THE STRUCTURE AND FUNCTION OF
CENTRIOLES AND THEIR SATELLITES IN
THE JELLYFISH PHIALIDIUM GREGARIUM

DANIEL SZOLLOSI, Ph.D.
From the Department of Biological Structure, University of Washington, Seattle

ABSTRACT

Testes of jellyfish Phialidium gregarium were fixed in 2 per cent OsO4 in Veronal-acetate
buffer at pH 7.4. Thin sections showed that in young spermatids the spindle fibers of the
last maturation division are attached to satellites of the filament-forming centriole. In more
mature spermatids this attachment is not observed. During the developmental phase, nine
satellites can be observed emanating from the interspaces between the nine tubular triplets
of this centriole. A circular region on each of the enlarged distal ends of the satellites at-
taches them to the cell membrane. The satellites apparently provide a firm anchor for the
axial filament. Each of the epithelial cells covering the testis produces a single long flagel-
lum. On the filament-forming centriole often a satellite can be observed to which tubules are
attached. These tubules are 180 Å in diameter and probably represent remnants of spindle
fibers. It is suggested that the distal centriole has the ability to form several satellites or
appendages at appropriate times during the cell cycle. These satellites are distinct from
the daughter centrioles in that they are supportive structures: in certain phases of cell life,
spindle fibers may attach to them, while in other instances the distal centriole and the
flagellum it is forming are anchored by them.

Basal bodies, cilia, and flagella have been de-
scribed in many different cell types. Morphological
evidence has suggested that basal bodies are
centrioles which give rise to cilia and flagella at
certain stages of differentiation (5, 6). Soon after
the first recognition of centrioles by means of elec-
tron microscopy and the description of their fine
structure (1), small, dense bodies were observed
which were connected to them by bridges (2, 3,
10). The terms pericentriolar body and satellite
have been applied interchangeably to these dense
bodies in the past. In this paper, evidence will be
presented from electron microscope studies that
two centrioles are present at the base of the sperm
tail of the hydromedusa Phialidium gregarium, a
distal or filament-forming centriole and a prox-
imal centriole. The distal centriole forms the flagel-
lum at a time when the spindle fibers of the last
meiotic division are still associated with satellites
projecting laterally from it. Further, the mor-
phology and the functional role of satellites will be
discussed.

MATERIALS AND METHODS

Male specimens of Phialidium gregarium1 were an-
esthetized in sea water by adding drops of 0.53 M
MgCl2 until they were immotile. The testes were
quickly removed, washed for a few minutes in fil-

1 The hydromedusae were collected at different
times during the spring and summer off the boat
pier at the Marine Biological Laboratories, Friday
Harbor, Washington. Many thanks are due to Dr. R.
Fernald, Director of the Laboratories, for the
laboratory facilities.
tered sea water, and fixed for 1 hour in a 2 per cent OsO₄ solution buffered by Veronal-acetate at pH 7.5. In some cases, the small hydromedusae were dropped directly into the fixative and the testes were dissected in 70 per cent ethanol. After ethanol dehydration was completed, small pieces of the tissue were embedded in Epon 812 (13). The specimens were mounted on metal chucks and oriented in such a manner that complete cross-sections could be obtained. Sections were cut, on a Huxley microtome with a diamond knife, at a thickness which gave silver to pale gold interference colors and mounted on

**Abbreviations for Figures**

af, axial filament  
bp, basal plate  
cr, ciliary rootlet  
dc, distal centriole  
f, flagellum  
M, mitochondrion  
N, nucleus  
pe, proximal centriole  
ps, perinuclear space  
sf, spindle fibers  
at, strands of satellites  
S, satellite

**Figure 1 a**  A spermatid with the proximal centriole (pe) and distal or filament-forming centriole (dc). Several spindle fibers (sf) converge toward them. × 61,000.

**Figure 1 b** The continuity is demonstrated of the tubules of the tail filament with the tubules within the wall of the distal centriole. × 58,000.
either 200 mesh or 75 X 300 mesh copper grids coated with a carbon film. All sections were stained with the alkaline lead technique of Millonig (15). Electron micrographs were taken with an RCA 2C electron microscope, and Dupont Ortho A lithographic sheet film with Cronar (polyester) base was used (23).

