

1985

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### Recommended Citation

Kleve, Maurice G. (1985) "Role of Centriolar Matrix and Striated Rootlets in Centriolar Pairing and Orientation During Spermatogenesis in *Hydractina echinata*," *Journal of the Arkansas Academy of Science*: Vol. 39 , Article 16.

Available at: <http://scholarworks.uark.edu/jaas/vol39/iss1/16>

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# THE ROLE OF CENTRIOLAR MATRIX AND STRIATED ROOTLETS IN CENTRIOLAR PAIRING AND ORIENTATION DURING SPERMATOGENESIS IN *HYDRACTINA ECHINATA*\*

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## ABSTRACT

Transmission electron microscopy of the spermatogenic stages of the hydroid, *Hydractinia echinata*, reveals a series of complex structural and positional changes in the centrioles of spermatocytes and spermatids. The newly generated centriolar pairs of spermatocytes form an unusual four-centriole aggregate that persists until cell division. The distal centrioles of this aggregate are shrouded with a very dense matrix that accumulates after centriolar replication. This matrix facilitates the mechanical attachment between distal centrioles and microtubular nucleating satellites, striated rootlets and pericentriolar processes. The association of these accessory structures occurs sequentially and is repeated in spermatocytes and spermatids. An electron dense plaque, which is an extension of distal centriolar matrix, is interposed between centriolar pairs of the aggregate. The plaque structurally maintains the centriolar aggregate and apparently facilitates the orientation of centrioles to prevent spacial interference while satellites, rootlets and pericentriolar processes associate with the distal centrioles. Striated rootlets are also involved in maintaining precise spacing and orientation between centriolar pairs. A single striated rootlet emanates from the base of each distal centriole of the aggregate and attaches with the opposite distal centriole. The attachment of rootlets to distal centrioles changes the spacing and orientation of centriolar pairs during the process of precocial flagellar development seen in *Hydractinia* spermatogenesis.

## INTRODUCTION

The scenario of spermatogenic centriolar propagation, movement and association with specialized structures is extremely complex in the Cnidaria (for review see Kleve, 1977). Several aspects of centriolar behavior and the formation of centriolar specializations which include microtubular nucleating satellites, striated rootlets, and pericentriolar processes have been described (Dewel and Clark, 1972; Summers, 1972; Hirsch and Clark, 1973; Clark and Dewel, 1974; Kleve and Clark, 1976). These studies have not dealt with the process of propagation and movement of centrioles and the formation of centriolar specializations in the same organism. Also, the studies have not related the various structures in a functional way or demonstrated mechanisms that might account for changes in centriolar pair orientation during spermatogenesis.

Centriolar satellites and their associated microtubules have been studied in many cell types (DeThe, 1964; Boisson et al., 1969; Tilney and Gibbons, 1969; Tilney and Goddard, 1970). In Cnidaria, satellites are involved in both the organization of division centers and the cytoskeletal phenomena of sperm differentiation (Kleve and Clark, 1976). Pericentriolar processes, which extend from the distal centriolar matrix of many invertebrate sperm, have been studied in the Cnidaria (Szollosi, 1964; Summers, 1972; Dewel and Clark, 1972; Kleve, 1977; Kleve and Clark, 1976, 1980). Pericentriolar processes have been shown to contain the contractile protein actin and are thought to be involved in the directed motility of chemotactically stimulated sperm (Kleve, 1977; Kleve and Clark, 1980). Centriolar or basal body rootlets and other similar striated centriolar structures such as rhizoplasts and kinetoplasts are ubiquitous components of ciliated eukaryotic cells and have been studied extensively at the ultra-structural level (for review see Wheatley, 1982). In the last decade striated rootlets have also been studied at the biochemical level (Stephens, 1975; Salisbury and Floyd, 1978). The general consensus has been that these structures are in some way responsible for anchoring cilia or flagella and absorbing the force of flagellar

beating. Several investigators have suggested a more dynamic role for rootlets involving chemical or motive functions. Since the presence of a calcium dependent contractile protein has been demonstrated (Salisbury, 1983) these possibilities are more likely.

