

CENTROSOME STRUCTURE IN *ANTHOCEROS LAEVIS* AND *MARCHANTIA POLYMORPHA*

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INTRODUCTION

Berlin and Bowen (2) observed paired centrioles adjacent to nuclei in vegetative hyphae, sporangiophores, and sporangia in the fungus *Albugo candida*. The centrioles were arranged end-to-end on the same axis "with little or no angle between them." Later these same authors suggested that the centrioles in an end-to-end orientation appeared to be in the process of replication (3). Turner (11) noted the appearance of centrioles immediately preceding the last division in spermatid mother cells of the bryophyte *Reboulia hemisphaerica*. Two centrioles were found aligned on a common axis with their proximal ends apposed. He observed that the "hub" of the cartwheel structure appeared to be continuous between the two centrioles. The work presented here considers the significance of an end-to-end orientation of centrioles in two other bryophytes.

MATERIALS AND METHODS

Antheridia of *Anthoceros laevis* L. and *Marchantia polymorpha* L. were fixed in 6% buffered glutaraldehyde and postfixed in 2% aqueous osmium tetroxide

(7), dehydrated through a graded series of ethyl alcohols, and embedded in Epon (8). Thin sections were cut with a diamond knife, and stained in uranyl acetate (12) and lead citrate (9). Sections were examined with a Hitachi HU-11a electron microscope.

OBSERVATIONS

In bryophyte antheridia the final mitotic division of each spermatid mother cell produces two daughter spermatids which mature to become biflagellate sperms. A two-part centrosome in each daughter spermatid produces the basal bodies necessary for the production of flagella. Figs. 1 and 2 show, respectively, spermatid mother cells of the hornwort *Anthoceros laevis* and the liverwort *Marchantia polymorpha*. Both cells are at a very early stage of division and reveal the presence of two centrosomes located on opposite sides of the nucleus. In Fig. 1 (*A. laevis*) spindle fibers are seen in the cytoplasm near each transected centrosome. The longitudinally sectioned centrosome in *M. polymorpha* is composed of two centrioles apposed end-to-end (Fig. 2, lower right). A longitudinal section through the centrosome of *A. laevis* shows that it also consists of two apposed centrioles

FIGURE 1 Spermatid mother cell of *A. laevis* showing transverse section of the centrosomes at the spindle poles. *N* (nucleus), *C* (centrosome), *P* (plastid), *S* (spindle fibers). $\times 19,800$.

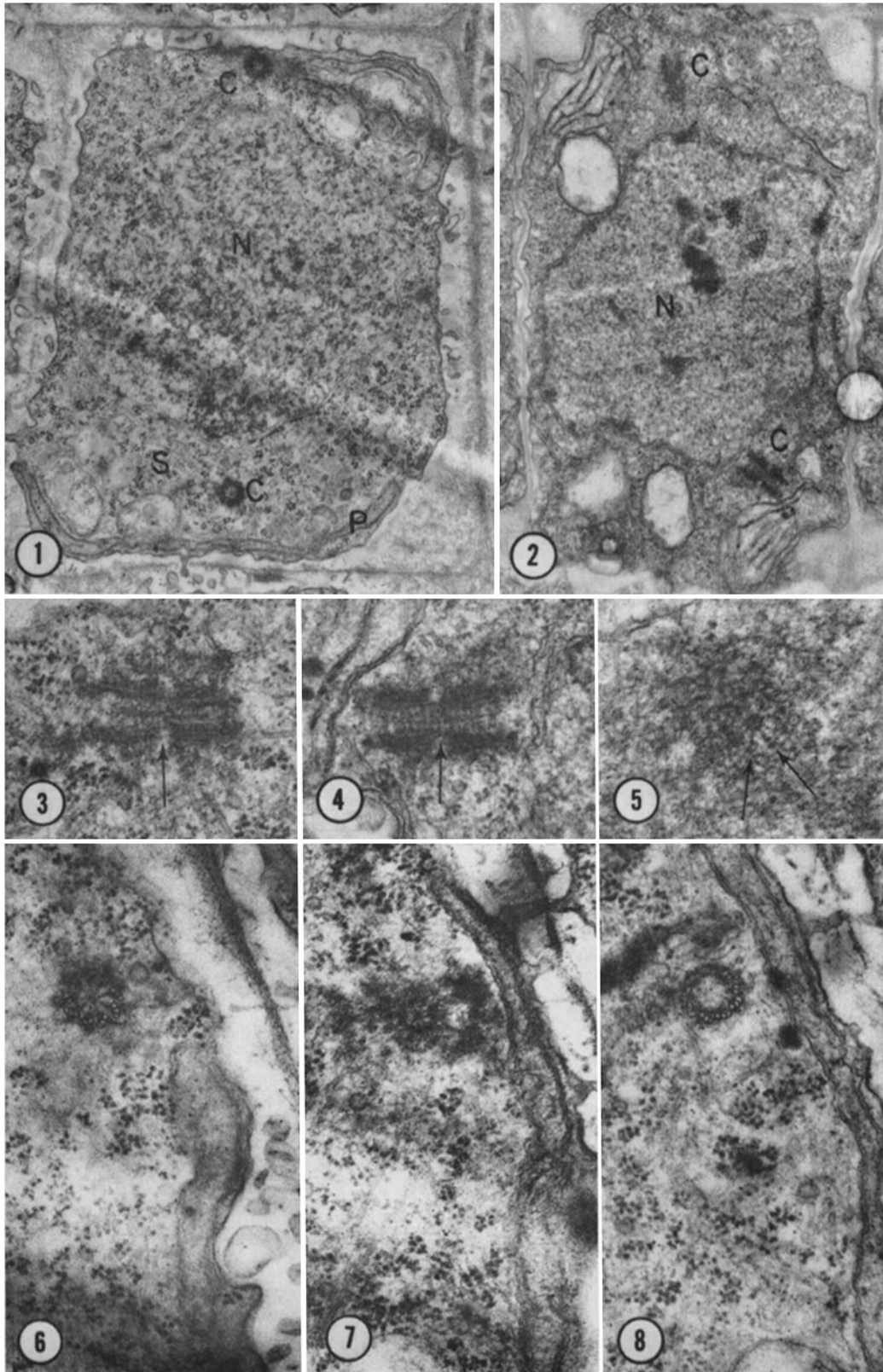
FIGURE 2 Spermatid mother cell of *M. polymorpha* showing longitudinal section of one centrosome (lower right) at the spindle pole. *C* (centrosome), *N* (nucleus). $\times 14,700$.

