CENTROSOMES AND MICROTUBULES DURING MEIOSIS IN THE MUSHROOM *BOLETUS RUBINELLUS*

DAVID J. McLAUGHLIN

From the Institute of Genetics, University of Copenhagen, Copenhagen, Denmark, and Department of Botany, University of Minnesota, Minneapolis, Minnesota 55455. The latter is Dr. McLaughlin's present address.

ABSTRACT

The double centrosome in the basidium of *Boletus rubinellus* has been observed in three planes with the electron microscope at interphase preceding nuclear fusion, at prophase I, and at interphase I. It is composed of two components connected by a band-shaped middle part. At anaphase I a single, enlarged centrosome is found at the spindle pole, which is attached to the cell membrane. Microtubules mainly oriented parallel to the longitudinal axis of the basidium are present at prefusion, prophase I and interphase I. Cytoplasmic microtubules are absent when the spindle is present. The relationship of the centrosome in *B. rubinellus* to that in other organisms and the role of the cytoplasmic microtubules are discussed.

Electron microscope studies have revealed a diversity of centrosome types in fungi (2, 4, 8, 9, 13, 16, 25, 27, 29). Centrioles are absent in the Ascomycetes and Basidiomycetes, both of which lack motile cells. In the Basidiomycetes two types of centrosomes have been reported: a simple, more or less spherical form (9, 15, 16), and a dumbbell-shaped form (4, 8). The dumbbell-shaped centrosome is here reported for meiosis in *Boletus rubinellus* Peck, and the role of cytoplasmic microtubules in this process is discussed.

MATERIALS AND METHODS

Fruitbodies were obtained on Modess' modification of Hagem's agar under controlled environmental conditions (12). For light microscopy pieces of hymenium were fixed and stained with hematoxylin according to Lu's procedure (9). For electron microscopy the tissue was fixed for 2 hr at room temperature or on ice in 4% glutaraldehyde prepared according to the method of Umphlett and Olson (24) to which $CaCl_2$ (1 ml of 0.01 M solution/100 ml fixative) was added and which was buffered at pH 6.4 or 7 with 0.067 M phosphate. The tissue was washed three times in buffer, postfixed in cold, phosphate-buffered, 1% osmium tetroxide for 2 hr or overnight, rinsed in buffer, dehydrated in ethanol and propylene oxide, and embedded in Epon (10) or Araldite-Epon mixture 1 (14). Silver sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome, mounted on Formvar-coated copper grids, and stained with uranyl acetate and lead citrate (21). Photographs were taken on a Zeiss EM9A, Siemens Elmiskop 1A, or Hitachi HU-11C. Sizes given are approximate. More than 300 micrographs of basidia at prefusion through meiosis I have been examined.

Terms for the parts of the centrosome are as follows: At interphase and prophase the centrosome consists of two components ("globular ends" of Girbardt, 4) connected by a middle part (4). At later stages in division, only one component is present.

RESULTS

Light microscope observations reveal that meiosis is not synchronous in *Boletus rubinellus*. All stages in meiosis may be found in any one piece of maturing hymenium. Exact correlation of stage of meiosis at the light and electron microscope levels is difficult

due to the small size of the nuclei (3–5 μ) and the lack of synchrony in division. Stages of basidial development were determined in part by nuclear size and condition and in part by the condition of the cytoplasm. The prefusion basidium is narrow and contains two nuclei, 2 × 3 μ in diameter, each with a nucleolus. Cytoplasmic microtubules parallel the long axis of the basidium but are not abundant. Lipid droplets are rare or absent. A few small vacuoles and a larger basal vacuole may be present. By prophase I a large fusion nucleus, 3 × 5 μ in diameter, has formed, cytoplasmic microtubules are abundant, and lipid droplets are beginning to accumulate.