This report will describe the role of centriolar matrix in propagation and movement of centriolar pairs and the association of specializations with centrioles of *Hydractinia echinata* spermatocytes and spermatids. The dense matrix of *Hydractinia* spermatocyte and spermatid centrioles and an extension of the matrix, the matrix plaque, facilitates aggregation and orientation of centriolar pairs to prevent special interference when the centrioles associate with satellites, rootlets and pericentriolar processes. Centriolar rootlets appear to further orient centriolar pairs to allow flagellar formation which occurs precociously in the spermatocytes of *Hydractinia*.

## MATERIALS AND METHODS

Colonies of *Hydractinia echinata* were collected from shells occupied by the hermit crab *Pagurus sp.* purchased from the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts. With few exceptions, the polyps on an individual shell are of one sex allowing easy isolation of male and female colonies. Synchronous gonadal development of male colonies was induced by exposure to continuous light for periods of one to seven days (Ballard, 1942).

Individual gonophores containing either synchronous primary and secondary spermatocytes or spermatids were fixed at room temperature for 60 min. in a glutaraldehyde-paraformaldehyde mixture (Karnovsky, 1965) buffered in 0.1 M sodium cacodylate (pH 7.2) or 0.1 M sodium phosphate (pH 7.3). Following a buffer wash, the tissue was post-fixed for 30 min. in 1.0% osmium tetroxide buffered as above, rapidly dehydrated in a graded acetone series, and embedded in a low viscosity epoxy resin (Spurr, 1969). All fixatives and buffers were osmotically adjusted by the addition of 6% W/V glucose. Thin sections were cut with glass or diamond knives on a Porter Blum MT-2 Ultramicrotome, picked up on uncoated grids, and stained with uranyl acetate (Watson, 1958) and lead citrate (Venable and Coggeshall, 1965).

\*Supported in part by the Office of Research in Science and Technology, College of Sciences, University of Arkansas at Little Rock and the Ben J. Alzheimer Foundation.

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All preparations were examined with a Hitachi HS-8 or RCA EMU 3 electron microscope.

Spermatocyte stages were determined by cell diameter, ratio of cytoplasm to nuclear volume, presence of a nucleolus, extent of chromatin condensation, the number and position of mitochondria, and the amount endoplasmic reticulum (Hanisch, 1970; Zihler, 1972; Dewel and Clark, 1972).

## RESULTS

The centrioles of *Hydractinia* spermatocytes and spermatids are similar to those found in most eukaryotic cells. They consist of nine microtubular triplets arranged in the typical 9 + 0 pattern (Fig. 1). The centrioles are 250 nm in diameter and approximately 400 to 500 nm in length. In the spermatids and mature sperm of *Hydractinia* a distal centriole can be distinguished from a proximal centriole by the perpendicular arrangement of the centriolar pair (Fig. 2). The distal centriole is oriented with the longitudinal axis of the sperm and the proximal centriole lies perpendicular to the sperm axis.

In addition to their orientation and location, structural differences exist between distal and proximal centrioles. The microtubular triplets of distal centrioles are embedded in an electron dense matrix which obscures the fibrous connectives seen between adjacent triplets (compare Figs. 1 and 2). The proximal centrioles of *Hydractinia* spermatids and sperm do not possess a matrix making the fibrous connectives readily visible. Distal centrioles often demonstrate nucleoids in their core while such structures are not observed in proximal centrioles (Fig. 3).

The differences between proximal and distal centrioles of spermatids are evidenced in the newly generated parent-daughter centriolar pairs of primary and secondary spermatocytes. In addition to the differences in density of matrix seen between parent and daughter centrioles, the daughter centriole of a spermatocyte centriolar pair contains a set of internal radial spokes located in the centriolar core (Fig. 4). This "cartwheel" structure is similar to that seen in other newly generated daughter centrioles (Anderson and Brenner, 1971). Cartwheels are rarely seen in mature centrioles and have not been observed in the matrix-clad distal centrioles of *Hydractinia*. Newly generated daughter centrioles are considered to form without a matrix which must accumulate as the centriole ages (Anderson and Brenner, 1971). This report will refer to the dense matrix-clad centrioles as distal and the matrix-less centrioles as proximal regardless of their age or location in spermatocytes or spermatids. This classifies the matrix-clad parent centriole of a pair as distal and the new matrix-less daughter centriole as proximal.

