THE CENTRIOLAR COMPLEX ISOLATED FROM STARFISH SPERMATOZOA

RYOKO KURIYAMA AND HARUO KANATANI
National Institute for Basic Biology, Okazaki 444, Japan

SUMMARY
Centrioles from spermatozoa of the starfish, Asterina pectinifera, were isolated and partially purified by solubilization of chromatin followed by sucrose density-gradient centrifugation. The ultrastructure of the isolated centriolar complex was investigated in whole mount preparations by electron microscopy. The complex unit was composed of a pair of centrioles and a pericentriolar structure, which associated with the distal end of the distal centriole by 9 spoke-like satellites extending radially to a marginal ring. Each satellite bifurcated at a dense node forming 2 fan-like shapes with a periodic striated pattern. The tubular structure of the centrioles easily disintegrated, leaving the pericentriolar structure or axonemal microtubules intact.

The distal centriole in a spermatozoon served as an initiating site for flagellar microtubule assembly; that is, a number of '9 + 2' axonemal tubules were observed adhering just beneath the distal end of the basal body. In experiments in vitro, polymerization of microtubule proteins purified from porcine brain was initiated by the structure at the ends of both proximal and distal centrioles, but not from the satellites or the marginal ring. Also, few if any microtubules were formed from the sides of each centriole, even in the presence of a high concentration of exogenous tubulin. On the other hand, centrioles of spermatozoa, when they were in mature ooplasm, could initiate the formation of sperm asters by microtubules. Therefore, centrioles in spermatozoa seem to be able to initiate microtubules in 2 ways. A possible explanation of the difference between the 2 types of microtubule organization in vivo, i.e. in the sperm cell itself and in the ooplasm, is discussed.

INTRODUCTION
The centriole or basal body has a characteristic cylindrical structure composed of 9 triplet microtubules. Several methods for the isolation and purification of this organelle from Tetrahymena pyriformis (Rubin & Cunningham, 1973; Heidemann, Sander & Kirschner, 1977), Chlamydomonas reinhardtii (Snell et al. 1974; Gould, 1975), rabbit oviduct (Anderson, 1974, 1977) or cultured Chinese hamster ovary cells (Blackburn, Barrau & Dewey, 1978) have already been devised, in order to learn more about the precise chemical or functional nature of this intriguing organelle.

The centriole or basal body has long been considered as a participant in the generation of spindle or ciliary and flagellar microtubules. There are 2 distinct methods of microtubule initiation from the centriole in vivo: one is the template and the other the astral type. The basal body located at the base of cilia or flagella serves as a template-organizing centre for ciliary or flagellar outer fibres (Fulton, 1971). The ninefold array of doublet microtubules grows directly from the tubular elements of the basal body. On the other hand, astral fibres in the mitotic spindle or cytoplasmic microtubule network in an interphase cell originate from all sides of centres (Wilson,
in which we can observe an amorphous cloud of pericentriolar material surrounding the centrioles. It has been suggested that the pericentriolar material rather than centrioles may act as a true initiating centre for spindle and cytoplasmic microtubules, both in vivo (De-Thé, 1964; Krishan & Buck, 1965; Robbins, Jentzsch & Micali, 1968; Endo, 1979) and in vitro (Weisenberg & Rosenfeld, 1975; Gould & Borisy, 1977; Telzer & Rosenbaum, 1979). In order to understand more precisely the role of the centriole or basal body as an assembly site for microtubules in vivo, the difference between these 2 types of microtubule organization should be clarified. Centrioles in a spermatozoon seem to be well-fitted for this purpose since these templates for axonemal microtubules can also nucleate an array of microtubules to form a sperm aster when they are introduced into mature eggs (Wilson, 1928; Longo & Anderson, 1968).

None of the data presented here concerning the isolation and characterization of centrioles from starfish spermatozoa have previously been reported. This paper describes the isolation and purification of the centrioles, and their ultrastructure. The capacity of the isolated centrioles to initiate microtubules is also investigated.

