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Meiosis in Basidiomycetous Fungi

I. Fine Structure of Spindle Pole Body Organization

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With 13 Figures

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Summary

Stages in meiosis have been studied in six basidiomycetes using the electron microscope. Monoglobular and diglobular spindle pole bodies are described. The monoglobular SPB was present at pachytene. The diglobular SPB was present at prekaryogamy, karyogamy and after meiosis. The relationship between the monoglobular and diglobular SPB's is discussed.

1. Introduction

Spindle pole bodies have been found in a variety of fungi (Girbardt 1968, 1971, Lu 1967, Motta 1969, Zickler 1970, Wells 1970, Moens and Rapport 1971, McLaughlin 1971, Raju and Lu 1973). They usually take the form of electron dense structures seen on the nuclear envelope during interphase and at the poles of the spindle during mitosis and meiosis. These structures have been variously named in the past. We prefer to use the term spindle pole body (SPB) in accordance with a decision made at the 1st International Mycological Congress, Exeter, England, 1971.

Two forms of the SPB (monoglobular and diglobular) are believed to exist at various stages of the life cycle of a basidiomycete. Raju and Lu (1973) believe that the diglobular form represents the duplicated form of the SPB and that this duplicated form is only present at certain points in meiosis. However, as McLaughlin (1971) clearly showed a diglobular form of the SPB can often appear as a monoglobular form unless serial sections of the organelle are examined.

McLaughlin (1971) reported the presence of the diglobular SPB of *Boletus rubinellus* at three stages of meiosis: prekaryogamy, prophase I and interphase I. Raju and Lu (1973) criticised McLaughlin's (1971) conclusion that a diglobular SPB occurred at prekaryogamy. Raju and Lu (1973) feel that

because of the asynchronous nature of meiotic divisions in *Boletus* it is difficult to distinguish late prekaryogamy basidia from prophase II basidia since in both cases two nuclei are found.

In *Coprinus* RAJU and LU (1973) could not detect SPB's from 10 to 15 hours before prekaryogamy until pachytene. During diplotene, metaphase, anaphase and telophase stages the SPB's were reported to be monoglobular but were diglobular at late diplotene and prophase II.

We have recently studied the ultrastructural aspects of meiosis in fruit bodies of some basidiomycetous fungi. We here report the ultrastructural characteristics of the SPB in six such fungi.

2. Materials and Methods

Six species of basidiomycete fungi were used in this study: *Coprinus cinereus, Panaeolus ater, Agrocybe (Pholiota) praecox, Agaricus bisporus, Hypholoma fasciculare* and a *Russula* species.

Coprinus cinereus (strain H 2 and H 5, kindly supplied by Dr. JANE NORTH) were obtained by fruiting a dikaryotic mycelium on minimal medium (LEWIS 1961). Fruit bodies of the other five basidiomycetes were collected from various points on the campus of the University of Kent.

Small pieces of gill tissue were removed and fixed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.2. Samples were postfixed in 1% osmium tetroxide in veronal/acetate buffer, dehydrated in a graded ethanol series and embedded in Spurr's resin (SPURR 1969). The tissue was flat embedded so that orientation of gills allowed longitudinal sections of basidia to be cut. Sections were cut on glass knives using an LKB ultratome III and stained with uranyl acetate and lead citrate. Sections were viewed in an AEI 801 A electron microscope at an accellerating voltage of 60 kV.