Centriolar replication occurs in primary and secondary spermatocytes immediately after cell division so four centrioles exist in each spermatocyte during interphase. The four centrioles form an aggregate (Fig. 5) that persists until separation to form spindle poles at the beginning of the next cell division. The distal centrioles of each pair in the aggregate lie in apposition to each other. Interposed between the distal centrioles is an electron dense plaque-like structure that has a density and texture similar to that of the distal centriolar matrix (Fig. 5). The plaque extends from the centriolar matrix along the longitudinal axis of the distal centriole. When viewed in cross section the plaque appears to be continuous with the centriolar matrix at one edge while the other edge extends into the cytoplasm (Fig. 6). Electron opaque fibrous material extends from the matrix of each distal centriole of the aggregate to the interposed plaque, providing what appears to be a framework for the maintenance of the four centriole aggregate (Fig. 5).

In the classic orthogonal parent-daughter arrangement of paired centrioles the axis of the parent is perpendicular to that of the daughter but lies in the same plane. This orientation is apparent in the centriolar pair of the early primary spermatocytes before the replication of new centrioles (Fig. 6). After centriolar replication and aggregation, the axes of the original distal-proximal centriolar pair have changed pitch and now lie in different parallel planes (compare the two distal centrioles of Figure 5, which were a parent-daughter [proximal-distal] pair in the previous cell cycle, with the pre-aggregation pair in Figure 6).

After aggregation, the four centrioles of primary and secondary spermatocytes migrate to a position near the plasma membrane. When the centriolar aggregate reaches this polar position, microtubular nucleating

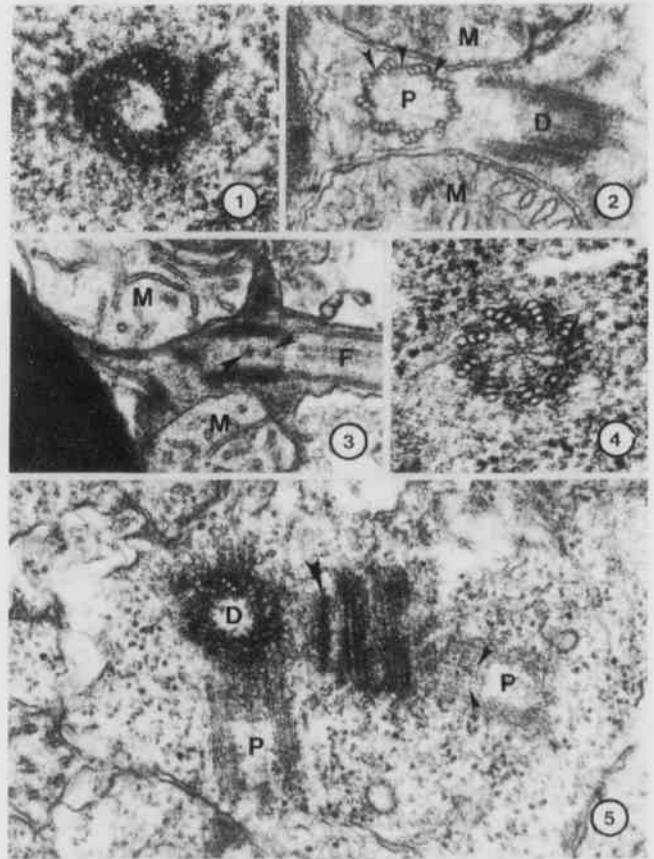


Figure 1. Cross section of a distal centriole found in a secondary spermatocyte. Note the very electron dense matrix in which the microtubular triplets of the centriole are embedded. X80,000