In the basidium of *B. rubinellus* before nuclear fusion, at prophase I and at interphase I of meiosis the double centrosome sits in an indentation of the nuclear membrane (Figs. 1, 6, 8). It is oriented toward the apex of the basidium. It is a dumbbell-shaped structure, 0.65–0.7 μ long, composed of two components connected by a middle part, 130 mμ long and 65 mμ thick. The components are flattened spheres, 240 mμ in average diameter and 180 mμ thick in their flattened dimension, appressed externally to the nuclear membrane. The components have a light core enclosed by a dense zone about 300 A thick and are composed of granular and fibrillar material. A ribosome-free zone surrounds the centrosome at prophase I (Figs. 6, 7). Dense material, probably chromatin, is present on the inner surface of the nuclear membrane adjacent to the centrosome (Figs. 1, 2). The membranes of the nuclear envelope remain closely adherent in the vicinity of the centrosome and enclose darker material than other regions of the nuclear envelope (Fig. 2). Double centrosomes occur on both nuclei before nuclear fusion which initiates meiosis (Figs. 1, 2).

At prophase I, interphase I, and before nuclear fusion, microtubules form a basket around the nucleus or nuclei (Figs. 3–5, 8). These microtubules are 250 A in diameter and surrounded by a light zone 100 A in diameter. Most of the microtubules are parallel to the longitudinal axis of the basidium. A few extend around the basidial apex or the nucleus in other directions. Seventy microtubules were found in a cross section of an interphase I basidium (Fig. 8). Microtubules are at times closely associated with the centrosomal component (Fig. 8).

At metaphase and anaphase I the spindle forms across the apex of the basidium and is seen in the light microscope as a horseshoe-shaped structure apparently attached at the poles to the cell membrane (Fig. 9). In the electron microscope these observations are confirmed. Here the spindle fibers radiate from a pole attached to the cell membrane and pass between the chromosomes (Figs. 10–12). The nuclear membrane has broken down at the pole, and fragments of nuclear membrane or endoplasmic reticulum border the spindle apparatus. The centrosomal components from prophase I apparently have separated to form the two spindle poles. The centrosome at the spindle pole, about 550 mμ in diameter, lacks the dense outer zone of a centrosomal component at prophase and has about twice its diameter.

At anaphase I, microtubules appear to be absent from the basidium except for the spindle. A similar phenomenon has also been reported in another Basidiomycete (4), algae, and higher plants (20). The spindle microtubules are similar in size to those found at prophase I in the cytoplasm. Each spindle pole contains 200–250 microtubules.

DISCUSSION

In its general appearance and size the double centrosome which is present at prefusion, prophase I, and interphase I in *Boletus rubinellus* agrees with the centrosome observed at mitosis in *Polystictus versicolor* (4) and at meiosis in *Coprinus radiatus* (8). The alteration of the nuclear membrane and presence of dense material, probably chromatin, at the point of centrosome attachment is found in both *B. rubinellus* and *P. versicolor*. In *C. radiatus* the centrosome is not attached to the nuclear membrane. The dense nuclear material near the centrosome may represent that reported in the prefusion and early fusion nucleus in *Neurospora* (18) where chromasomes gather at a dark-staining region on the nuclear membrane. Pickett-Heaps (20) considers close association of chromosomes with the nuclear membrane, a situation also found in dinoflagellates (7), to be a primitive condition. Girbardt (4) and Lerbs and Thielke (8) report that the two components of the prophase centrosome separate and form the poles of the spindle. Whether the two components of a prophase centrosome in *B. rubinellus* also separate to form the poles at anaphase I remains unproven, but seems probable.

In *B. rubinellus* the size of the centrosomes at anaphase is more than twice the size of the centrosomal components at prophase, a phenomenon

