MEIOSIS IN COPRINUS

VIII. A Time-Course Study of the Fusion and Division of the Spindle Pole Body during Meiosis

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ABSTRACT
The time-course study of meiosis in the fungus Coprinus cinereus (C. lagopus) by electron microscopy reveals that two monoglobular spindle pole bodies (SPB's) of prekaryogamy nuclei come together during karyogamy and are fused. The fusion SPB of postkaryogamy nucleus persists through zygotene and pachytene as evidenced by the presence of axial components and synaptonemal complexes. At early diplotene, the SPB divides. The divided SPB takes on a diglobular form, which grows in size to form two daughter SPB's. These separate and move to opposite poles at metaphase I.

KEY WORDS Coprinus • meiosis • time-course • spindle pole body

In a previous study on the structure and behavior of the spindle pole bodies (SPB's) of Coprinus (13), we were confronted with the problem as to whether the SPB is synthesized de novo, as claimed by McLaughlin (9), or is a self-duplicating organelle. The argument for de novo synthesis was based on negative evidence that the SPB had not been seen between karyogamy and pachytene. We argued that the absence may be due to lack of a thorough search. In the present study, I have performed a systematic search for this organelle and have established its presence in all stages and a time sequence of its division during meiosis.

MATERIALS AND METHODS
Fruiting bodies were obtained from the dikaryotic culture of C. cinereus (JR52 × PR2301) used in previous studies (12, 13). The basidiocarp has synchronous meiosis and contains ~100 gills, each of which is at an identical stage of development. A few gills were removed at selected time intervals for electron microscopy and it was possible to follow the time sequence of meiotic events. The same technique has been used previously to study the time of meiotic stages by light microscopy (12). Because the period of different stages is known and can be predicted, samples were taken at the most appropriate times, as shown in Fig. 1.

Gill samples were fixed for both light and electron microscopy. Light microscope observations were the same as those reported earlier (7, 12) and were used only to confirm stage identification for electron microscope observations. For electron microscopy, samples were fixed in 5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 for 3 h and washed in three changes of buffer at 3-h intervals; the last wash lasted overnight, and postfixation was done in 2% OsO₄ in the same buffer for 1 h. The fixed and washed samples were stained with 2% uranyl acetate in 50% ethanol and were dehydrated in an ethanol series and embedded in Spurr's low viscosity epoxy resin (14) using propylene oxide as the carrier. The sections were stained with 2% uranyl acetate in methanol at pH 7.2 for 3 h and washed in three changes of buffer at 3-h intervals; the last wash lasted overnight, and postfixation was done in 2% OsO₄ in the same buffer for 1 h. The fixed and washed samples were stained with 2% uranyl acetate in 50% ethanol and were dehydrated in an ethanol series and embedded in Spurr's low viscosity epoxy resin (14) using propylene oxide as the carrier. The sections were stained with 2% uranyl acetate in methanol for 20 min and then with 0.3% commercial lead citrate for 3 min. Photographs were taken with a Philips EM200 electron microscope.

RESULTS
The first sample was taken from the basidiocarp for electron microscopy at 1½ h when ~35% of
basidia had started karyogamy as monitored by light microscopy. In this sample, most of the basidia are at prekaryogamy stage; some are at karyogamy stage but no synaptonemal complexes (SC) have been formed. Single, unpaired axial components are observed in some postkaryogamy nuclei, presumably at leptotene. Observations were focused on the basidium where karyogamy was imminent and where karyogamy had taken place. Fig. 4 shows two nuclei before karyogamy, each containing one SPB in the indentation of the nuclear envelope. The SPB's are positioned at equidistance from where the nuclei meet. This arrangement could be taken as accidental, but repeated observations suggest that this juxtaposition is associated with incipient fusion of the two SPB's. This suggestion has been borne out by serial sections through a postkaryogamy nucleus where two monoglobular SPB's are found side by side (Fig. 2a-f). This configuration should not be taken as a diglobular structure because there is no connection between them. Nor should it be taken as early separation of the SPB, because the separation is not expected in another 10-h time, and no separation configuration has been observed in the intervening pachytene stage. In another postkaryogamy nucleus an oblong SPB is seen, suggesting that two monoglobular SPB's have fused. The above observations have provided compelling evidence that the SPB's from two sexually compatible nuclei come together at karyogamy and are fused. A near-median and a median section of such an oblong SPB are shown in Fig. 3a and b.

The second sample taken at 5 h was expected to be at early pachytene for most basidia, and this was confirmed by light microscopy. Electron microscopy reveals the presence of synaptonemal complexes (Fig. 5); some basidia, however, are still at zygote (photograph not shown). The two axial components of a homologous pair of chromosomes are aligned as reported previously (5). An SPB is seen in an indentation of the nuclear envelope (Fig. 5) at both stages. Thus, the fusion SPB persists as meiosis progresses through zygote and pachytene.