FIGURE 3 Longitudinal section of the centrosome of *A. laevis* showing discontinuity of triplets (arrow). $\times 50,000$.

FIGURE 4 Longitudinal section of the centrosome of *M. polymorpha* showing cartwheel spokes located in middle of discontinuity between triplets (arrow). $\times 50,000$.

FIGURE 5 Transverse section through axial midpoint of the centrosome of *M. polymorpha* showing the hub and radiating cartwheel spokes (arrows). $\times 87,000$.

FIGURES 6-8 Transverse sections through the same centrosome in a spermatid mother cell of *A. laevis*. Fig. 6 shows centriole triplets imbricating in a direction opposite that of the centriole triplets in Fig. 8. Fig. 7 is a section through the axial midpoint of the centrosome and shows a continuity of cartwheel structure. Figs. 6-8, $\times 50,000$.



(Fig. 3). The centrosome has a total length of about 0.5μ and a diameter of about 0.2μ . The two centrioles are aligned on a common axis with their proximal, or cartwheel, ends closest to the axial midpoint. In Fig. 3 the hub of the cartwheel is a thin, dense line about 0.28μ long. Although the hub is longitudinally continuous at this stage, the triplets of the centriole pairs are discontinuous, with an interval of about 400 \AA separating each set (Fig. 3, arrow).

The centrosome of *M. polymorpha* (Fig. 4) is like that of *A. laevis*. About 13 parallel lines appear perpendicularly between the triplets in Fig. 4 and are arranged about 0.1μ from the distal end of each centriole. The parallel lines are the cartwheel spokes which radiate from the hub and attach to triplets (Fig. 6, *A. laevis*). One such line of spokes is located exactly in the middle of the discontinuity between the two sets of triplets (Fig. 4, arrow). A transverse section through this same midpoint shows the continuous hub and radiating spokes (Fig. 5, *M. polymorpha*). The hub is about 330 \AA in diameter and has a darkly stained center surrounded by an area of lesser density. Each spoke is about 400 \AA long and terminates without attachment to a triplet (Fig. 5, arrows). Three serial but nonconsecutive transverse sections through a single centrosome in a spermatid mother cell of *A. laevis* are shown in Figs. 6–8. Fig. 7 is a section through the axial midpoint of the centrosome. Fig. 6 shows the cartwheel region of one of the centrioles, and Fig. 8 shows the distal end of the other centriole. The triplets of the centriole in Fig. 6 imbricate in a direction opposite those of the other centriole (Fig. 8).