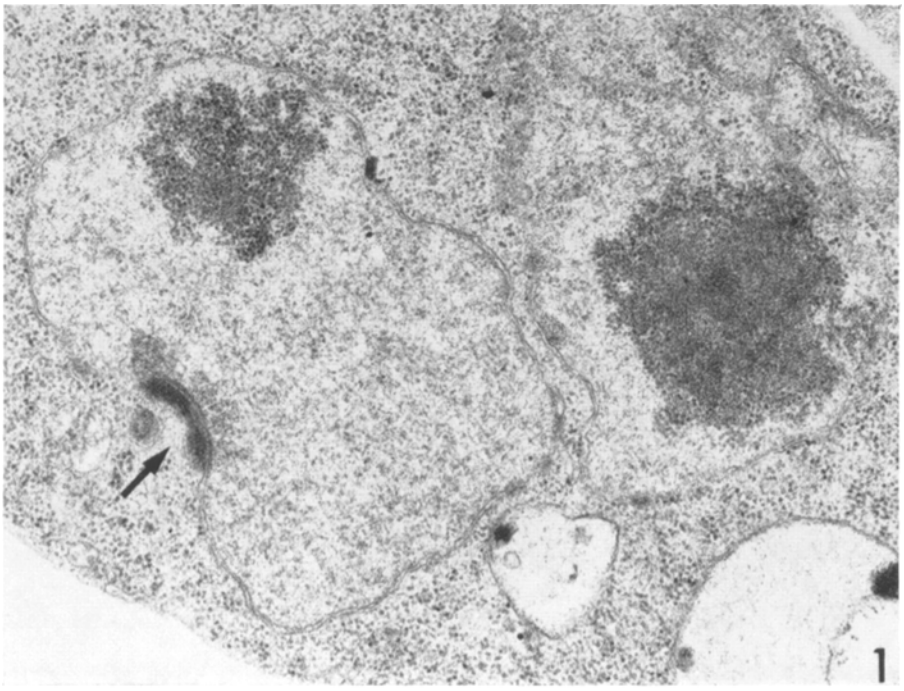
3. Results

3.1. The Monoglobular Spindle Pole Body

The monoglobular SPB is illustrated in Figs. 7–9 and 10. In *Panaeolus* (Figs. 7 and 10), *Agrocybe* (Fig. 8), and *Coprinus* (Fig. 9) the monoglobular SPB is located outside the nuclear envelope. In *Coprinus* it was sometimes located in an invagination of the nuclear membrane (Fig. 9). In each of these fungi the monoglobular SPB was situated close to the nucleolus (Figs. 7–9 and 10).

3.2. The Diglobular Spindle Pole Body

The diglobular form of the SPB is illustrated in five fungi: *Agaricus, Agrocybe, Panaeolus, Hypholoma,* and *Russula* (Figs. 1, 3–6, 11, and 13). The SPB's differed slightly in shape between fungi; the true diglobular form is seen in Fig. 3 for *Panaeolus*, Fig. 6 for *Hypholoma* and Fig. 11 for *Russula.* In these micrographs the SPB is seen to have two main components ("globular ends") which are connected by a strip of similar electron dense material.



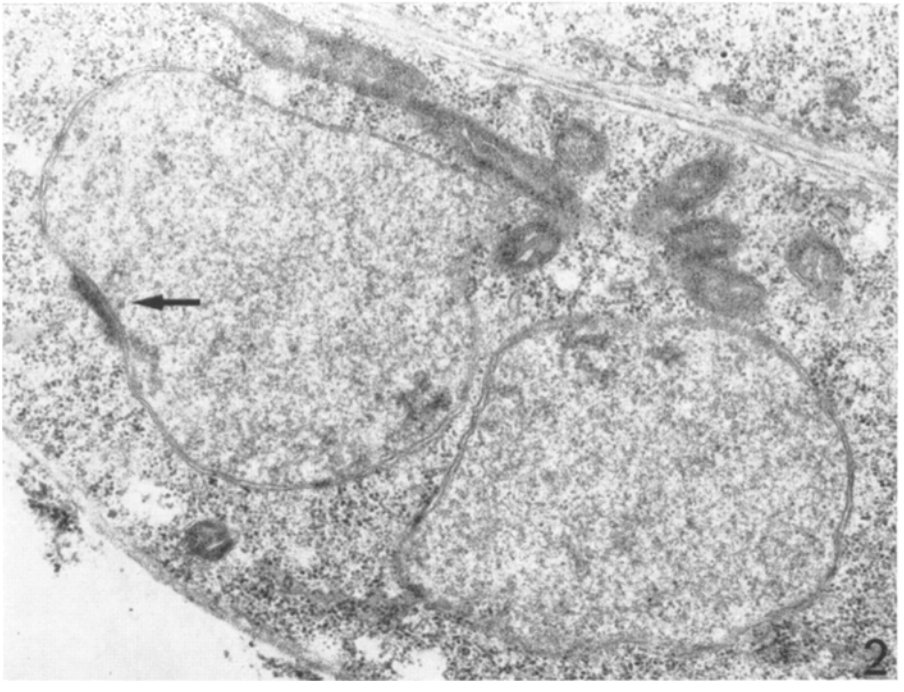


Fig. 1. *Agaricus*. Diglobular SPB. ×25,800
Fig. 2. *Panaeolus*. Diglobular SPB. ×25,800