**Observations**

Centrioles and Flagellum of the Spermatid

Spermatocytes and spermatids of *Phialidium* are favorable objects for the study of centrioles because the cells are small and develop nearly synchronously in clusters; there is, hence, a good opportunity for locating in thin sections large numbers of centrioles in different functional stages. Spermatogenesis proceeds in the testis radially and peripherally from the gastrodermis, and the developmental stages can be determined accurately (19). The protruding sperm-tail filament serves as an accurate marker for locating the centrioles in thin sections.

Fig. 1a shows a cross-section of the proximal centriole of a *Phialidium* spermatid demonstrating cytoplasmic tubules converging toward this centriole. Figs. 1a and b show the distal centriole cut longitudinally and demonstrate that the peripheral tubules of this filament-forming centriole are continuous with the peripheral tubules of the flagellum. The flagellum emerges from a cup-shaped fold of the cell membrane at the distal end of the centriole, and at this level the basal plate can be seen. An appendage projects laterally at an angle from the filament-forming centriole (Fig. 2) and shows a slightly club-shaped termination.

![Figure 2 A young spermatid, showing three spindle fibers (sf) making contact with a cross-striated satellite (S). X 35,000.](image)
This appendage will be referred to as a satellite. Tubular elements 180 A in diameter are associated with the enlarged terminal end of this satellite (Figs. 2 and 7). These and several other tubules also observed in the cytoplasm probably represent the spindle fibers or astral rays of the last maturation division. The satellite shows a cross-striation in cross-section (Figs. 1 a, 3 a) the typical pinwheel-shaped arrangement of triplets of tubules can be recognized in the walls of both the proximal and distal centrioles (8, 16). Each one of the interspaces between the nine tubular triplets has a dense matrix which gives rise to a satellite displaying the same 120 A periodicity (Figs. 2 and 4).

**Figure 3 a** Cross-section of spermatid distal centriole with its nine tubular triplets and nine satellites. Two of the satellites demonstrate the circular profile (arrows) from which three strands (st) originate. One satellite (S) originates from the dense matrix between adjacent tubular triplets. X 78,000.

**Figure 3 b** A spermatid centriole with two satellites and their strand-like (st) arborizations. At the arrow is seen the circular profile where the strands attach. X61,000.

with a 120 A separation between two dense components. This periodicity differs from that of the cross banding of ciliary rootlets in which the prominent bands are reported to be 850 to 900 A apart. Spindle fibers at metaphase of the second meiotic division attach to a dense structure near a centriole which is probably the club-shaped enlargement of a satellite (Fig. 14).

The array of satellites also assumes a pinwheel configuration. Gibbons and Grimstone (8) reported, in their study on flagellates, the presence of a small fiber connecting the inner tubule (A) of one triplet with the outermost tubule (C) of the neighboring triplet. Due to the dense matrix between the tubular complexes within the wall of the centrioles in the spermatid of *Phialidium*, the
FIGURE 4  An oblique section through the spermatid filament-forming centriole (dc) with several satellites whose origin can be traced to the dense matrix between the tubular triplets. × 56,000.

FIGURE 5  Metaphase of the second maturation division of a spermatocyte. The centriole at the spindle pole shows the fine fibrils between the A and C components of the adjacent tubular triplets (arrows). × 67,000.
fine fibrous connecting elements between the A and C components of adjacent triplets were not seen. In the centriole of a spermatocyte found in metaphase of the second maturation division, however, such fibers are observed (Fig. 5). In more mature spermatids fine strands project from circular profiles at the distal end of the satellite into the cytoplasm. These strands may extend along the cell membrane or, in other cases, may be firmly attached to the cell membrane. A dense granule is found in the center of the circular profiles (Figs. 3 and 6). A periodicity of 120 A is also observed in some of the strands (Fig. 4). This complex, that is, the circular profiles and the fibrils emanating from them, seems to anchor the filament-forming centriole to the plasma membrane. In longitudinal sections, the satellites often form an angle of 55 to 60° with the long axis of the centrioles as they extend toward the cell membrane.

In a slightly tangential cross-section (Fig. 6) through the cup-shaped region where the flagellum emerges from the cytoplasm, the relationship of the satellite attachment complex is particularly clear. The circular profiles apparently attach to the cell membrane while their fine strand-like arborizations bend back toward the cytoplasm. In most cases, three fine fibrils arranged like pigeon toes are seen per satellite. Since three is quite consistent for the number of fine strands observed thus far in many different planes of section, it is assumed that this is the usual case, though a larger number cannot be excluded. The plane of section in Fig. 6 is probably very close to the basal plate, the transition zone between the distal centriole and the sperm-tail filaments (see Fig. 8 and cross-section E in text; Fig. 3 in Gibbons and Grimstone, reference 8). Only two doublets of peripheral tubules are present and these are connected to one another by a continuous double membrane. This circular connecting membrane corresponds to the cylindre on manchon fibreux intraflagellaire of Noirot-Timothé (16). Each double tubular complex is linked with the flagellar membrane by a homogeneous dense mass or by small fibers. At the axis there are remnants of two tubular elements, the proximal ends of the central flagellar tubules. The cell membrane forming the basal cup of the flagellum and the flagellar membrane itself are scalloped to form nine semicircles, corresponding to the nine subunits of the centrioles and the nine satellites. The scallopings of the adjacent membranes coincide. Attachments of the satellites and their fibrils are