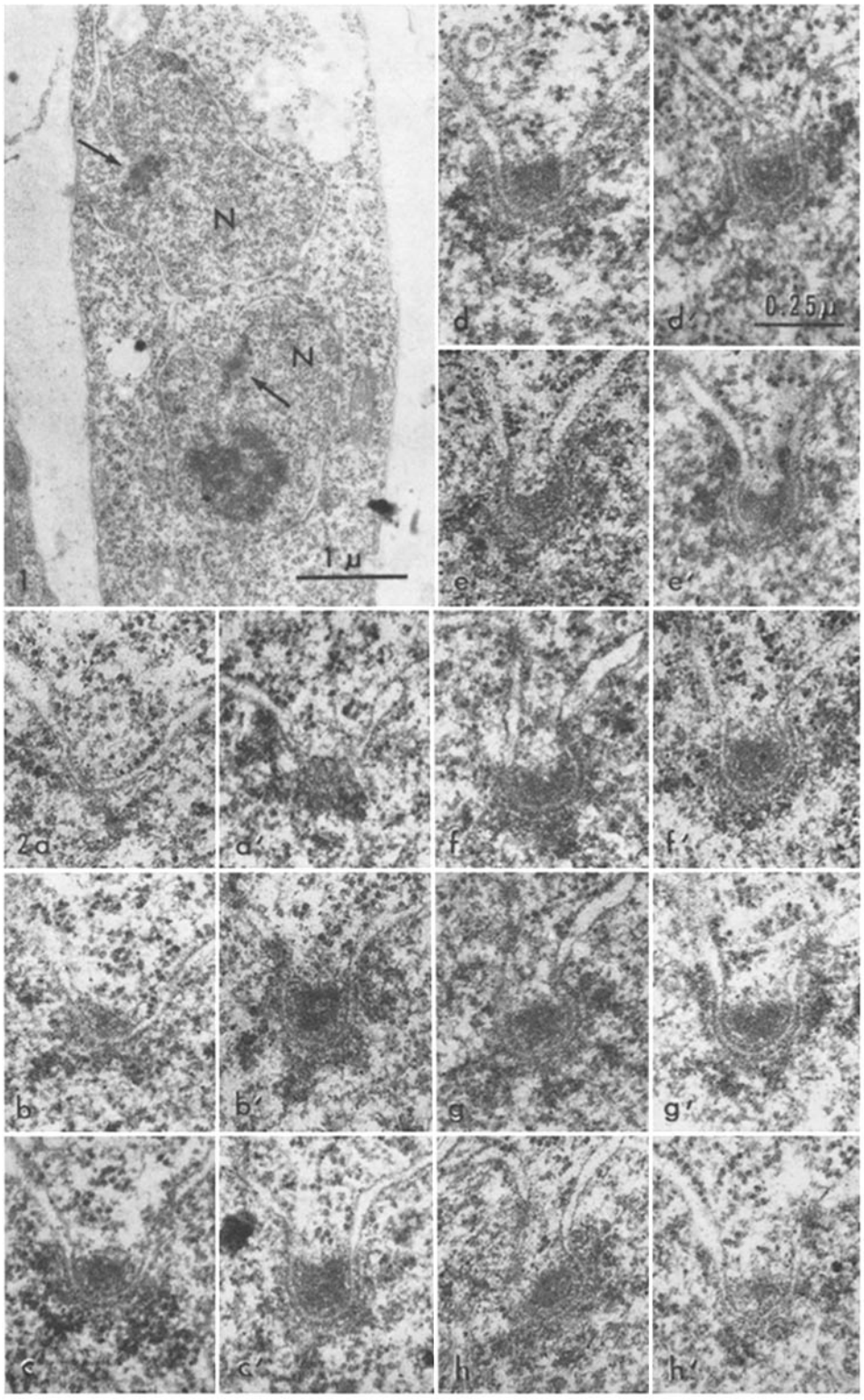

FIGURE 1 Longitudinal section through a prefusion basidium with a cross section of the double centrosome (arrows) in an indentation in each nucleus. The basidial apex is toward the top of the figure. All figures are from material fixed at room temperature unless otherwise indicated. *N*, nucleus. × 16,000.

FIGURE 2 A series of eight cross sections through the double centrosomes in Fig. 1: upper nucleus, a–h; lower nucleus, a′–h′. Note the flattening of the centrosome at the middle part in e and e′. The nuclear membranes are closely appressed and dense nuclear material is present near the centrosome. × 52,500.