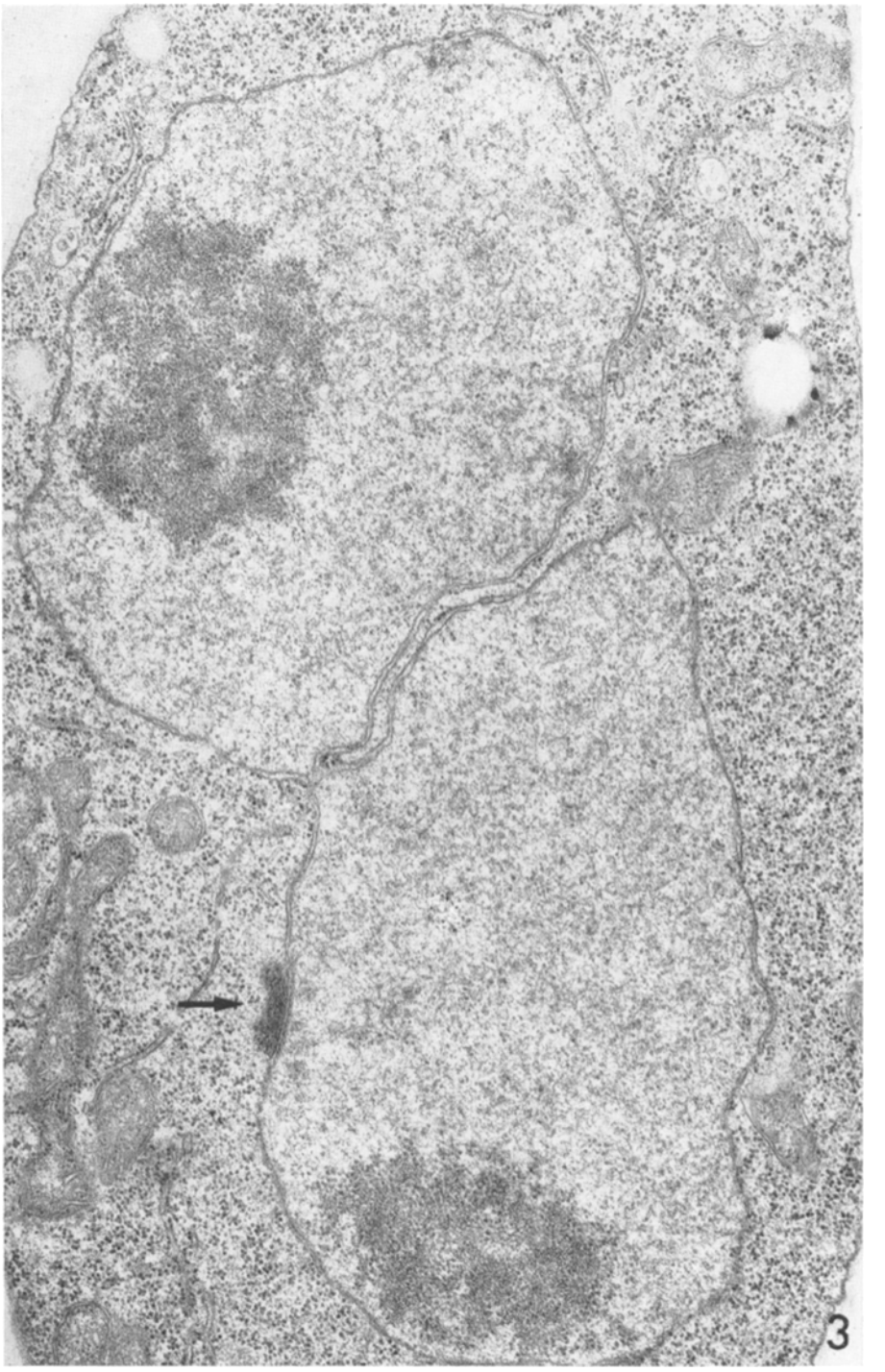
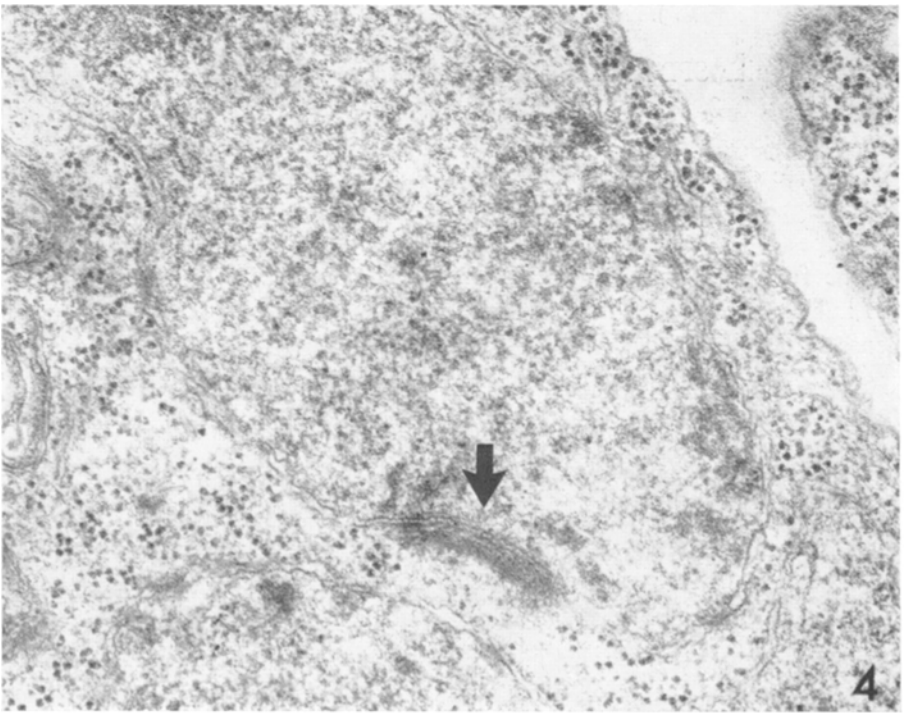
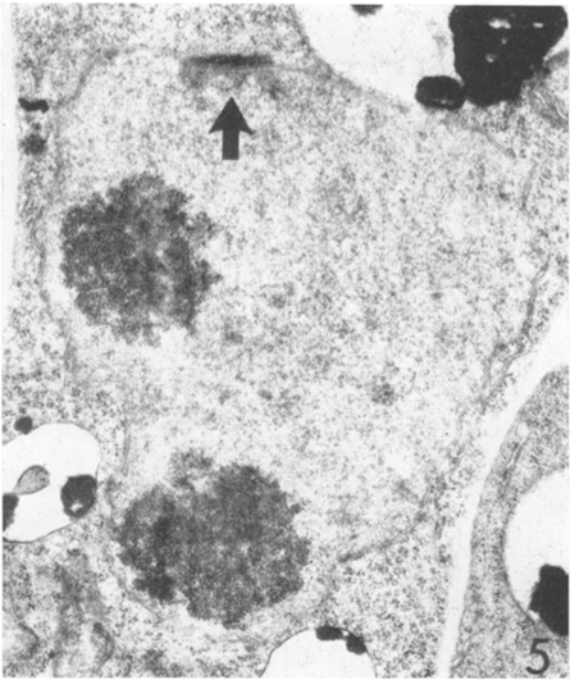