Figure 6 The circular profiles (arrows) of the satellites and their strands (st) are near the obliquely cut cell membrane of the spermatid. The tail filament (af) is sectioned close to the point where it emerges from the cytoplasm. X 56,000.
**Figure 7 a** A young spermatid, showing spindle fibers (sf) attached to a satellite (S) of the filament-forming centriole (dc). × 64,000.

**Figure 7 b** The spindle fibers (sf) of the spermatid converge to the club-shaped end of a satellite (S) and attach to it. × 66,000.
found in the valleys between the outer semicircles of the basal cup, whereas the tubules of the tail filament are situated at the level of the corresponding arcs.

In very young spermatids the tail-forming centriole can be recognized to possess a satellite (Figs. 2, 7a). It is clear that the spindle fibers or astral rays converge toward the enlarged distal portion of the satellite and that they are not continuous with the tubular elements of the centrioles themselves (Figs. 2, 7a and b). If a section just grazes the satellite in a plane parallel to the long axis of the centriole, a star-shaped figure is formed by the converging spindle fibers (Fig. 8).

**Centrioles, Flagellum and Ciliary Rootlet of Epithelial Cells**

The testes of *Phialidium* are covered by large epithelial cells, each of which possesses a single long cilium which also may be termed a flagellum (Fig. 9). The point of emergence is, as in the spermatid, a cup-shaped indentation of the cell membrane at the basal body. Two centrioles are found at this point. The ciliary rootlet emerges from the filament-forming centriole and has typical cross-striations about 850 to 900 Å apart.

The ciliary rootlet is similar to those described in the literature (5, 6, 17, 20) (Figs. 9, 11, and 12). It often splits into two components. The largest rootlet diameter measured in this study was 1600 Å, but often rootlets or branches were seen to be narrower. One branch of the rootlet bends at the level of the proximal centriole and projects laterally. This rootlet shows 8 to 12 longitudinal fibrillar subunits. The number of such subunits varies, but they are always separated by a space of about 150 Å. Thus, in the thicker region of the rootlet more fibrillar subunits are found.

In electron micrographs of the fresh water mussel *Elliptio*, a basic periodicity of 550 to 700 Å in ciliary rootlets has been reported, this was established by prominent alternating dense and light bands, the light bands being transected by two to four less prominent dense lines (5, 6). The banding pattern of the ciliary rootlets of *Phialidium* is even more complicated. The center-to-center distance between the “major periods” of prominent bands is about 850 to 900 Å. Each major dense band, however, can be seen to consist of three distinct components: two denser bands limit laterally a fainter central band. This complex measures 250 Å across. Between the prominent dense bands five faint

---

**Figure 8** The spermatid spindle fibers (sf) form a star-shaped figure as they converge toward a satellite (S) (8), × 64,000.
cross-lines can be resolved, with every second band being slightly more dense. In the case of Phialidium, the bands of the ciliary rootlet are perpendicular to the long axis of the rootlet. The dimension of the general banding pattern reported here coincides well with the periodicity on ciliary rootlets in the electron micrographs published by Olsson (17) and is diagrammatically represented in Fig. 13.

These tubules are of the same dimensions as spindle fibers.

An incidental observation deserves mention here. In longitudinal sections of the sperm tail and the epithelial cell’s flagellum the contained tubules show periodically recurring lateral projections which apparently interlink adjacent pairs of tubules as well as the peripheral pairs with the central pair of tubules. This periodicity is approximately 300 A (sperm tails, Fig. 15 a and b; flagellum of epithelial cell, Fig. 9).

