MEIOSIS IN COPRINUS
IV. MORPHOLOGY AND BEHAVIOUR OF SPINDLE POLE BODIES

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SUMMARY
Meiotic synchrony in the genus Coprinus has permitted the sequential study of spindle pole body (SPB) behaviour through the meiotic process. The SPBs are monoglobular in the young basidia immediately after the last premeiotic mitosis. From 10 to 15 h before karyogamy until pachytene, spindle pole bodies are not found. They become conspicuous in diplotene and persist until the completion of meiosis. During diplotene, metaphase, anaphase and telophase stages the spindle pole bodies are monoglobular but at late diplotene they duplicate and become diglobular with an isthmus connecting the 2 globular elements. The spindle pole bodies remain in a diglobular state until diakinesis when the isthmus breaks separating the 2 daughter spindle pole bodies. The diglobular SPBs in late diplotene and prophase II are believed to represent the duplicated form of the monoglobular state. The spindle pole bodies in Coprinus contain no centrioles. In thin sections the SPBs appear to be fibrillar amorphous structures with a dense inner core surrounded by a less-dense outer zone.

INTRODUCTION
In a comparative study with light and electron microscopy, Lu (1967a) reported the fine structure of centrosomes in Coprinus lagopus. Since then a number of ultrastructural studies revealed different centrosomal forms in Ascomycetes and Basidiomycetes. In the latter, for example, 2 centrosomal types have been reported: a monoglobular or spherical form (Lu, 1967a; Motta, 1967, 1969) and a diglobular or dumbbell form (Girbardt, 1968, 1971; Lerbs & Thielke, 1969; McLaughlin, 1971). Both forms may be found in a single species at different times during the nuclear cycle. Whether the diglobular structure is a duplicated form of the monoglobular centrosome is still not clear.

In Coprinus the close synchrony of meiotic divisions facilitated the sequential study of meiosis (Raju & Lu, 1970) and made it possible to correlate the centrosome behaviour observed with light and electron microscopy to the meiotic sequence. Since the meiotic process and the centrosome behaviour in 2 species of Coprinus (C. lagopus and C. comatus) are similar, the sequence of events is illustrated with the best evidence obtained from both the species.

The centrosome is variously named in the literature (e.g. centriole: Lu, 1967b; centriolar plaque: Robinow & Marak, 1966; Zickler, 1969; Wells, 1970; Centriole ähnliche körper: Lerbs & Thielke, 1969; centrosomal plaque: Zickler, 1970; spindle plaque: Moens & Rapport 1971a, b; Van Winkle, Biesele & Wagner, 1971; centro-
Materials and Methods
Basidiocarps of *C. lagopus* were obtained from cultures on horse dung or a synthetic medium devised by Rao & Niederpruem (1969). The culture dishes, inoculated with a dikaryotic mycelium, were incubated at 35 °C for 5-6 days until the substrate was covered by mycelium. They were then incubated at 25 °C under a regime of 16 h light and 8 h dark where they subsequently produced basidiocarps in 4-5 days. The basidiocarps of *C. comatus* were collected from Guelph gardens during the summer and fall months. They were brought into the laboratory with a block of undisturbed soil and kept in a moist chamber at room temperature where they developed and matured normally. A few gills from the developing basidiocarps were taken at various time intervals until meiosis was completed and processed for light and electron microscopy as described below.

Light microscopy
Gills were fixed in modified Lu’s fixative (Lu, 1962) containing 9 parts ethanol, 6 parts propionic acid and 2 parts 10% aqueous chromic acid. Since fungal cells are highly basophilic, acid hydrolysis was important for proper staining of chromosomes and spindle pole bodies. A simplified iron haematoxylin method (Henderson & Lu, 1968) was used with slight modifications (Lu & Raju, 1970).

Electron microscopy
Samples were fixed for 2-4 h in 5% glutaraldehyde in either 0.1 M cacodylate buffer at pH 7.2 or 0.067 M phosphate buffer at pH 6.0. They were washed overnight with the same buffer and postfixed in 2% OsO₄ for 1-2 h, dehydrated in an ethanol series and embedded in a low viscosity resin mixture (Spur, 1969). Figs. 3 and 7 were taken earlier using OsO₄ fixation and Araldite embedding. Sections were cut with a Porter-Blum MT-2 ultramicrotome using a glass knife and examined with a Philips EM 200 or a Zeiss EM 9 at either 60 or 80 kV.