MATERIALS AND METHODS

Spermatozoa of the starfish, Asterina pectinifera, obtained at Hashirimizu (Kanagawa) and Asamushi (Aomori) were used. Isolated testes fragments were incubated with buffered artificial sea water (ASW: 26-9 g NaCl, 0-754 g KCl, 1-35 g CaCl₂, 2H₂O, 7-30 g MgCl₂, 6H₂O, 4-31 g MgSO₄, 7H₂O, 1-24 g H₃BO₃ in 1 litre at pH 8-2, adjusted with NaOH) containing 1·2 × 10⁻⁷ M i-methyladenine (i-MeAde; Sigma, St Louis, Mo., U.S.A.) to release spermatozoa. Calcium-free artificial sea water (CFSW) is ASW minus calcium. After three times repeated centrifugations at 140 g for 5 min the spermatozoa in the supernatant were sedimented at 1500 g for 10 min at room temperature. Subsequent steps were carried out at 0-4 °C.

Isolation and purification of the centriolar complex from spermatozoa

The packed pellet of spermatozoa was resuspended in cold ASW and homogenized with a motor-driven Teflon homogenizer using 10 strokes. The suspension of decapitated spermatozoa was centrifuged at 1000 g for 5 min to separate heads from tails. Subsequent steps of the preparation were monitored by phase-contrast microscopy. Sedimented heads were resuspended in ASW and subjected to centrifugation several times to remove residual tails. Heads freed from mitochondria could be prepared according to the method originally developed for sea-urchin spermatozoa (Morisawa, 1969) with some modifications. The final pellet of purified head fraction was resuspended in 10-20 vol. of ASW, followed by addition of trypsin (Boehringer, Mannheim, West Germany) to a final concentration of 10 μg/ml. Five minutes after incubation at 35 °C, trypsin was inhibited with 50 μg/ml soybean trypsin inhibitor (Sigma, St Louis) and the suspension was cooled on ice. The head fraction was then homogenized for 5 min using more than 10 strokes, followed by centrifugation at 1000 g for 5 min. The pellet was washed once by suspension in ASW and resuspended in cold 10 mM Tris-HCl, 0-5% Triton X100 at pH 8-0. Heparin (Katayama, Nagoya, Japan; final concentration, 200-500 μg/ml) was added to the suspension to solubilize the chromatin (Bornens, 1973). The resulting viscous solution was treated with DNase I (Sigma, St Louis; final concentration, 10-25 μg/ml) to reduce viscosity. Five minutes after incubation with DNase I at 35 °C in the presence of magnesium, the solution was centrifuged at 1000 g for 10 min to sediment the remaining nuclear fragments. The supernatant was then centrifuged at 35,000 g for 10 min to pellet a fraction rich in centriolar complexes, and containing some other cytoplasmic contaminants. This was followed by resuspension in a medium containing 10 mM Tris-HCl, 2 mM EDTA,
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0.1% Triton X100, pH 8.0. The suspension was layered on a discontinuous sucrose density gradient made of equal volumes of 0.3 M and 1.0 M sucrose dissolved in 10 mM Tris-HCl, 2 mM EDTA at pH 8.0. The gradient was centrifuged at 6000g for 20 min (Hitachi swinging-bucket rotor, RPRS 14) and the 0.3 M/1.0 M sucrose interface was collected and diluted with 5–10 vol. of 10 mM Tris-HCl, 2 mM EDTA to reduce the sucrose concentration. The partially purified fraction was again layered in a 10 ml tube over a discontinuous sucrose gradient, made of 2 ml each of 1.0, 1.5 and 2.0 M solutions in 10 mM Tris-HCl 2 mM EDTA. After centrifugation for 60 min at 25,000g, material at the 1.0 M/1.5 M sucrose interface was withdrawn and examined by electron microscopy.

Formation and isolation of sperm asters from starfish eggs

Starfish oocytes were obtained by tearing isolated ovaries in ASW with forceps. After washing several times with CFSW, they were transferred to ASW and then inseminated by the addition of an excess amount of spermatozoa in order to induce polyspermy. No membrane elevation was observed. In order to increase the motility of spermatozoa, 10-4 M histidine was added to ASW just before insemination. Fertilized eggs were sedimented and washed once with ASW, and treated with 10-6 M 1-MeAde. Polyspermy artificially induced by this procedure (Hirai, unpublished) resulted in the formation of many sperm asters within 1 egg, so is pertinent to the observation and isolation of many asters.