Fig. 3. *Panaeolus*. Nuclear fusion in a basidium. One nucleus possesses a diglobular SPB. ×30,000





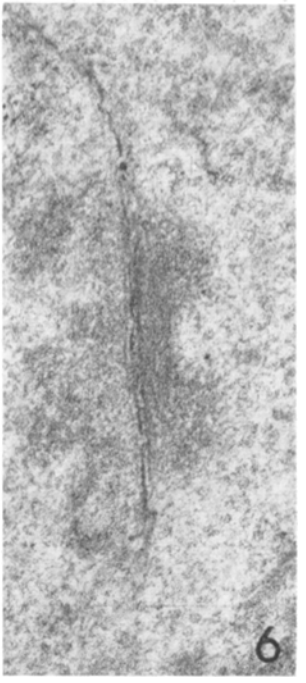


Fig. 4. *Agrocybe*. Diglobular SPB. ×57,000
Fig. 5. *Agaricus*. Postkaryogamy nucleus containing two nucleoli and a diglobular SPB. ×17,800
Fig. 6. *Hypholoma*. Diglobular SPB. ×63,000

The diglobular SPB always appeared adpressed to the outer nuclear membrane (Figs. 1, 2, 4, and 6). At this point the two membranes of the nuclear envelope were always evenly spaced and parallel to each other. No nuclear pores were ever observed in the length of nuclear envelope adpressed to the SPB (Figs. 1–4 and 6) although they were present elsewhere in the nuclear envelope (Figs. 2 and 4).

In the fungi studied electron dense material was consistently found inside the nuclear envelope near the SPB (Figs. 1, 4–6, and 11–13). This electron dense material showed the ultrastructural characteristics of chromatin. In some cases the diglobular SPB was found in a slight indentation of the nuclear envelope (Figs. 1–3).

3.3. Form of the Spindle Pole Body during Meiosis

We have been particularly interested in ascertaining the form of the SPB around the period of karyogamy. It is difficult to tell a prekaryogamy basidium from a prophase II basidium as both contain two nuclei. We have, however, consistently found the diglobular form of the SPB in binucleate basidia of *Agaricus* (Fig. 1), *Panaeolus* (Fig. 2), and *Agrocybe* (Fig. 4). We feel that some of these nuclei and basidia do represent prekaryogamy stages. We have also been able to obtain two other stages in early meiosis, both showing a diglobular SPB associated with a nucleus. Fig. 3 shows a stage in the process of nuclear fusion in *Panaeolus*. The two nuclei are joined by a small connection but the nucleoli have not yet fused. A diglobular SPB is present in this micrograph, adpressed to the nuclear envelope. The stage of karyogamy immediately after this would involve a basidium containing one nucleus but that nucleus having two nucleoli (nuclear fusion having occurred but not nucleolar fusion). This stage is illustrated in a basidium of *Agaricus* in Fig. 5; a diglobular SPB is present at this stage. The SPB, therefore, appears to be present during karyogamy and is in the diglobular form.