Results
Light-microscope observations revealed spindle pole bodies at the last premeiotic mitosis as well as during meiosis in the basidia of *Coprinus*. The spindle pole bodies are well stained and appear to be spherical at the mitotic metaphase (Fig. 1). These spherical structures were also seen in the young basidia immediately following mitosis (Fig. 2). However, during the period from 10 to 15 h before karyogamy to pachytene, extensive studies with light and electron microscope failed to reveal any structure analogous to a spindle pole body. This organelle can be seen again from late pachytene/diplotene to the completion of meiosis. At late pachytene/early diplotene a spherical spindle pole body was seen attached to an indented portion of the outer membrane of the nuclear envelope (Fig. 3). Identification of this stage as late pachytene/diplotene was confirmed by the fact that on the same sections synaptonemal complexes were found. The spindle pole bodies become quite conspicuous during diplotene and
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were readily seen by light or electron microscopy (Fig. 4.) In some cells the spindle pole bodies may be found at a distance from the nuclear material since acid hydrolysis in the staining procedure destroys the nuclear envelope. At late diplotene the spindle pole bodies are usually diglobular with 2 globular elements (daughter SPB) connected by SPB isthmus (Figs. 4, 9). The isthmus stretches and breaks at diakinesis separating the 2 daughter spindle pole bodies (Fig. 9), which are spherical during the subsequent metaphase, anaphase and telophase stages (Figs. 5, 6, 8, 10). At metaphase I, the spindle pole bodies occupy opposite poles at right angles to the basidium. Figs 5 and 6 show the ultrastructure of the organelle in the lateral view and the polar view respectively. The spindle pole bodies are fibrillar amorphous structures. In the polar view, however, a dark band was seen around the inner dense sphere which is surrounded by a less-dense outer region (Fig. 6). In Coprinus the nuclear envelope is disrupted at metaphase I (Fig. 5) and reformed in telophase. The spindle pole bodies are spherical for a short time at the end of the first meiotic division but soon become diglobular in interkinesis or in prophase II (Figs. 7, 10, 11). The size of spindle pole body at metaphase I and at the end of the first division is approximately the same (Figs. 8, 10). In prophase II, however, the diglobular spindle pole body is almost twice as large as the monoglobular one of metaphase I and telophase I suggesting that its duplication involves the synthesis of some SPB material. During the second metaphase and anaphase stages the spindle pole bodies are monoglobular (Fig. 12). At the end of second division, there is one spindle pole body associated with each nucleus. At this stage, it appears to be monoglobular although the photomicrograph does not clearly illustrate this (Fig. 13). Electron microscopy at this stage revealed only the monoglobular form.

DISCUSSION

Since the spindle pole bodies exist either in monoglobular or diglobular form depending upon the state of the nucleus (Girbardt, 1971) it is important to understand its behaviour in relation to the meiotic sequence. In Coprinus the diglobular spindle pole bodies observed in late diplotene and prophase II are believed to represent the duplicated form.

Although spindle pole bodies are observed in young basidia, their continued presence up to late pachytene/early diplotene could not be demonstrated in the present study. Similarly, Zickler (1970) and McLaughlin (1971) failed to find any structure between karyogamy and pachytene that resembled a spindle pole body. During late pachytene and early diplotene, Lu (1967 a) showed a monoglobular amorphous structure as the Coprinus spindle pole body. However, as cautioned by Girbardt (1971), unless serial sections are examined even a diglobular form may appear as monoglobar if the sections are cut perpendicular to the axis of the diglobular spindle pole body.

McLaughlin (1971) reported diglobular spindle pole bodies in the basidia of Boletus rubinellus at prekaryogamy, prophase I and interphase I. In our opinion McLaughlin's observation of a diglobular form before karyogamy is not conclusive. Meiotic divisions in Boletus are not synchronous and the stage of basidial development was estimated by
nuclear size and condition of the cytoplasm, both of which are unreliable criteria. In electron micrographs it is difficult to distinguish between late prekaryogamy basidia and those at prophase II since in both cases 2 nuclei are found without much change in the size and shape of basidia.

Recent observations of Moens & Rapport (1971a) on *Saccharomyces cerevisiae* support our observations in *Coprinus*. Since meiotic stages could not be identified in this yeast, time course studies were made after the cells had been transferred to the sporulation medium. Moens & Rapport noted that during the first 4–5 h period the cells have only one indistinct spindle pole body which they called a spindle plaque. After about 8 h on the sporulation medium many of the larger yeast cells have 2 plaques, side by side, connected by an isthmus (plaque bridge). In a more recent paper, they showed the presence of polycomplexes in the cells after 8 h on the sporulation medium – which suggested that the cells were in diplotene (Moens & Rapport, 1971b). Thus, in yeast, the spindle pole body is single in the early stages of meiosis but becomes double at a later stage (diplotene?). In our opinion the diglobular spindle pole body reported in Basidiomycetes should be considered a duplicated one. Furthermore, in *Coprinus* the high frequency of diglobular spindle pole bodies in late diplotene suggests that there is considerable time between their duplication and separation.