Figure 2. Longitudinal section of a late spermatid showing the distal centriole (D) and the proximal centriole (P) situated between two midpiece mitochondria (M). The arrows indicate the fibrous connectives between the A and C triplet tubules of the proximal centriole. These connectives are obscured by dense matrix in distal centrioles. X60,000

Figure 3. Longitudinal section of a late spermatid showing a distal centriole lying between two midpiece mitochondria (M). The centriole is associated with a flagellum (F) and two nucleoids (arrows) which are situated in the core of the distal centriole. These nucleoids are not seen in proximal centrioles. X40,000

Figure 4. A cross section of a proximal centriole in a secondary spermatocyte seen shortly after centriolar replication. This centriole demonstrates a thin matrix around the microtubular triplets. The core of the centriole contains a cartwheel structure, composed of nine radial spokes which are typical of newly generated daughter centrioles. X80,000

Figure 5. A thin section passing through two aggregated distal-proximal centriolar pairs of a secondary spermatocyte. The distal centrioles (D) demonstrate a dense matrix and a dense plaque is situated between them (large arrow). A profile view of the central cartwheel (small arrows) can be seen in the half of each proximal centriole (P) adjacent to the distal centriole. The two centrioles marked distal (D) are the parent-daughter pair from the preceding cell cycle. X60,000

## The Role of Centriolar Matrix and Striated Rootlets in Centriolar Pairing and Orientation During Spermatogenesis

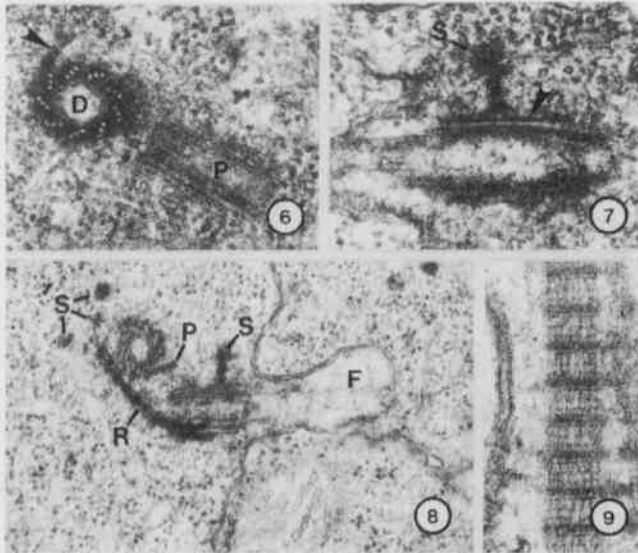


Figure 6. A thin section electron micrograph of a distal-proximal centriolar pair found in a secondary spermatocyte. Note the distinct difference in density between the distal (D) and proximal (P) centriolar matrix. The arrow indicates a dense plaque which emanates from the distal centriolar matrix. X60,000

Figure 7. Electron micrograph of a distal centriole found in a secondary spermatocyte. A satellite (S) can be seen attached to the distal centriole by means of a tapered stalk on which the satellite is situated. The stalk appears to connect with the centriole by means of a plaque (arrow). X80,000

Figure 8. Electron micrograph depicting the event of precocial flagellar (F) formation in a secondary spermatocyte. Two distal centrioles are visible with interspersed plaque and striated rootlet. Several satellites (S) are seen either attached to the distal centrioles or lying close to them. X25,000

Figure 9. High magnification micrograph of the striated appearance of the rootlet associated with distal centrioles. X180,000

satellites become attached to the distal centrioles and striated rootlets and pericentriolar processes form. Each of these centriolar specializations appear in primary and secondary spermatocytes and then disappear as the centriolar pairs separate for spindle formation and cell division. These specializations reappear in spermatids and again disappear as spermiogenesis proceeds with the exception of pericentriolar processes which persist in the mature sperm. There is a discernible sequential pattern to the appearance and disappearance of centriolar specializations. Satellites become attached to distal centrioles first followed by rootlets and then pericentriolar processes.