The third sample taken at 9 h was identified as the transition from late pachytene to early diplotene by light microscopy. In the basidium at early diplotene, in which the SC has dissolved leaving only the remains of the axial components, several diglobular SPB's are observed (Fig. 6). It should be noted that the two daughter SPB's are small and very close to one another, suggesting that duplication of this organelle has begun.

The fourth sample was taken at mid-diplotene at 11 h. It is evident that the daughter SPB's have grown in size. As shown in Fig. 7, the two daughter SPB's are still associated. Light microscopy has also established the division and separation of daughter SPB's. Detailed observations have already been reported (13).

The fifth sample taken at 14 h was found to be at or near metaphase I as determined by light microscopy. In a basidium at metaphase I, a monoglobular SPB is seen associated with the spindle tubules. The division and separation of SPB's at diplotene, and their association with spindle tubules at metaphase, have already been shown by light and electron microscopy (5, 13) and therefore will not be repeated here.

**DISCUSSION**

Several important and still unanswered questions concerning the SPB's in meiosis of higher fungi are: (a) Do the SPB's from two compatible nuclei fuse at karyogamy or do they disappear and later resynthesize de novo before nuclear division? (b) If the SPB persists, at what stage of meiosis does it divide? Observations of this organelle by electron microscopy have usually been a matter of chance. For this reason, we failed to see this organelle between karyogamy and late pachytene in our previous studies (13). Such negative evidence is insufficient to support the claim made by McLaughlin (9) that the SPB disappeared at karyogamy and resynthesized de novo at a later stage. The demonstration of (a) an SPB in each prekaryogamy nuclei, (b) two SPB's standing side-by-side after karyogamy, and (c) an elongated SPB in the postkaryogamy nucleus can be taken as evidence for fusion of the organelle at the time of karyogamy. The elongated shape (Fig. 3) may represent the "fusion state." Evidence of nuclear fusion occurring at the region of the spindle plaques has been demonstrated in yeast (1, 2).
Flou~ 2 Postkaryogamy nucleus (N) showing serial sections through the SPB's (a-f) just before their fusion. Two monoglobular SPB's are seen standing side by side in an indentation of the nuclear envelope (NE). × 62,000.

Figure 2 Postkaryogamy nucleus showing serial sections through the SPB's (a-f) just before their fusion. Two monoglobular SPB's are seen standing side by side in an indentation of the nuclear envelope (NE). × 62,000.

Flmu~ 3 Postkaryogamy nucleus showing a fusion SPB. The elongated form is believed to be a fusion state. (a) Near-median section. (b) Median section. × 62,000.

Figure 3 Postkaryogamy nucleus showing a fusion SPB. The elongated form is believed to be a fusion state. (a) Near-median section. (b) Median section. × 62,000.
Figure 4  Karyogamy imminent, two nuclei are seen, each containing an SPB in an indentation of the nuclear envelope. Bar, 1 μm. × 62,000.

Figure 5  Zygotene and pachytene. An SPB is seen in an indentation of the nuclear envelope of a pachytene nucleus showing the synaptonemal complex (Syn). N, nucleus; NE, nuclear envelope; MC, membrane complex; PM, plasma membrane; ac, axial component; W, cell wall; R, ribosomes. × 95,000.
The persistence of the SPB through all stages of meiosis has been clearly demonstrated by the time-course study. Its presence at zygotene and pachytene is corroborated by the presence of paired axial components and synaptonemal complexes. The coexistence of the synaptonemal complex and the SPB has also been shown by Gull and Newsam (4) in another Coprinus species.

The division of the SPB was shown to commence at early diplotene, ~9 h after the onset of karyogamy, and the daughter SPB's are fully divided at late diplotene. The separation of the daughter SPB's has already been well documented by light microscopy as well as by electron microscopy by Raju and Lu (13). The division of SPB's has also been reported in yeast (10, 11), in fission yeast (8) and in Gelasinospora (6).