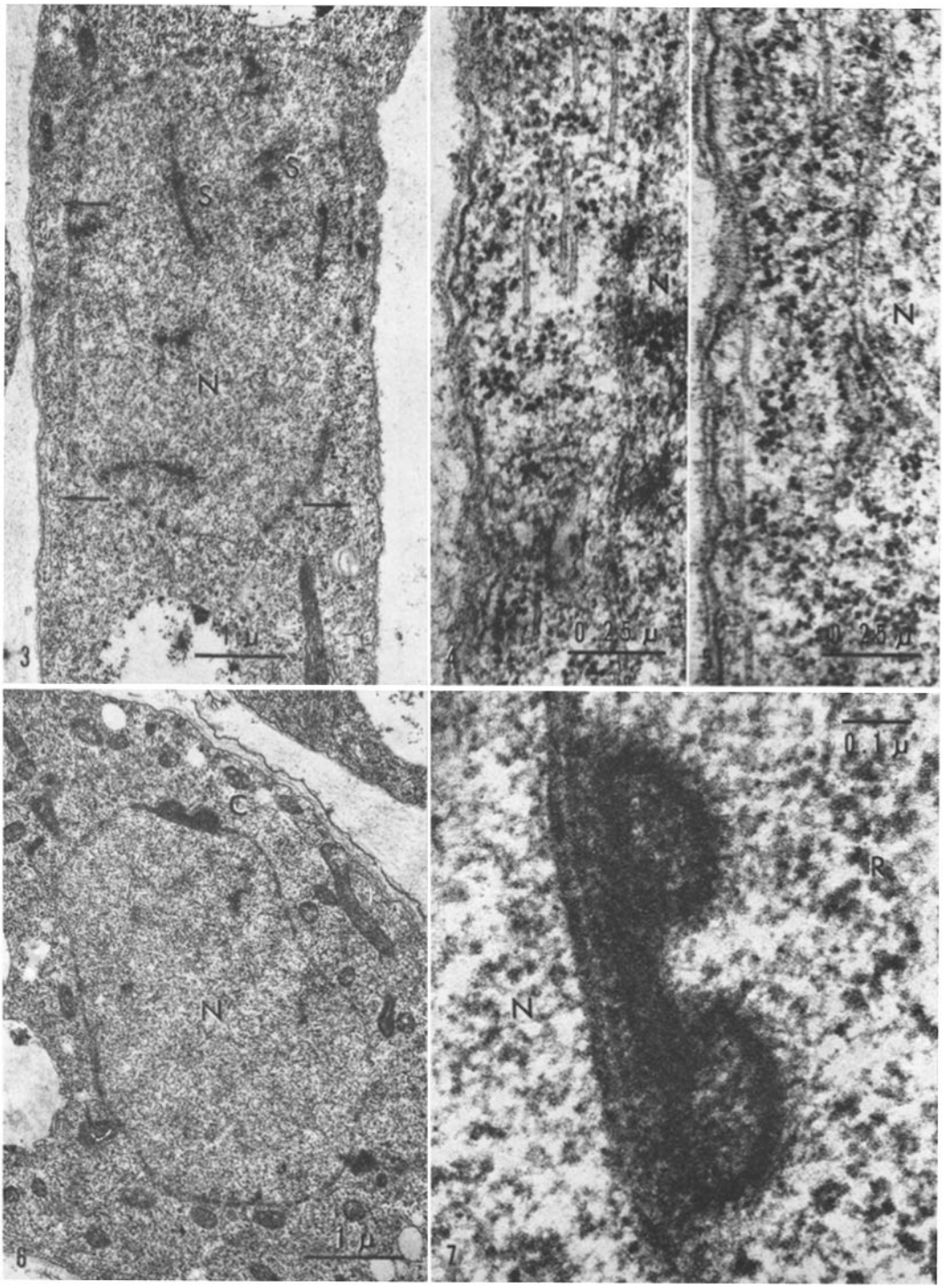

FIGURE 3 A longitudinal section through a basidium, apex upward, at prophase I of meiosis containing synaptonemal complexes. Cytoplasmic microtubules (arrows) parallel the long axis of the basidium. *N*, nucleus; *S*, synaptonemal complex. × 14,300.