**DISCUSSION**

The general features of centrioles in the spermatid and the testicular surface epithelial cell of jellyfish Phialidium gregarium are similar to those described by Gibbons and Grimstone (8) in ciliated protozoa Trichonympha and Pseudotrichonympha. One of the
new observations in the present study has been the finding that the satellites originate from the dense matrix between the tubular triplets of the distal centriole. Although only a few perfect cross-sections are available, it can be stated that, at least in some cases, the number of satellites is nine. One satellite originates from the matrix between adjacent triplet structures of the centriole. Bernhard and de Harven (2) take issue with Bessis's claims (3) that nine dense masses are found regularly

**Figure 10** Epithelial cell showing the filament-forming centriole (dc) with a club-shaped satellite (S) to which spindle fibers (sf) attach. × 64,000.

**Figure 11** Epithelial cell showing the filament-forming centriole with one satellite having typical cross-banding, while other smaller centriolar appendages attach to the cup-shaped fold of the cell membrane at the base of the flagellum. × 35,000.
around centrioles, and they point out that this number may depend on the particular physiological state of centriolar activity. No serial sections of centrioles at spindle poles have been analyzed; therefore, it is not known how many satellites actually exist or whether the number varies. It is undeniable, however, that in certain periods of physiological activity nine satellites are present. This is documented in Fig. 3 of the present paper; as well as in a micrograph presented by Maillard (Reference 14, Plate G-d) of a centriole in the A-cell of the anterior pituitary gland in fetal rats.

In these cases, the connections to the centrioles cannot be traced exactly. Satellites have not been demonstrated, thus far, on the proximal centriole.

A periodicity on the satellite has been previously described by Sorokin (21). The satellites he described were also associated with the filament-forming centriole. Similarly, a repeating periodic banding is evident in electron micrographs of ciliary derivatives published by several other investigators (see e.g., Figs. 5 and 6 of Bernhard and de Harven, reference 2; Plate VI of Carasso, reference 4; Hendrickson, reference 11; and Kelly, reference 12).

It is not known how long the spindle fibers remain attached to the satellites after meiotic or mitotic divisions, nor whether spindle fibers are associated with all satellites. This latter point might, however, reflect difficulties with the alignment of this small organelle for sectioning in a preferential plane. When the spermatid chromatin starts to compact and the distal centriole reaches the proximity of the cell membrane, the spindle fibers apparently lose their contact with the satellite. Many tubular elements 180 A in diameter can be found in the cytoplasm, to be sure, but they can no longer be traced to the club-shaped enlargements.
FIGURE 14 A longitudinal section of a centriole at metaphase of the second maturation division of spermatocyte. The spindle fibers converge to a dense structure lateral to the centriole. This dense structure may be the club-shaped enlargement of a satellite. × 64,000.

FIGURE 15 a and b Longitudinal sections of the sperm tail demonstrating the periodically recurring lateral projections of the individual tubules. These projections apparently interlink the adjacent pairs of tubules. × 56,000.
of the satellites. It may be necessary to find a
different system for the study of these relation-
ships; i.e., one in which the formation and disper-
sion of the spindle can be controlled or timed
better.

The fine strands emanating from the club-
shaped enlargement of the satellite were never
found to exist simultaneously with spindle fiber
attachments. They seem to appear only after the
flagellum has emerged from the cytoplasm and
the centrioles become located near the cell mem-
brane. It is suggested that the circular profiles at
the distal end of the satellites and their newly
formed strands establish an attachment with the
cell membrane around the base of the flagellum.
Such an arrangement would lend a great struc-
tural rigidity to the centriole and the flagellum it
is forming. The scalloped cell membrane, shown
in Fig. 6, would therefore be a reflection of a firm
attachment between the circular profiles of the
satellite and the plasma membrane, thus reinforc-
ing the interpretation that the satellite serves, in
this case, a structural role. An attempt has been
made to reconstruct diagrammatically the centriole
in this phase of activity (Fig. 16).

The centriolar organization found in the ciliated
epithelial cells covering the testes is similar to that
described above for the spermatid. Certain lateral
appendages of these centrioles are associated with
tubular elements; some others make contact di-
rectly with the cell membrane. Micrographs of the
ciliary rootlets disclose a great complexity of their
organization but do not add to our understanding
of their function. The cilia of the testicular surface
epithelium are very long, and some investigators
call them flagella. It is impossible to decide, on a
morphological basis alone, which term should be
used. One distinction apparently is the type of beat
(5, 6), but no observations have been carried out
on the testes of living Phialidium. The rootlet is
usually referred to as a "ciliary rootlet," but in
multiciliated cells in which rootlets have been de-
scribed previously only the filament-forming cen-
triole is present. In isolated instances, a rootlet has
been found also in spermatids, where it is found to
be compressed between the cell membrane and one
of the gigantic mitochondria at the base of the
nucleus, making morphological distinction even
more difficult. Cilia and flagella may differ mor-
phologically in that only the filament-forming
centriole is found in case of cilia, while two cen-
trioles, at right angles to each other, are present at
the base of flagella.