Sperm asters that had begun to grow from about 20 min after addition of 1-MeAde could be isolated in glycerol/Mg2+/Triton X100 medium (Sakai, Shimoda & Hiramoto, 1977). Eggs were cultured in ASW at room temperature until many asters appeared in most of the eggs. The eggs were then sedimented and washed twice with 1 M dextrose or glycerol to remove ASW. The packed pellet of eggs was then resuspended in about 10–30 vol. of isolation medium, which contained 10 mM 2(2-aminoethylether)tetraacetic acid (EGTA), 1 mM MgCl2, 2 mM ethylene glycol bis(2-aminohethyl)ether tetraacetic acid (EGTA), 1 mM MgCl2, 0.05% Triton X100 at pH 6.15. After 1 min at room temperature, the suspension was shaken to rupture the eggs. Isolated asters were collected by centrifugation at 1000g for 10 min.

Preparation of microtubule protein and polymerization onto the centriolar complex

Microtubule protein was purified from porcine brain (Kuriyama, 1975; Borisy et al. 1975) and stored at −80°C before use without losing any of its ability to polymerize. An equal volume of purified microtubule protein in polymerization buffer [0.1 M piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES), 0.5 mM MgCl2, 1.0 mM EGTA, 2.0 mM GTP at pH 6.7] was added to the freshly prepared centriolar fraction at a final concentration of 0.5–1.0 mg/ml. After incubation at 35°C for 5–15 min, polymerization was stopped by addition of 0.5 vol. of 3% glutaraldehyde containing 10 mM PIPES, 1 mM EGTA, 0.5 mM MgCl2 (pH 6.7).

Microscopy

Whole mounts of samples were prepared as described by Gould & Borisy (1977) with some modifications (Kuriyama & Borisy, 1981). Several drops of fixed samples were sedimented, at 500–1000g for 5–10 min in a centrifuge, onto ionized Formvar-coated 400-mesh grids that had been heavily coated with carbon. After washing with distilled water, the grids were stained with 2% phosphotungstic acid, adjusted to pH 6.5 with NaOH, and examined in a JEM 100CX or Hitachi H-500 electron microscope operated at 75–100 kV.

For preparation of thin sections, samples were fixed with 2.5% glutaraldehyde, postfixed in 1% OsO4, and stained with 0.5% uranyl acetate for 2 h at room temperature. After dehydration through an ethanol series, they were infiltrated and embedded in an Epon formulation of Spurr according to the standard technique. Ultrathin sections for spermatozoa, or 25 µm sections for egg samples, were cut on a Sorvall Porter-Blum II ultramicrotome with glass knives, picked up on Formvar-coated grids and stained with uranyl acetate and lead citrate.
RESULTS

Observations on the centriolar complex isolated from starfish spermatozoa

The starfish spermatozoon is composed mainly of acrosome, nucleus, mitochondrion, centrioles and flagellum. Fig. 1A, B shows electron micrographs of a thin section and a negatively stained sample of whole spermatozoa. The acrosome occupies most of the anterior part of the nearly spherical sperm head and the posterior region is characterized by the attachment of a mitochondrion located just beneath the nucleus. The mitochondrion surrounds a set of centrioles behind which is the flagellum (Hagiwara, Dan & Saito, 1967).