FIGURES 4 and 5 Microtubules indicated by arrows at the left in Fig. 3 are shown at higher magnification. *N*, nucleus. Fig. 4, × 57,200. Fig. 5, × 58,600.

FIGURE 6 A longitudinal section through the double centrosome attached to a late prophase I nucleus. The basidium with the apex toward the top of the figure is sectioned obliquely. *C*, centrosome; *N*, nucleus. × 14,650.

FIGURE 7 A higher magnification of another section of the double centrosome shown in Fig. 6 showing in longitudinal section the two components connected by the middle part and surrounded by a ribosome-free zone. *N*, nucleus; *R*, ribosome. × 102,400.

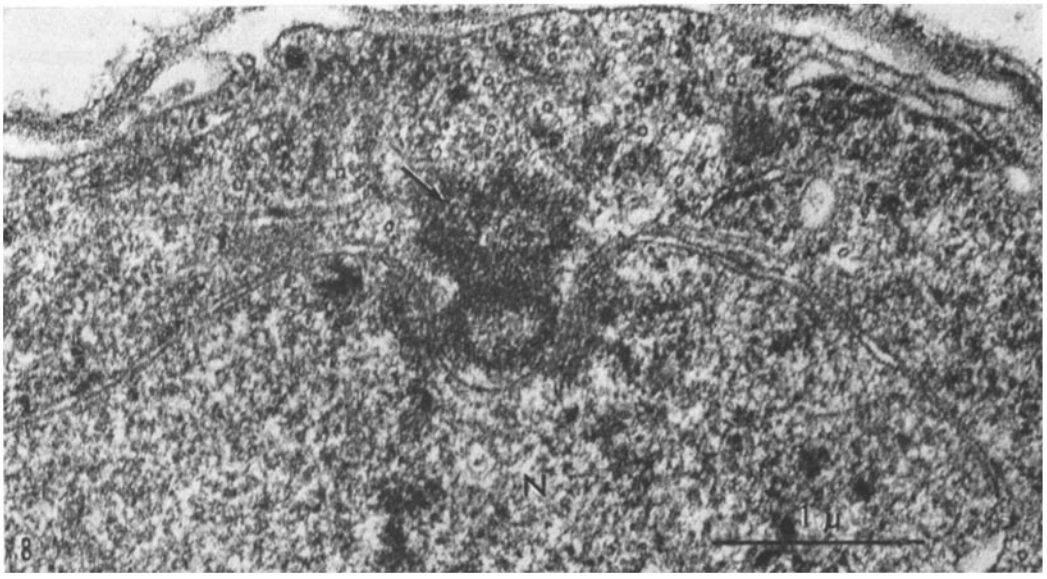

FIGURE 8 A cross section through a binucleate basidium at interphase I with the double centrosome sectioned tangentially and revealing the band-shaped middle part. Numerous cytoplasmic microtubules are shown in longitudinal and cross section including one (arrow) adjacent to a globular end. *N*, nucleus. × 28,250.

also encountered in *C. radiatus* (8). In *P. versicolor* the centrosome is reported to be continually present (4). Centrosomes attached to nuclei containing synaptonemal complexes have not been seen in *B. rubinellus*. In Ascomycetes, also, the centrosome seems to be absent at this stage (29). The presence of one double centrosome on each prefusion nucleus in *B. rubinellus* presents a question as to the fate of these centrosomes at nuclear fusion. Three possibilities exist: (*a*) at the time of karyogamy both double centrosomes disappear and one double centrosome reforms during prophase; (*b*) one double centrosome forms either by fusion of the two or by elimination of one; (*c*) each double centrosome enlarges to form one spindle pole as encountered at anaphase. The apparent absence of the centrosome when synaptonemal complexes are present suggests that the first possibility is correct. The fate of the centrioles at fertilization in other organisms is pertinent to this point. In most cases the cells obtain a centriole from the male gamete, the egg centriole being suppressed; in a few cases the centriole is derived from the egg, and in one case from both egg and sperm (26).