The significance of the banding pattern of the
satellite is not clear at the moment. On cursory
examination the periodicity is different from that
of the ciliary rootlet. It is possible, however, that
the basic elements of the satellites are essentially
similar to those of the ciliary rootlet and that the
difference is to be sought in the degree of aggrega-
tion or polymerization. The cross-banding pattern
of the satellites is certainly the most prominent
pattern, for only rarely are there indications of
longitudinally oriented fibers.

The electron micrographs presented in this
study show that in the case of Phialidium the
spindle fibers converge to one of the satellites (or
possibly to a few satellites) rather than to the
centrioles directly (Figs. 7 a and b). At this time
nothing can be added to describe the details of
this attachment or the nature of its origin that
would not be purely speculative. It seems clear,
however, that the spindle fibers do not just end
blindly near the centrioles but rather that they
make contact with the club-shaped enlargement
of the satellites. The mode of attachment of the
spindle fibers to the centrioles has puzzled many
microscopists in the past. In previously published
electron micrographs, it has been shown that the
spindle fibers reach only the vicinity of the cen-
trioles or end a certain distance from them (2, 4, 9,
The axes of the centrioles at the spindle pole are arranged in such a manner that the spindle fibers could not be continuous with the tubular elements of the centrioles themselves.

Thus far, satellites have been implicated in the role of centriolar duplication. Bernhard and de Harven (2, 10) and Gall (7) showed clearly that this is the case for at least some of the lateral projections. Examination of their published micrographs discloses that certain lateral projections do look like small centrioles (reference 2, Fig. 7), while others seem to be club-shaped (reference 2, Figs. 4 to 7). The material for their studies consisted of young, rapidly dividing cells, and it is reasonable to presume that two different centriolar functions were being expressed simultaneously: the club-shaped satellites for spindle attachment and other projections representing stages in the production of daughter centrioles. Spermatids do not usually divide and, accordingly, in no case of Phialidium spermatids were daughter centrioles observed as lateral projections. In the case of a viviparous snail, however, it has been shown that the atypical spermatids show daughter centrioles which correspondingly form several sperm-tail filaments (7). Two distinct functions may be performed simultaneously by the centrioles of young Phialidium spermatids and the centrioles of the flagellated epithelial cells: the formation of the sperm tail or the flagellum, respectively, and the attachment of the spindle fibers. Spindle fibers may be replaced by a fibrous arborization of the satellites to form the specialized anchoring system at a more advanced stage of differentiation. Thus, the significance of lateral projections described by other authors as being involved in centriole duplication appears to be quite different from that of lateral projections found in Phialidium spermatids.

The idea is attractive that a relationship between spindle fibers and satellites is a general feature of dividing cells. It must be emphasized, however, that the present observations were limited to a few meiotic and some post-meiotic cells in a single species and that such a conclusion may not be borne out by further investigation.

Centrioles may be capable of forming a large number of satellites or lateral projections in different physiological states. The proximal centriole of photoreceptors, for example, forms a satellite with a large club-shaped ending, but, at the same time, on a different level several small fibers may form anchorage to the cell membrane (11, 12, 22). A most important property of centrioles thus may be the ability to form one to several satellites or projections at appropriate times during the cell cycle to serve different functions.

Organelles associated with centrioles are then of at least two kinds. The term “satellite” should be reserved for the supportive or structural elements involved in anchoring either flagella, cilia, or spindle fibers, while the side projections in replication phases of the centriole should be called “daughter centrioles.”

I want to thank Dr. Edward Roosen-Runge for allowing me to use several of his Phialidium testes preparations and for his interest during the development of this study.

My thanks are also due to Dr. J. H. Luft, Dr. R. L. Wood, and Dr. D. E. Kelly for reading this manuscript, for long discussions, and for their suggestions which contributed significantly in shaping the ideas expressed in this article.

Figs. 13 and 16 were prepared by Mrs. Marjorie Domenowske, Department of Medical Illustration, School of Medicine, University of Washington.

This investigation was supported in part by Public Health Service Research Grant No. 1HE-02989 from the National Heart Institute and by a State of Washington Initiative 171 grant.

Received for publication, August 21, 1963.