One centrosome is found in each spermatid following the division of the spermatid mother cell. During late telophase in *M. polymorpha* the two centrioles undergo a separation and displacement relative to the original end-to-end orientation (Figs. 9 and 10). The displacement ultimately results in a side-by-side alignment of the distal and proximal ends of the two centrioles. A similar displacement occurs in *A. laevis* and accounts for a nearly parallel alignment of the two centrioles in the spermatid (Fig. 11). Transverse sections through the two centrioles show the triplets of both imbricated in the same direction (Fig. 12, *A. laevis*; Fig. 13, *M. polymorpha*). Figs. 12 and 13 have been printed to correspond to the clockwise orientation of triplets described by Gibbons and Grimstone (5). A drawing depicting a three-

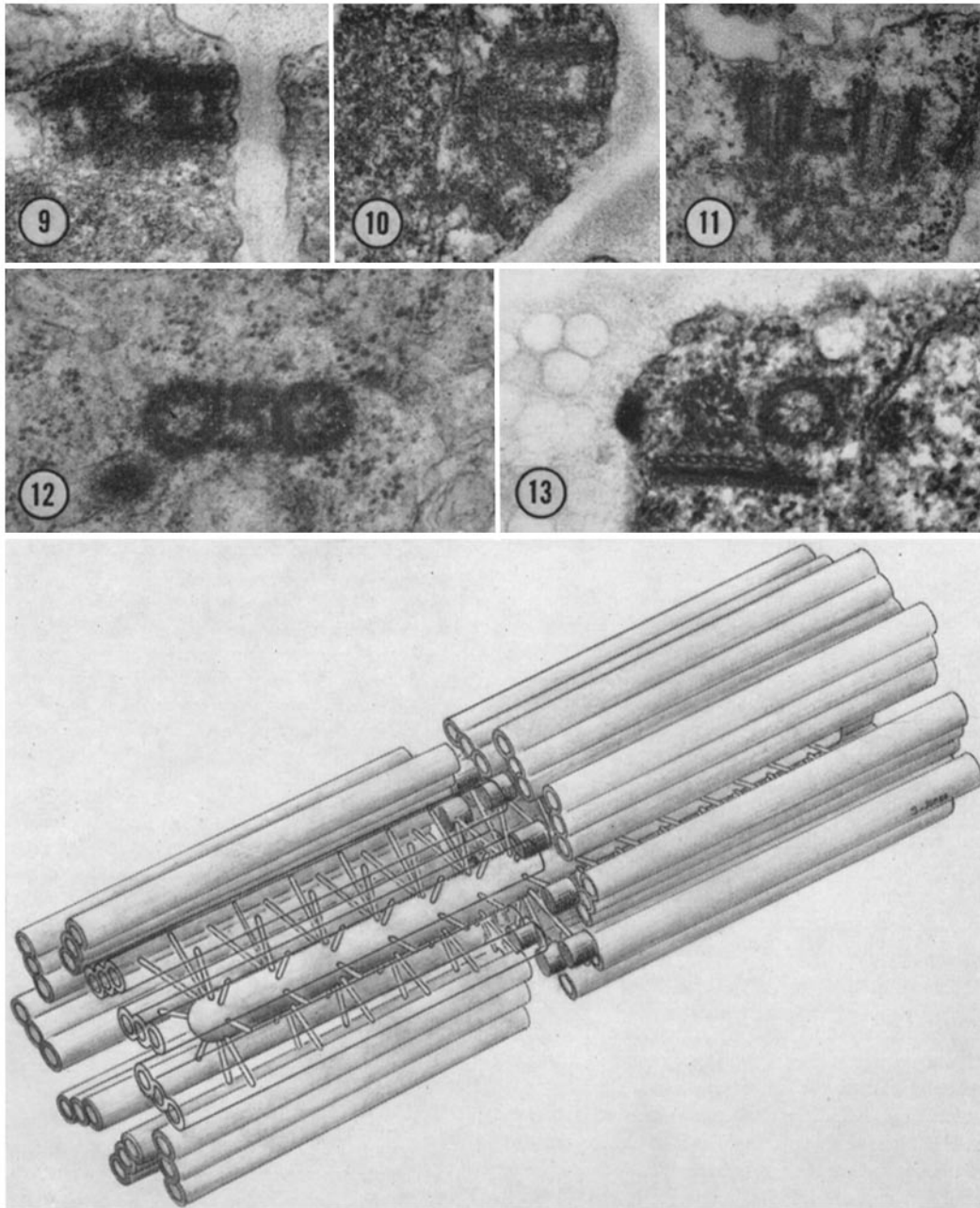
dimensional interpretation of the centrosome in *Anthoceros* and *Marchantia* is presented in Fig. 14.