When a starfish spermatozoon was incubated in a low-ionic strength medium such as 1 or 10 mM Tris-HCl at pH 8.0, the flagellum was detached from the head parts but remained attached to its centrioles and consequently formed a line with a small knob at the end. This was easily observed under the phase-contrast microscope (Fig. 2). The addition of heparin greatly increased the number of these structures. Observation by electron microscopy of whole mount preparations demonstrated the fine ultrastructure around the knob where the typical continuity between the basal body and the axoneme can be clearly seen (Fig. 3). There were 2 centrioles (proximal and distal), which contained 9 sets of triplets. This was observed in thin-sectioned samples as well as negatively stained preparations. The tubules of the distal centriole were continuous with the outer doublet tubules of the flagellum, therefore serving as a template. This centriole was about 0.25 μm in diameter, 0.4 μm long and parallel to the long axis of the tail. On the other hand, the length of the proximal centriole
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(0.2 μm) was shorter than its diameter (0.25 μm). When the centriolar complex was removed from the spermatozoon and observed in whole-mount preparations, the proximal centriole was found to lie at a slant to the distal one; in some cases they were situated almost perpendicular to each other. Both centrioles were occasionally located in an almost straight line. This was also confirmed by many views of the centriolar complex sectioned at various planes (data not shown).

Fig. 2. Phase-contrast light micrographs of flagella attached to the centriolar complex. They appear as lines with small knobs (arrow) at the end which correspond to the complexes. ×1540.

The centriolar complex could be isolated and partially purified because of the structural simplicity of the spermatozoon. As described in Materials and methods, homogenization, trypsin digestion and heparin treatment caused the separation of the flagellum from the mitochondrion and the head. Repeated centrifugation and fractionation in a sucrose density-gradient then separated the centriolar complex from most of the other particulate contaminants. The negatively stained electron micrograph of Fig. 4 shows material taken from the interface between 1.0 M and 1.5 M sucrose after the second centrifugation. The presence of Triton X100 and EDTA was indispensable for the production of uncontaminated centriolar complex preparations. Treatment of the sperm heads containing centrioles with heparin had also to be complete, otherwise the recovery and the purity of the isolated centriolar complex was poor. The morphology of the fractionated centriolar complex revealed by high magnification electron microscopy is illustrated in Fig. 5A; this led us to conclude that a set of centrioles and a pericentriolar structure were isolated as a structural unit of the complex. The pericentriolar structure consisted of 9 projecting spoke-like satellites plus a marginal ring to which the radiating satellites were attached. It was located at the distal end of the complex and the satellites emanated laterally from the edge of the distal centriole. Generally the basic structure of the complex was quite well preserved and almost unaltered from the state in situ; that is, it retained
Fig. 3. Whole-mount electron micrographs of an axoneme attached to the centriolar complex. B shows a high magnification of the area outlined in A. The axoneme in C has begun to break up. The axoneme with centriolar complex in D coils round the head; such samples were frequently obtained. A, ×10200. B, ×41400. C, ×10800. D, ×8900.
almost all of its normal morphological characteristics. This can be seen by comparing the isolated centriolar complex and the native one in a spermatozoon (Figs. 5, 6). Moreover, isolated and negatively stained whole-mount preparations enabled us to see the ultrastructure of the complex in more detail (Fig. 5A, B). Each satellite emanating from the matrix of the distal end of the distal centriole branched at a dense node into forks inserted into the marginal ring. The two forks branched out and unfolded into a fan-like structure through a part of the striated and narrow stalk, and a periodic cross-banded pattern was clearly visible from the point of this bifurcation. Each stripe appeared to have about a dozen longitudinal fibrillar subunits becoming clearer and clearer towards the bifurcated brim because of their expanding shape. The marginal ring was composed of amorphous material and appeared to contain no microtubules. No special differentiated structures connecting the 2 centrioles were detected.

The centriolar complex could be stored at $-80^\circ$C in a deep-freeze without any morphological changes for at least several months. Storage at $-20^\circ$C was also effective in sucrose or glycerol, as in the case of tubulin solutions (Kuriyama, 1975). Surprisingly enough, centriolar tubules were found to be less stable than axonemal doublet microtubules. This was more striking for the proximal centriole than for the distal centriole; it seems that the shorter the centriole, the faster its breakdown. This phenomenon was also confirmed by separate experiments on centrioles extracted from cultured Chinese hamster ovary cells (Kuriyama & Borisy, 1981). After keeping the partially purified complex fraction at $0^\circ$C for 1 or 2 days, the centrioles disintegrated and remained as indefinite amorphous structures. We do not have any data available from sectioned preparations of aged centriolar fractions, therefore it is impossible to say yet whether the centrioles have disintegrated, or rather the glue, if there, has weakened resulting in disintegration. On the other hand, the satellites and the marginal ring, as well as a few of the outer doublet contaminants, retained their characteristic