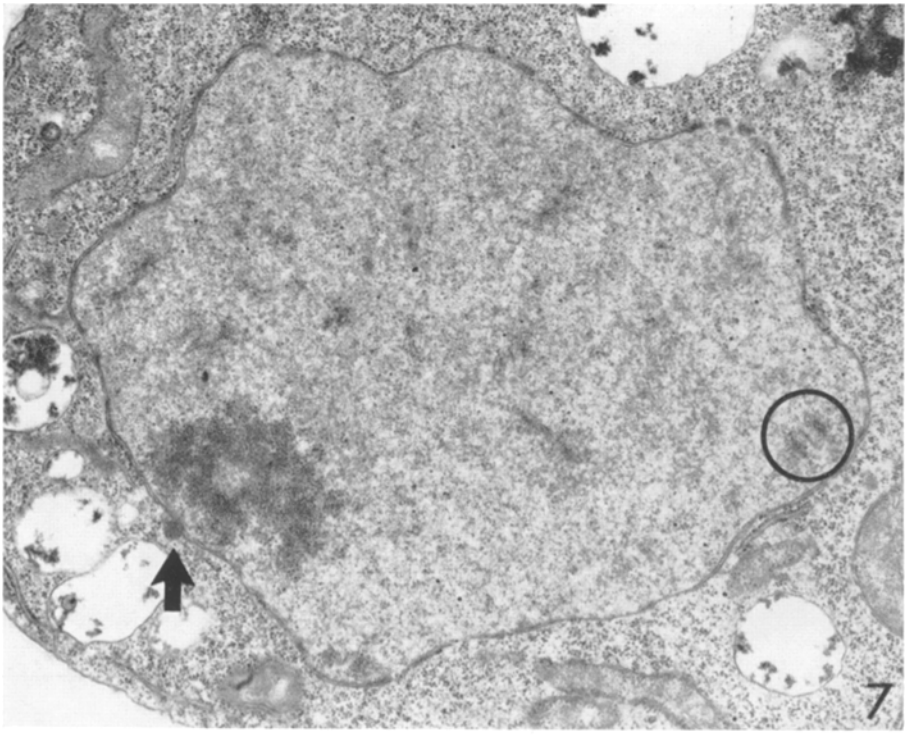
The next readily identifiable stage of meiosis studied was pachytene where synaptonemal complexes were found in some of the fungi studied. At this stage a monoglobular SPB was found in *Coprinus* (Fig. 9), *Agrocybe* (Fig. 8), and *Panaeolus* (Fig. 7).

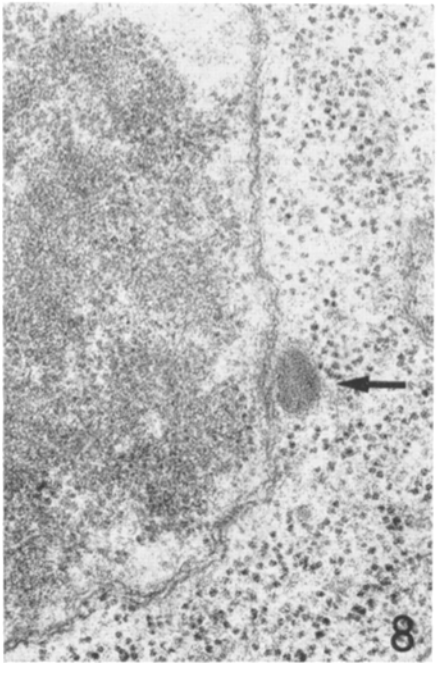
The completion of meiosis is clearly marked by the presence of four nuclei in a basidium. At this stage a diglobular SPB was seen associated with the nuclei of *Panaeolus* (Figs. 12 and 13) and *Russula* (Fig. 11).