The breakdown of the nuclear membrane during division, here reported in *B. rubinellus*, has been observed in other Basidiomycetes (4, 8, 9, 15, 16, 23). No membrane intervenes between the centrosome and the spindle as occurs in Phycomycetes and Ascomycetes where division is intranuclear (2). The spindle pole is attached to the cell membrane in *B. rubinellus*, a configuration similar to that shown in *Coprinus* (9, Fig. 25). The association, of the centrosome with the cell surface may lend support to the spindle.

Numerous terms have been applied to centrosomes in the fungi which lack true centrioles; for example, in Ascomycetes: archontosome, centriolar plaque, centrosome, centrosomal plaque (A. Beckett, personal communication; 22, 25, 27, 29); in Basidiomycetes: kinetochore equivalent, centrosome, centriole-like body, peripheral body (4, 8, 9, 15, 16, 23). Westergaard and von Wettstein (27) point out the inappropriateness of using the term centriole for any structure which lacks the nine triplet tubules arranged in a circle, and they prefer the term centrosome, or polar cap as used in higher plants, for the polar structure involved in nuclear division. The term centrosome as used in this paper is not that of earlier authors who defined it as a special region of the cytoplasm enclosing a pair of centrioles which are now known to contain a characteristic microtubule pattern

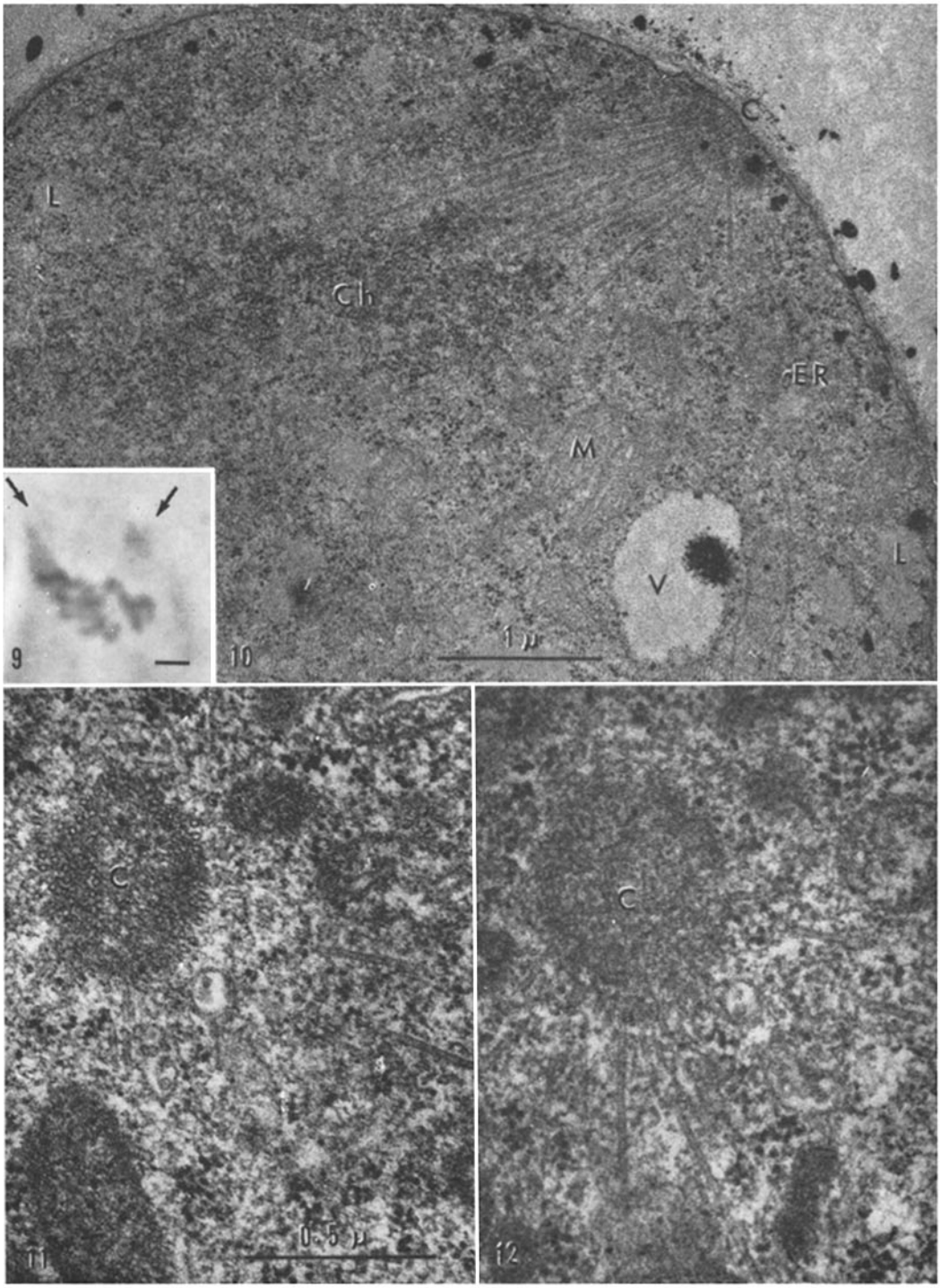

FIGURE 9 A light micrograph of the apex of a late metaphase I or early anaphase I basidium with spindle poles (arrows) attached to the cell surface. Scale indicates 1 μ. × 5,000.

FIGURE 10 A longitudinal section through a basidial apex at anaphase I showing the spindle pole attached to the cell membrane. *C*, centrosome; *Ch*, chromosomes; *ER*, endoplasmic reticulum; *L*, lipid droplets; *M*, mitochondrion; *V*, vacuole. × 24,500.

FIGURE 11 A cross section through the surface of the centrosome forming the pole at metaphase or anaphase I with spindle microtubules arising from a single, spherical centrosome. Fixed at 4°C. *C*, centrosome. × 57,000.