![Fig. 4. Whole-mount electron micrograph of the isolated centriolar complex fraction stained with 2% phosphotungstic acid. $\times 11,200$.](image)
morphologies. The micrograph in Fig. 5B, showing a distinct striation in each satellite, was taken after 1 day of storage of the isolated complex in 10 mM Tris-HCl (pH 8.0) medium. It is clear that all the constituents in the complex except the centrioles were preserved. The structural stability of the complex also appears to depend on the spawning season of the starfish. Some of the substances used in the isolation and purification of the complex from spermatozoa, such as trypsin (10 μg/ml, 35 °C, 5 min), heparin or DNase, had no effect on the morphology. Moreover, Mg²⁺, chelating reagents or pH changes between 6.5 and 8.5 seemed to have no striking effect on the structure of the complex, but sucrose helped to stabilize the structure of the complex.

Microtubule-initiating activity of the centrioles of the spermatozoon

As is clearly shown in Figs. 3A, 3, one of the centrioles in the spermatozoon served as a template for flagellar microtubules, and we could count up to ‘9+2’ tubules from just beneath the distal end of the basal body (Fig. 7). When the isolated centriolar complex from the starfish spermatozoon was incubated with porcine brain microtubule protein in vitro, microtubules were initiated by the structure from not only the distal centriole but also from the proximal one. This is shown in Fig. 8B–D. Prolonged incubation of centrioles with tubulin caused a bidirectional assembly of microtubules, which was also dependent on the concentration of exogeneous microtubule protein (Fig. 8C). Though this phenomenon was similar to those reported previously for
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Fig. 6. Electron micrographs of the centriolar complex sectioned from several planes of a whole spermatozoon. A, x 41,400. B, x 68,000. C, x 62,100. D, x 75,600.

heterogeneous assembly of brain tubulin onto flagellar axonemes or basal bodies from sea-urchin spermatozoa or *Chlamydomonas* (Snell et al. 1974; Allen & Borisy, 1974; Binder, Dentler & Rosenbaum, 1975), the proximal centriole in this complex showed only a biased directional growth of microtubules. This may result, at least partly, from the fact that the proximal centriole lay just on the proximal side of the distal centriole. Fig. 8D shows microtubule polymerization from centriolar tubules as well as from doublet tubules that were still attached to the distal centriole. Under the staining conditions used here, the proximal centriole in the spermatozoon did not appear to show any distinct structural differentiation like the dense cap at the distal end already demonstrated in basal bodies of *Chlamydomonas* (Snell et al. 1974) or centrioles of Chinese hamster ovary cells (Kuriyama & Borisy, 1981). Since the structural polarity of the basal body or the centriole is reflected in the biased direction of the assembly microtubules onto it, it seems reasonable to conclude that the preferred end for growth is the distal end. No further kinetic analysis of microtubule assembly onto centrioles of the spermatozoon has been done. During the course of our polymerization experiments, we had many chances to see micrographs like the one
Fig. 7. Whole-mount electron micrograph of an isolated centriolar complex with short axonemal microtubules. A number of microtubules (9 + 2 = 11) are attached just at the end of the distal centriole. When the homogenization for sperm decapitation was incomplete, the centriolar complex with attached axonemal tubules, as shown in this figure, could be obtained. × 28,000.

shown in Fig. 9, in which a free proximal centriole with assembled brain microtubules is illustrated.

With a high concentration of tubulin subunits, the number of microtubules added at the distal end from both the proximal and distal centrioles exceeded 9, which is the maximal number present in these template structures. This resulted from the assembly of singlet microtubules from the B tubule as well as from the A tubule of the triplet microtubules, as first reported in Chlamydomonas flagella (Allen & Borisy, 1974). No doublet microtubules were formed even from the basal body of the spermatozoon.