As these centriolar specializations appear, precocial flagellar biogenesis also begins. These flagella are complete and form in spermatocytes as well as spermatids. Flagellar biogenesis begins as an outgrowth or bulge in the plasma membrane adjacent to the posterior end of the distal centriole (Fig. 8). Both distal centrioles of the aggregate acquire a flagellum. The result of this precocial synthesis of flagella in spermatocytes are secondary spermatocytes which are flagellated at the time of cell division. When the second meiotic division is complete, each of the newly formed spermatids already possesses a flagellum.

Satellites are often seen in the cytoplasm near distal centrioles before replication and aggregation. After aggregation, satellites are also seen attached to distal centrioles. These attachments are accomplished by

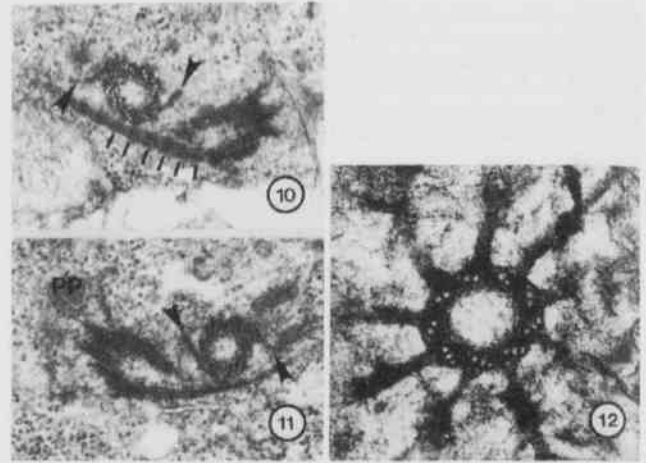


Figure 10. Electron micrograph of two distal centrioles associated with a striated rootlet. Two plaques (arrows) can be seen projecting from the matrix of the distal centriole seen in cross section. Note the attachment of the distal centriole (shown in cross section) to the rootlet by the 5th and 6th striated bands (bars). X25,000

Figure 11. Electron micrograph of a tangential section showing three centrioles. Note the twin centriolar plaques (arrows) which extend from the middle distal centriole and the forming pericentriolar processes attached to the rootlet bearing distal centriole. X25,000

Figure 12. Electron micrograph of a cross section of the posterior region of a spermatid. The section passes through the distal centriole at the level of the pericentriolar processes. Nine primary processes can be seen extending from the centriolar matrix between triplets. X80,000

means of dense tapered stalks (Figs. 7 and 8). The stalks and satellites are attached to distal centrioles *via* the centriolar plaque (Fig. 8).

A single striated rootlet (Fig. 8) associates with each of the distal centrioles of the centriolar aggregate just prior to the initiation of precocial flagellar synthesis. Rootlets have not been observed in association with proximal centrioles. The rootlets project from the matrix at the anterior end (the flagellar end being considered posterior) of each distal centriole (Fig. 8). They project into the cytoplasm at an angle 20 to 30 degrees from the centriolar axis, extending 800 to 1000 nm. They are approximately 80 nm in width at their base where they emanate from the distal centriolar matrix and gradually taper to a point at their termination. The rootlets have a striated banding pattern similar to that of other cnidarian cell types (Westfall, 1965) which consists of alternating major and minor bands (Fig. 9). The striated rootlet extending from the anterior end of one distal centriole is associated with the matrix of the second distal centriole in the aggregate (Figs. 10 and 11). This association appears as a solid and consistent connection between the extended matrix of the second distal centriole and the fifth and sixth major bands of the rootlet counting out from the rootlet base.

The centrioles of the aggregate lie in a single plane before the appearance of the rootlets making it possible to acquire all four centrioles in a single thin section (Fig. 5). As the rootlet appears there seems to be a general repositioning of the centrioles in the aggregate making it impossible to cut a thin section that will pass through all four centrioles. Most sections will reveal only two centrioles (Fig. 10) but in some fortuitous tangential sections three centrioles are seen (Fig. 11). Serial sections of the aggregate have revealed that all four centrioles are still present.