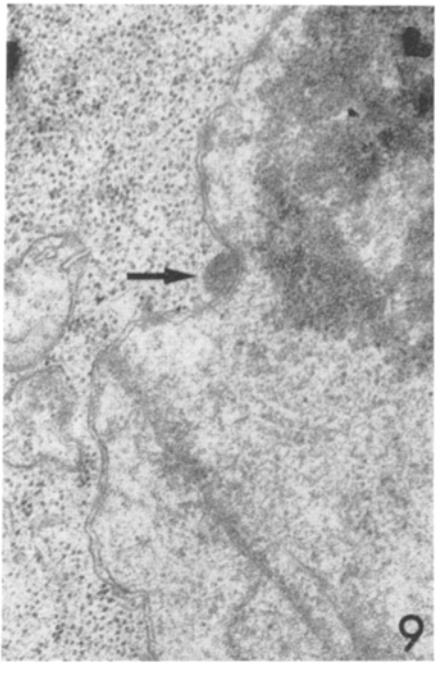
Fig. 7. *Panaeolus*. Monoglobular SPB on the nuclear envelope close to the nucleolus. Note the cross section of the synaptonemal complex (circled). ×19,600

Fig. 8. *Agrocybe*. Monoglobular SPB. ×50,000

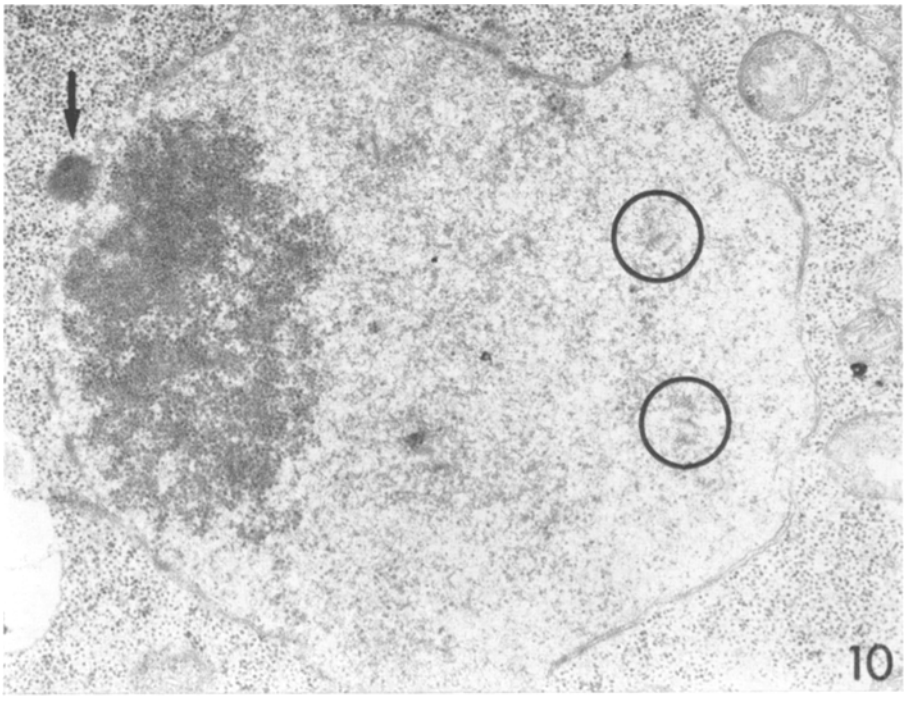
Fig. 9. *Coprinus*. Monoglobular SPB in a pocket of the nuclear envelope, near the nucleolus. A glancing section of a synaptonemal complex is visible in the nucleus. ×39,900