In the mitotic cell cycle of *Polystictus versicolor*, Girbardt (1971) showed that the kinetochore equivalent (spindle pole body) becomes diglobular in interphase as early as 30–40 min following the previous nuclear division. He suggested that the change to the diglobular condition in interphase was an indication that DNA synthesis had started. The apparent correlation of SPB duplication and DNA synthesis is fortuitous since the SPB can duplicate in the absence of gross DNA synthesis in interkinesis or prophase II (Figs. 10, 11).

McLaughlin (1971) suggested that at karyogamy both the spindle pole bodies disappear and one reforms during prophase. He drew parallels between the fate of the centrosome at fertilization in animals and karyogamy in *Boletus*. Although the failure to find spindle pole bodies at karyogamy and pachytene is believed to be due to the disappearance of this organelle, which is synthesized de novo when needed as suggested by Fulton & Dingle (1971), other explanations are equally attractive. The structure may actually be present at this time in the monoglobular form but probably in an indistinct state. Unless a thorough search for the SPB or its precursor was carried out as Fulton & Dingle did in *Naegleria*, this latter possibility cannot be ruled out.

The spindle pole bodies in Ascomycetes and Basidiomycetes lack the 9 triplet tubular structure that is characteristic of centrioles in animals, algae, and lower fungi (see reviews by Went, 1966; Pickett-Heaps, 1969; Fulton, 1971). Nevertheless, in *Ascobolus* and *Podospora*, Zickler (1970) showed that at metaphase and anaphase the spindle pole body consisted of 2 zones situated on each side of the nuclear envelope: an electron-opaque outer zone and a less-dense inner zone in which most of the microtubules end. Some form of zone differentiation is evident, although to a lesser extent, in other Ascomycetes (Moens & Rapport, 1971a; McCully & Robinow, 1971). The presence of a darker band in the central core of the *Coprinus* spindle pole body
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is noteworthy although its significance cannot be established at this time. Its similarity to the dark band of the spindle pole body in Ascomycetes may be of some phylogenetic significance.

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REFERENCES

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ABBREVIATIONS ON PLATES

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<tr>
<td>c</td>
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<tr>
<td>ch</td>
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Figs. 1-4. Spindle pole body behaviour during meiosis in C. lagopus.

Fig. 1. Metaphase of premeiotic mitosis. Spindle pole bodies are spherical (monoglobular). × 2500.

Fig. 2. Young basidia 15 h before karyogamy. One spindle pole body is visible in each basidium. × 2500.

Fig. 3. Electron micrograph of a basidium at early diplotene. Spindle pole body is attached to an indented portion of the nuclear envelope. OsO₄ fixation. × 60 000.

Fig. 4. Electron micrograph at late diplotene. Spindle pole body is diglobular. Glutaraldehyde-OsO₄ fixation. × 46 000. The inset is a photomicrograph of a basidium at late diplotene. × 2500.
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1. Scale: 10 μm

2. Scale: 0.5 μm

3. Scale: 5 μm
Fig. 5. Lateral view of a section through the spindle at metaphase showing spindle pole bodies. Glutaraldehyde-OsO₄ fixation. × 40,000.

Fig. 6. Polar view of a section through a spindle pole body at metaphase I. Note the dark band (arrowed) around the inner dense sphere. Glutaraldehyde-OsO₄ fixation. × 60,000.

Fig. 7. Electron micrograph of a section through a diglobular spindle pole body at prophase II. OsO₄ fixation. × 60,000.
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Figs. 8–13. Photomicrographs showing spindle pole body behaviour during meiosis in Coprinus comatus (Fig. 12, C. lagopus). All figures are ×2500.

Fig. 8. Diplotene to metaphase I: 1, monoglobular spindle pole body at diplotene; 2, diglobular spindle pole body at diplotene; 3, monoglobular spindle pole body at metaphase I. Note the size of the nucleolus in the 3 basidia.

Fig. 9. Daughter spindle pole bodies separating at diakinesis showing SPB isthmus (arrowed).

Fig. 10. The basidia (1 and 2) are at the end of the first meiotic division. Spindle pole bodies are monoglobular.

Fig. 11. Prophase II. Spindle pole bodies are diglobular.

Fig. 12. The spindle pole bodies at anaphase II are monoglobular (C. lagopus).

Fig. 13. Tetrad stage. One spindle pole body is associated with each of the 4 nuclei.
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10 μm