membered centrioles can give rise to giant centrioles, one wonders whether the reverse can also take place. *Sciara* somatic tissues of both embryos and larvae appear to contain only 9-membered centrioles. Since 9-membered centrioles are not present in *Sciara* sperm (26, 27), they must arise ultimately from giant centrioles, 9-membered centrioles of the egg, or centrioles which arose de novo. We have not attempted to search for centrioles in the large amount of cytoplasm of the mature egg, but we do find giant centrioles, albeit of the smaller type, in oogonia of second instar larvae and in primary oocytes of fourth instar larvae, and four giant centrioles are found sometimes in one cell. We have never observed more than four centrioles in any germ cell, male or female, so it is unlikely that female germ cells with four giant centrioles also contain 9-membered centrioles. It also is unlikely that oocytes receive 9-membered centrioles from nurse cells since nurse cells are a germ line tissue and can also be found with four giant centrioles.

The 9-membered centrioles of somatic cells of *Sciara*, therefore, probably derive either from 9-membered centrioles which arose de novo or from 9-membered centrioles which arose in association with giant centrioles.

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