Figs. 7–9



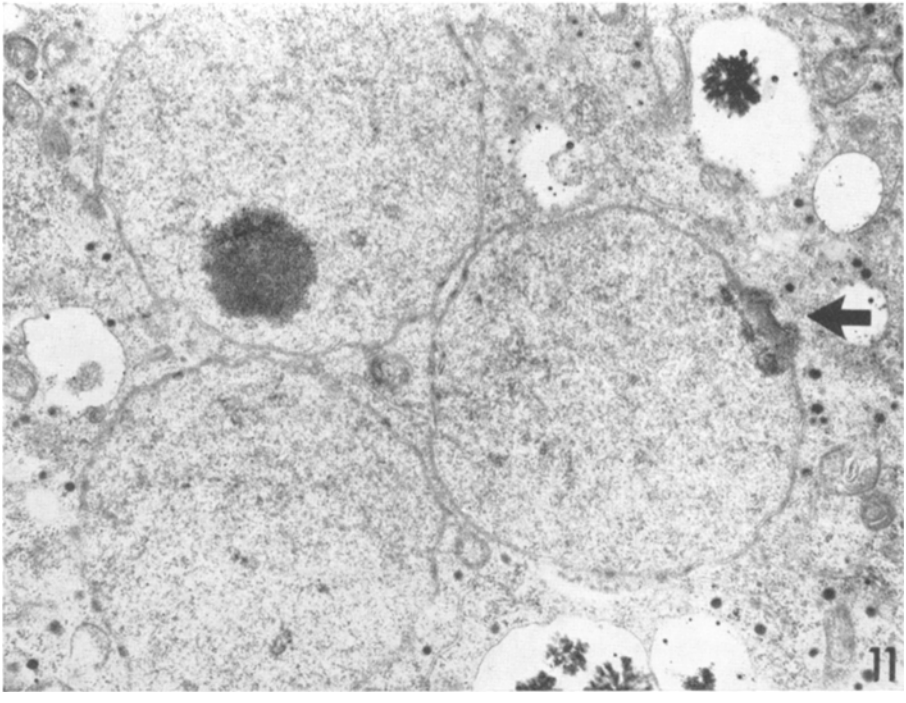
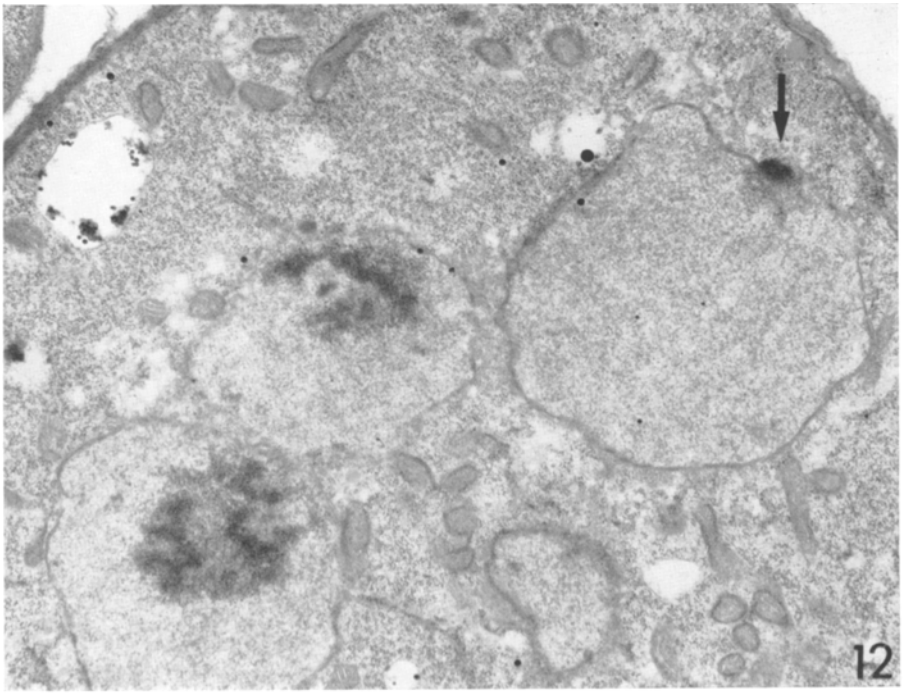


Fig. 10. *Panaeolus*. Monoglobular SPB. Note the cross sections of synaptonemal complexes (circled). ×26,600

Fig. 11. *Russula*. Basidium with three distinct nuclear profiles. One nucleus shows a diglobular SPB. ×15,900



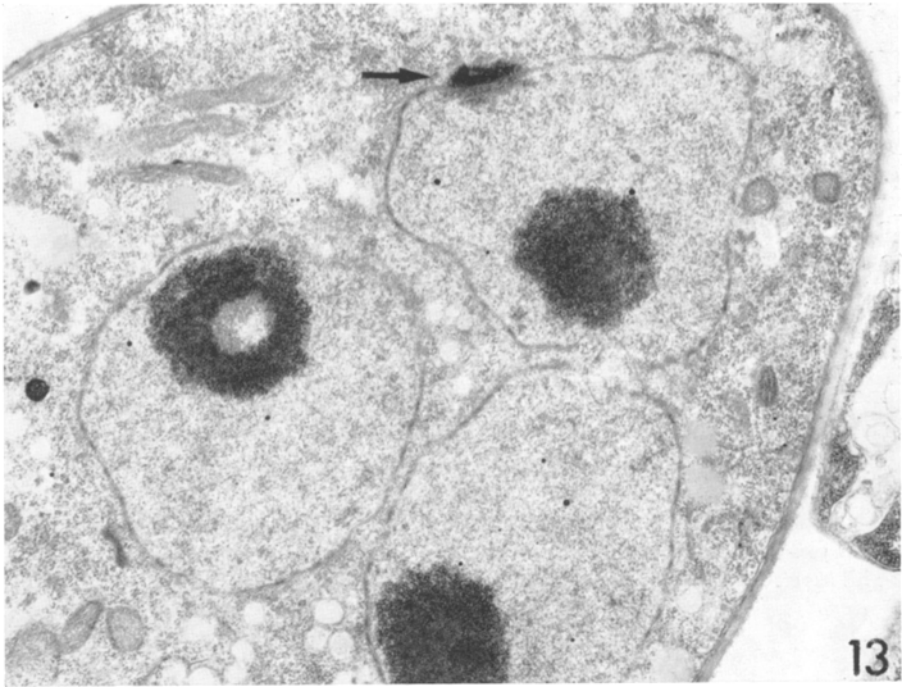


Fig. 12. *Panaeolus.* Basidium with four nuclear profiles. One nucleus has a diglobular SPB. ×16,600