The problem of monoglobular vs. diglobular appeared to be controversial. McLaughlin (9) claimed to have demonstrated diglobular structure by serial sections. His claim was supported by Gull and Newsam (4) who reported that they observed some monoglobular and some diglobular structures in the same species. There is no denial, from the electron micrographs reported by Gull and Newsam (4), that a diglobular structure exists. I, too, have reported both monoglobular and diglobular structure in Coprinus in a previous paper (13) and the present paper. The question is: How are these structures related to stages of meiosis? McLaughlin (9) provided no reference to stages; thus, his observations cannot be assessed. Gull and Newsam (4) did make some reference to some stages. They reported an elongated SPB (not truly diglobular) in a postkaryogamy nucleus where two nucleoli are not yet fused. The elongated SPB can be related to what appeared to be a fusion state as I have shown in Fig. 3. They have also demonstrated a diglobular SPB at the four-nucleate stage when meiosis was complete. Since I did not extend my time-course studies to that late stage, I therefore am not in a position to assess its stage relationship. However, I would like to point out that the four-nucleate stage is an interphase before the postmeiotic mitosis which results in binucleate spores. According to Girbardt (3), the transition from a monoglobular form to a diglobular form in the mitosis of Polystictus takes place during interphase, and according to Moens and Rapport (10) and Byers and Goetsch (1, 2), the division of the SPB in mitosis of yeast takes place at interphase when DNA is being replicated. I would not be at all surprised that what Gull and Newsam (4) have seen are duplicated SPB's.

The structures seen in Fig. 2 cannot be considered a diglobular form as there is no connection between them. I believe that they are two monoglobular SPB's from two compatible nuclei shortly before they are fused. Thus, before fusion, the SPB's are monoglobular as demonstrated clearly by serial sections. Apart from the transient fusion state, the postkaryogamy SPB at zygotene and pachytene may also be a monoglobular form.
The demonstration of a time sequence of SPB division supports our earlier contention (13) that the diglobular SPB represents a duplicated form. Our whole-mount light microscopy (13) and the studies of Peterson and Ris (11) using high voltage electron microscopy unequivocally supported this claim. It is true, as put forth by Gull and Newsam (4), that a diglobular SPB may be seen as a diglobular or monoglobular form depending on the section plane. If the SPB were diglobular as McLaughlin (9) claimed, the probability of sectioning through one or both subunits should be equal. In the present study, no diglobular form was found in over 20 samples in a synchronous population of zygotene and pachytene basidia. In contrast, equal numbers of monoglobular and diglobular forms were observed at diplotene during which stage the SPB has duplicated.

It is concluded therefore that (a) the SPB is monoglobular in structure except during fusion and division stages, (b) two monoglobular SPB's fuse during nuclear fusion, and (c) the fusion SPB divides at early diplotene with the two daughter SPB's maturing and separating at mid to late diplotene.

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REFERENCES

1. Byers, B., and L. Goetsch. 1974. Duplication of spindle plaques and integration of the yeast cell cycle. Cold Spring Harbor Symp. Quant. Biol. 39:123-131.
2. Byers, B., and L. Goetsch. 1975. Behavior of spindles and spindle plaques in the cell cycle and conjugation of Saccharomyces cerevisiae. J. Bacteriol. 124:511-523.
3. Gérardt, M. 1971. Ultrastructure of the fungal nucleus. II. The kinetochore equivalent (KCE). J. Cell Sci. 9:453-473.
4. Gull, K., and R. J. Newsam. 1975. Meiosis in basidiomycetous fungi: I. Fine structure of spindle pole body organization. Protoplasma. 83:247-258.
5. Lu, B. C. 1967. Meiosis in Coprinus: A comparative study with light and electron microscopy. J. Cell Sci. 2:529-536.
6. Lu, B. C. 1967. The course of meiosis and centriole behavior during Ascus development of the Ascomycete Gelasinospora calospora. Chromosoma (Berl.). 22:210-226.
7. Lu, B. C., and N. B. Raju. 1970. Meiosis in Coprinus. II. Chromosome pairing and the lambrush diplotene stage of meiotic prophase. Chromosoma (Berl.). 29:305-316.
8. McCully, E. K., and C. F. Robinow. 1971. Mitosis in the fission yeast Schizosaccharomyces pombe: A comparative study with light and electron microscopy. J. Cell Sci. 9:475-507.
9. McLaughlin, D. L. 1971. Centrosomes and microtubules during meiosis in the mushroom Boletus rubinellus. J. Cell Biol. 50:737-745.
10. Moens, P. B., and E. Rapport. 1971. Spindles, spindle plaques and meiosis in the yeast Saccharomyces cerevisiae (Hansen). J. Cell Biol. 50:344-361.
11. Peterson, J. B., and H. Ris. 1976. Electron-microscopic studies of the spindle and chromosome movement in the yeast Saccharomyces cerevisiae. J. Cell Sci. 22:219-242.
12. Raju, N. B., and B. C. Lu. 1970. Meiosis in Coprinus. III. Timing of meiotic events in C. lagopus (sensu Buller). Can. J. Bot. 48:2183-2186.
13. 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.
14. Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-41.