FIGURE 12 A section within the spindle pole adjacent to the section shown in Fig. 11. Microtubules radiate from the periphery of the centrosome. Fixed at 4°C. *C*, centrosome. × 57,000.

(26). The usage of centrosome here is in accord with earlier studies in which this term was applied to the polar body when centrioles could not be detected within it (26). Centrosome is accepted here as a generic term to designate the polar body. Variations in the centrosomal structure justify special descriptive terms for each type. Kinetochore equivalent (4) is an unsatisfactory term because the kinetochore is a chromosomal region and is not clearly related to the spindle pole, although such a relationship has been suggested (20). The term kinetochore equivalent does not characterize the distinct structure found in *P. versicolor*, *C. radiatus*, and *B. rubinellus* and may be taken to imply that kinetochores are absent in the fungi, centrosomes assuming their role. Basidiomycete centrosome is a more descriptive term for the structures found in these fungi. Lu (9) and Motta (16) report chromosomal microtubules in Basidiomycetes which implies that kinetochores exist although they may not be sharply defined. In the Ascomycetes, centrosomes have been reported in *Neottiella* (27) and have been seen in various light microscope studies (18). Kinetochores are often diffuse or structureless in higher plants (20): thus, the absence of well-defined kinetochores in many fungi does not justify the assumption that the centrosomes assume their function.

Two types of centrosomes have been reported in Basidiomycetes: those with two components (*B. rubinellus*; 4, 8) and those with one component (9, 15, 16). If the single centrosome proves to be actually single (rather than a sectional view of a two-component type), then two forms of Basidiomycete centrosomes can be distinguished: the single and the double. If only one type occurs in the Basidiomycetes, it can be called the Basidiomycete centrosome. The single Basidiomycete centrosome should not be confused with the individual components of the double Basidiomycete centrosome found at certain stages of division.