Fig. 13. *Panaeolus.* Basidium with three nuclear profiles, one nucleus possesses a distinct diglobular SPB. ×16,600

4. Discussion

Spindle pole bodies have been more intensively investigated in *Ascomycetes* than in *Basidiomycetes*. In *Basidiomycetes* there appears to be a change in form of the SPB during meiosis. In this study we have found both the diglobular and monoglobular SPB at different stages of meiosis in the same fungus. In *Panaeolus*, *Agrocybe*, and *Coprinus* we find the monoglobular SPB at late pachytene when the nucleus contains synaptonemal complexes. As has been pointed out previously, however, it is difficult to tell a monoglobular SPB from a diglobular SPB unless it has been serially sectioned. MCLAUGHLIN (1971) has shown that the diglobular SPB of *Boletus* is an elongated structure. If the basidial nucleus undergoes a change in orientation during prophase I (for example a movement through 180° with respect to the long axis of the basidium), then a monoglobular SPB could well result from a cross section of a diglobular SPB. We feel that a more intensive study using serial section reconstruction techniques needs to be attempted before the true relationship of the monoglobular and diglobular SPB's is established. Such a study would also clarify the fate of the two SPB's, assuming that each prekaryogamy nucleus has one SPB.

MCLAUGHLIN (1971) observed a diglobular SPB prior to karyogamy, at prophase I and interphase I. RAJU and LU (1973) have recently critizised MCLAUGHLIN's interpretation of the presence of a SPB just prior to karyogamy; on the grounds that it is impossible to tell a late prekaryogamy basidium from a prophase II basidium. Our results seem to support MCLAUGHLIN's view. We find a diglobular SPB in basidia containing two nuclei. A diglobular SPB is present at the time of karyogamy in *Panaeolus* also, at a slightly later stage in karyogamy in *Agaricus*.

In all six fungi studied we have found the close association of what appears to be chromatin with the SPB area (see also MCLAUGHLIN 1971). Nuclear staining techniques at the light microscope level appear to stain the SPB (RAJU and LU 1973). It is, however, important to bear in mind the close proximity of this chromatin to the SPB. Its presence or absence at certain times in the meiotic process may influence the shape of the presumed SPB in the light microscope.

Acknowledgements

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References

GIRBARDT, M., 1968: Ultrastructure and dynamics of moving nucleus. In: Aspects of Cell Motility, 22nd Symp. Soc. exp. Biol., pp. 249—259 (P. L. MILLER, ed.). Cambridge: University Press.

— 1971: Ultrastructure of the fungal nucleus II. The kinetochore equivalent (KCE). J. Cell Sci. **9**, 453—473.

LEWIS, D., 1961: Genetic analysis of methionine suppressors in *Coprinus.* Genet. Res. (Camb) **2**, 141—155.

LU, B. C., 1967: Meiosis in *Coprinus lagopus:* a comparative study with light and electron microscopy. J. Cell Sci. **2**, 529—536.

MCLAUGHLIN, D. J., 1971: Centrosomes and microtubules during meiosis in the mushroom *Boletus rubinellus.* J. Cell Biol. **50**, 737—745.

MOENS, P. B., and E. RAPPORT, 1971: Spindles, spindle plaques and meiosis in the yeast *Saccharomyces cerevisiae* (Hansen). J. Cell Biol. **50**, 344—361.

MOTTA, J. J., 1969: Somatic nuclear division in *Armillaria mellea.* Mycologia **61**, 873—886.

RAJU, N. B., and B. C. LU, 1973: Meiosis in *Coprinus.* IV. Morphology and behaviour of spindle pole bodies. J. Cell Sci. **12**, 131—141.

SPURR, A. R., 1969: A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**, 31—43.

WELLS, K., 1970: Light and electron microscope studies of *Ascobolus stercorarius* I. Nuclear divisions in the ascus. Mycologia **62**, 761—790.

ZICKLER, D., 1970: Division spindle and centrosomal plaques during mitosis and meiosis in some *Ascomycetes.* Chromosoma **30**, 287—304.

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