DISCUSSION

Centrosome or centrosome-like bodies have been seen by Ikeno (6), Schaffner (10), and Gavaudan (4) in the spermatid mother cells of *Marchantia polymorpha*. In each case they were observed in diagonally opposite corners of the cell at the spindle poles of the final mitotic division. Our investigation confirms the presence of centrosomes in the spermatid mother cells of *M. polymorpha*. Bagchee (1) did not report centrosomes in spermatid mother cells of *Anthoceros laevis* but observed a nuclear origin of the blepharoplast in the spermatid. We have shown here that centrosomes are present at the spindle poles of the spermatid mother cell in that species, and that they persist in the spermatid to become flagellar bases.

The centrosomes of *A. laevis* and *M. polymorpha* are comprised of two centrioles apposed end-to-end and joined by a continuation of their cartwheel structures (Figs. 3, 4 and 14). This arrangement of centrioles and continuity of the cartwheel was observed by Turner (11) for centrosomes in the spermatid mother cell of the liverwort *Reboulia hemisphaerica*. Berlin and Bowen (2 and 3) also saw an end-to-end alignment of centrioles near the nuclei of developing zoospores in the fungus *Albugo candida*. Neither Turner nor Berlin and Bowen commented on the structural significance of centrioles apposed at their proximal ends. Our work shows that an end-to-end positioning results in a reversed imbrication of triplets in the two centrioles when the whole centrosome is viewed from one end to the other end (Figs. 6–8 and 14). This situation has been determined by serial sections of centrosomes in two spermatid mother cells of *Anthoceros laevis*. Serial sections have not yet been obtained to determine with certainty a reversed imbrication of the triplets of *Marchantia polymorpha*. In both organisms studied, the size of the centrosome and the requirement of precise transverse sectioning makes serial sections difficult to obtain. Reversed imbrication is assumed for the centrosome of *Marchantia* because of its similarity to the centrosome of *Anthoceros* (Figs. 3 and 4).

In the spermatid of *A. laevis* and *M. polymorpha* apposed centrioles undergo a separation and angular displacement of about 180° . The result is a near parallel arrangement of centrioles with their



FIGURES 9 and 10 Longitudinal sections of *M. polymorpha* centrosome at late telophase of the final mitotic division showing two stages of separation and angular displacement of the centrioles. $\times 50,000$.

FIGURE 11 Longitudinal section of centrioles in a spermatid of *A. laevis* showing nearly parallel orientation of centrioles. $\times 50,000$.

FIGURE 12 Transverse section of centrioles in a spermatid of *A. laevis* showing clockwise imbrication of triplets in both centrioles. $\times 50,000$.

FIGURE 13 Transverse section of centrioles in a spermatid of *M. polymorpha* showing clockwise imbrication of triplets in both centrioles. $\times 50,000$.

FIGURE 14 A three-dimensional, interpretive drawing of the centrosome in *A. laevis* and *M. polymorpha*. Some triplets have been rendered transparent to reveal details of cartwheel structure.

proximal ends side-by-side. The significance of this positioning of centrioles is seen in the fact that, after the centrioles become basal bodies, flagella grow out from them in the same direction in accordance with mature sperm morphology. Turner (11) observed "parallel" centrioles in the spermatid of *R. hemisphaerica* and noted cartwheel structure at their proximal ends. This observation and others concerning subsequent flagellar growth confirm for the centrioles of *R. hemisphaerica* an angular displacement like that seen in *A. laevis* and *M. polymorpha*. Fig. 5 in the work by Berlin and Bowen (2) shows flagella extending from the developing zoospores of *Albugo candida* in essentially opposite directions. Therefore, separation of centrioles from an original end-to-end alignment is not followed by a great angular displacement.

The origin of the centrosome has not been determined for *A. laevis* and *M. polymorpha*. No stages have been seen prior to the location of centrosomes at the spindle poles. Bagchee (1) failed to report centrosomes in the spermatid mother cells of *A. laevis*. Ikeno (6) described centrosome origin in *M. polymorpha* as an extrusion of a dark-staining mass from the spermatid mother cell nucleus. The mass divided and each part migrated to opposite sides of the nucleus. Woodburn (13) observed dense bodies at the spindle poles of the spermatid mother cell of *M. polymorpha* but could not verify a nuclear origin for these bodies.

Turner (11) suggested three possibilities for the origin of centrosomes in *R. hemisphaerica*. The centrioles all appear simultaneously and migrate in pairs to the spindle poles, or the centrioles appear, migrate to their respective spindle poles, and replicate, and, lastly, the centrioles appear directly at the spindle poles either simultaneously or consecutively. Turner was not able to verify any of the possibilities for *Reboulia*. In rare instances he did observe three centrioles in a cluster but did not state whether any two of the three were apposed end-to-end. Berlin and Bowen (3) suggested that an end-to-end orientation of centrioles represented replication, but their conclusion was

not verified. Work is being done by us to determine centrosome origin in *Anthoceros* and *Marchantia*.

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