After rootlets form, and the centrioles change orientation, pericentriolar processes appear. These processes, which have been studied in detail at both the ultra-structural (Kleve and Clark, 1976) and



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biochemical level (Kleve and Clark, 1980), appear after precocial flagellar biogenesis has begun. The distal centriolar matrix provides the structural media for attachment of pericentriolar processes to the centriole (Fig. 12). The pericentriolar processes of mature sperm are extensive and complex with three orders of structure. They consist of primary processes which attach directly to the centriolar matrix; secondary processes which emanate from the first order primaries; and tertiary processes which emanate from secondaries (Kleve and Clark, 1976). Only rudimentary primary processes develop in the spermatocytes of *Hydractinia*. Secondary and tertiary processes appear only during final differentiation of spermatids.

## DISCUSSION

Several structural markers can be used to distinguish parent and daughter centrioles (Anderson and Brenner, 1971). The centriolar matrix, which accumulates as centrioles age, is always much more dense in the parent centriole. The perpendicular position of daughter centrioles make it only possible to view both parent and daughter centriole in thin section when the parent is cut in cross section. The cartwheel found only in newly generated daughter centrioles provides another marker. Using these markers, the daughter centrioles synthesized in spermatocytes can easily be distinguished from their parents. If it is possible to judge the age and thus the parent-daughter relationship of a centriolar pair by the amount of dense matrix accumulated, then the distal centrioles of mature sperm should be parents to the matrix-less proximal centrioles. Sperm distal and proximal centrioles, although they are arranged perpendicular to each other, are the opposite arrangement to parent-daughter pairs found in spermatocytes. In mature sperm, both distal and proximal centrioles are only visible at the same time in thin section when the dense matrix-clad distal centriole is cut in profile and the proximal is cut in cross section. Before aggregation and appearance of matrix plaques and striated rootlets in spermatocytes, the only section that shows both distal and proximal centrioles is one in which the distal centriole is in cross section and the proximal is in profile. It appears that the orthogonal parent-daughter arrangement of newly generated centrioles is rearranged to the opposite configuration before flagellar synthesis.

Most early researchers, studying ciliated epithelia, concluded that striated rootlets performed an anchor function to absorb the stress of ciliary action (Fawcett, 1958; Gibbons, 1966). Werner (1966) suggested that rootlets might aid in the separation and positioning of centriolar pairs. Striated centriolar rootlets have been shown to contain ATPase activity (Anderson, 1977), a calcium dependent contractile protein (Salisbury, 1983) and actin has been localized in rootlets (Gordon et al., 1980). The intimate association of rootlets with distal centrioles prior to flagellar synthesis, and the concomitant change in centriolar pair orientation, suggests they are in some way involved in the maintenance and rearrangement of centriolar pairs during spermatogenesis. An anchor function in the case of sperm flagella is unlikely since rootlets are transient structures during spermatogenesis and are not present during the period of actual flagellar function.

The distal centrioles of spermatocytes and spermatids are functionally different from proximal centrioles. Distal centrioles associate with satellites, rootlets and pericentriolar processes and form the basal bodies for flagella while proximal centrioles do not. The observation that only distal centrioles are clad with a dense matrix suggests the unique nature of distal centrioles may be due, in part, to their extensive matrix. The centriolar plaque, which appears to facilitate the attachment of specializations to distal centrioles, may be an extension of the matrix material. Ultrastructurally, the plaque material is indistinguishable from that of the matrix. The plaque appears to be involved in a variety of activities. Structural continuity between satellites and the centriolar matrix is afforded by the centriolar plaque. The plaque appears to be involved in some way in the relationship of the two daughter-parent or proximal-distal centriolar pairs that make up the aggregates seen in spermatocytes. The position of the plaque between the apposed distal centrioles suggests that it may affect the maintenance of the aggregate or the spacial relationship of the aggregated centrioles. The observa-

tion that the first appearance of plaques coincides with the change in centriolar orientation in the aggregate leads credence to the contention that plaque as well as striated rootlets may function in a dynamic way to orient centriolar pairs.

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