The satellites and the marginal ring were incapable of nucleating microtubules in
Fig. 8. For legend see opposite.
Fig. 9. Whole-mount electron micrograph of a free proximal centriole with assembled microtubules. The final tubulin concentration was 0.9 mg/ml. × 16200.

Fig. 10. Phase-contrast light micrograph of an isolated sperm monoaster from a starfish egg. × 2680.

vitro under the conditions tested. Moreover, few microtubules were formed from the sides of the centrioles even after prolonged incubation with high concentrations of microtubule protein. Therefore, it could be concluded that the centriolar complex in the starfish spermatozoon serves only as a template for the formation of axonemal microtubules in vivo (in the sperm cell), and for the assembly of exogenous brain microtubules in vitro.

Almost immediately after the entrance of a spermatozoon into an egg, it is well-documented that the sperm monoaster appears from the region of the mid-piece within the ooplasm (Wilson, 1928; Longo & Anderson, 1968). Fig. 10 is a phase-contrast micrograph of an isolated sperm aster formed within a starfish egg after artificially induced polyspermic entry. This procedure increased the probability of finding or forming asters, which were easily identified by their simple structure.
The fibrous structure can be clearly seen originating from the dot at the centre. The electron micrograph of a sectioned whole egg (Fig. 11) shows the presence of microtubules in these sperm asters and microtubules radiating from every side of the centre where the centriole had been.

**DISCUSSION**

Pericentriolar structure in the spermatozoon has been shown to have a wide distribution among invertebrate and vertebrate species (Colwin & Colwin, 1961; Szollosi, 1964; Summers, 1970, 1972; Fontaine & Lambert, 1976). In mature spermatozoa of the starfish, *Asterina pectinifera*, the centriolar complex is composed of distal and proximal centrioles, satellites and a marginal ring. On the other hand, some morphological variability has been reported among different animal species and also the complex structure has been known to exhibit many changes during the process of cell differentiation (Summers, 1972). Preliminary observations on the isolated flagellar apparatus from sea-urchin and rainbow trout spermatozoa revealed that they have their own characteristic morphologies, which are different from that of the pericentriolar region of the starfish (Kuriyama, unpublished).

Isolation of centrioles from cnidaria or sea-urchin spermatozoa has been attempted (Kleve & Clark, 1975; Ishikawa, Ohta & Kato, 1979). In the present paper, it is clearly demonstrated that a pair of centrioles together with a pericentriolar structure was isolated as a structural unit from a starfish spermatozoon, making possible the observation of the ultrastructure of this complex in more detail by electron microscopy of whole-mount preparations. The radiating pericentriolar structure, suggestive of satellites, displays a distinct periodicity in its banded pattern. The marginal ring...
is connected with the distal centriole by bridges of 9 satellite projections. Although it has been suggested that the marginal ring contains several microtubules in mature holothurian and ophiuroid spermatozoa (Fontaine & Lambert, 1976), we failed to observe any microtubules around the ring in either the intact or the isolated complex of starfish spermatozoa. No apparent structural continuity was evident between the distal and proximal centrioles and they appeared to be independent of each other. This may result in the situation often encountered (shown in Fig. 9) in which only a free proximal centriole was seen. We failed to find any thin strands of pericentriolar material, which were suggested as connecting the 2 centrioles in hydroid spermatozoa (Summers, 1970). Therefore, it would be interesting to know how the 2 centrioles actually associate with each other and are, consequently, isolated as a pair. The same mechanism of adhesion may be involved in the interaction between parent and daughter centrioles in animal cells, and in some centriolar events such as disorientation, nucleation or separation (Kuriyama & Borisy, 1981).