The homology of centrosomal structures, within the fungi and with other groups of organisms, is unclear. If, as Margulis (11) postulates, all eukaryotic organisms have a 9 + 2 flagellated ancestor and retain the genetic information associated with this organelle, then a relationship may exist between centrioles and centrosomes in various eukaryotic organisms. Furthermore, the origin of the centriole in *Labyrinthula* (19) shows that a complex polar structure may be derived from a simple one and suggests that the structurally simple centrosomes of other organisms may be related to centrioles. Certain homologies are suggested by the structure of the double Basidiomycete centrosome. The double nature of the centrosome suggests a relationship to paired true centrioles. The mechanism of duplication proposed by Girbardt (4) wherein a single centrosome gives rise to a second centrosome adjacent to itself also suggests a relationship to true centrioles. Motta (16) points out the similarity in size between a single Basidiomycete centrosome and a centriole. The centrosome in Ascomycetes may be homologous with the middle part of the double Basidiomycete centrosome which it resembles in thickness and in its close association with and indentation in the nuclear membrane (25, 27, 29). Zickler (29) portrays the centrosomal plaque as band-shaped, on the basis of light and electron microscope evidence. The middle part of the centrosome in *B. rubinellus* is also band-shaped. A relationship between Ascomycete and Basidiomycete centrosomes is also favored by Pickett-Heaps (20). A tubular substructure in the centrosome has been reported in a number of Ascomycetes (22, 25, 29) which suggests a relationship to centrioles. An increase in the size of the centrosome occurs in some Ascomycetes during meiosis (29). The change in size of the spindle pole in *B. rubinellus* and *C. radiatus* (8) may be comparable. In both of these organisms the centrosome is oriented toward the basidial apex which may indicate that it is involved in nuclear movement (8).

The relationship between the Basidiomycete centrosome and the electron-transparent, organelle-free polar cap or centrosome of higher plants is uncertain particularly in view of the results obtained with *Chlamydomonas* (6), which is often assumed to be an ancestor of higher plants. In this alga the spindle pole and the centriole are functionally independent of each other, the former being involved in nuclear division, the latter in cell division. Pickett-Heaps (20) proposes that centrioles and "Microtubule-organizing centres" (MTOC) are independent of each other, an argument based heavily on the absence of centrioles in higher plants and the variations in centrosomes in the fungi. The absence of a centriole involved in nuclear division in one or two lines of evolution, e.g., that leading to the angiosperms, does not justify a generalization for all lines of evolution. Moreover, the fungi are poor candidates upon which to base a generalization since they are not

evolutionarily related to the higher plants, nor are they a monophyletic group (28). In *Labyrinthula* (19) the "MTOC" and the centriole-forming region of the cell must be intimately related if not identical. This intimate relationship makes it difficult to deny with certainty that the Basidiomycete centrosome is derived from or related to the centriole. The possibility that the "MTOC" evolved from the centriole must also be considered. We will not clearly understand the interrelation of types of centrosomes until more is known of the structure, origin, and chemical organization of centrosomes in a wide variety of organisms.

Microtubules in *B. rubinellus* agree, in size and structure and in the presence of a clear zone around them, with those of other organisms (2, 17, 25). The small number of microtubules comprising the spindle in *B. rubinellus* agrees with that found in *Saprolegnia* (5), and confirms Olive's conclusion (18) on the small size of the spindle in fungi in general. By contrast, the spindle in *B. rubinellus* is no larger than one or two chromosomal fibers of the African Blood Lily, *Haemanthus katherinae*, and is dwarfed by the 5000–10,000 microtubules in its spindle (1).

In *B. rubinellus* and *P. versicolor* (4), microtubules at interphase and prophase are mostly oriented parallel to the longitudinal axis of the basidium and the terminal cell of the vegetative hypha. A variety of roles has been assigned to microtubules (17). Cytoplasmic microtubules are implicated in the orienting of microfibrils in higher plant cell walls (17), but no evidence for such a role exists for fungal cells (2). The presence of a rigid cell wall makes it unlikely that the cytoplasmic microtubules function as a cytoskeleton (17). Girbardt (4) also believes that cytoplasmic microtubules do not serve this function but are involved in nuclear movements through sol or gel transformations of the cytoplasm. These microtubules have been implicated in the orientation of events within the cell, particularly in predetermining the plane of spindle formation in algae and higher plants (3, 20). The cytoplasmic microtubules in *B. rubinellus* may be involved both in nuclear movement and in the orientation of the meiotic spindle in the basidium.

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