In spite of the lack of available data to define the role of the pericentriolar structure in the spermatozoon, several possibilities have already been postulated (Szollosi, 1964; Summers, 1970, 1972; Fontaine & Lambert, 1976). Szollosi (1964) proposed the pericentriolar construction as a supporting element for the flagellum by anchoring the distal centriole via the satellite. Another possibility is the participation of the complex in the coordination of flagellar movement. This idea seems particularly intriguing in the light of the recent report concerning flagellar movement of Chlamydomonas (Hyams & Borisy, 1978). The waveform or direction of movement of isolated flagellar apparatus was changed by manipulation of the calcium ion concentration. Different types of motion shown within an identical structure appeared to arise from a change in the degree of flagellar bending resulting from a change in the angle between the 2 basal bodies. The rhizoplast, the striated fibrous structure associated with the flagellar apparatus in a quadriflagellate green alga, could be made to contract and extend cyclically by incubation of the organism in a solution containing calcium and ATP (Salisbury & Floyd, 1978). This contraction generated a force sufficiently strong to displace the basal bodies out of the flagellar pit. In starfish spermatozoa, the presence of ATPase activity has been reported in the region of the centriolar complex (Mabuchi & Mabuchi, 1973). Therefore the pericentriolar structure may be functionally linked to flagellar activity by controlling the arrangement of the centrioles.

Basal bodies or densely stained amorphous structures called microtubule organizing centres (MTOCs) (Pickett-Heaps, 1969) have long been considered to be the nucleating sites for microtubule formation in vivo. One of the most interesting problems on which the attention of many investigators is being focused is how the basal body or the MTOCs assemble microtubules onto themselves. Outer doublet microtubules in cilia or flagella are formed from the ninefold triplet in the basal body which is composed of microtubules; that is, the basal body serves as a seed for the axonemal microtubules (Fulton, 1971). On the contrary, microtubules in the mitotic spindle originate from all sides of the centrosome (Wilson, 1928), which is composed of a pair of centrioles and pericentriolar material. Work on microtubule assembly in vitro has made possible a new approach to test the ability to nucleate microtubules in isolated structures such
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as: basal body (Snell et al. 1974), centrosome (Gould & Borisy, 1977; Telzer & Rosenbaum, 1979), kinetochore (Telzer, Moses & Rosenbaum, 1975; Gould & Borisy, 1978), spindle-pole body in yeast (Hymas & Borisy, 1978a); MTOCs in surf-clam egg (Weisenberg & Rosenfeld, 1975) and in the quadriflagellate alga Polytomella agilis (Stearns, Connolly & Brown, 1976); or the astral centrosphere in the sea-urchin egg (Kuriyama & Borisy, 1978). The results revealed by those experiments led to the suggestion that the pericentriolar material rather than the centrioles could give rise to the great majority of the microtubules. This conclusion is not inconsistent with many reports that MTOCs need not contain centrioles. Therefore, the 2 distinct methods of microtubule formation in vivo (templating, and astral types from basal body and MTOCs, respectively) might depend only on whether pericentriolar material is present surrounding the centrioles or not.

The isolated basal body or partially fractionated sperm-head fraction was reported to be involved in the process of aster formation in frog eggs (Heidemann & Kirschner, 1975; Maller et al. 1976; Heidemann et al. 1977); this was confirmed by the results obtained in the present paper. In vivo, and also in vitro, centrioles in the spermatozoon can nucleate microtubules only by the template method. On the other hand, when they are in mature ooplasm, they can polymerize microtubules by the astral method. The 2 types of microtubule initiation, either in or out of the egg, suggest the possibility of the presence of some material surrounding the centrioles (Gould & Borisy, 1977; Endo, 1979). And it is this material, rather than the centrioles themselves, that can assemble microtubules from the pericentriolar region radially to form the sperm monoaster. Though the structural identification of pericentriolar material with true MTOCs is still far from complete in sperm asters, it seems possible that newly synthesized or already stored material is activated and accumulates around centrioles in the egg cytoplasm after maturation. Heidemann et al. (1977) found an inhibitory effect of RNase on the ability to induce aster formation by basal bodies. However, RNase-treated centrosomes isolated from cultured mammalian mitotic cells did not have an inhibited ability to form microtubules by the astral method in vitro (Kuriyama, unpublished), so RNase may interfere with the process of accumulation of the pericentriolar material around the centrioles. The identification and isolation of this intriguing substance would be a useful way to advance our understanding of microtubule organizing centres.

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