SPINDLES, SPINDLE PLAQUES, AND MEIOSIS IN THE YEAST *SACCHAROMYCES CEREVISIAE* (HANSEN)

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ABSTRACT

The intranuclear spindle of yeast has an electron-opaque body at each pole. These spindle plaques lie on the nuclear envelope. During mitosis the spindle elongates while the nuclear membranes remain intact. After equatorial constriction there are two daughted nuclei, each with one spindle plaque. The spindle plaque then duplicates so that two side-by-side plaques are produced. These move rapidly apart and rotate so that they bracket a stable 0.8 μm spindle. Later, during mitosis, this spindle elongates, etc. Yeast cells placed on sporulation medium soon enter meiosis. After 4 hr the spindle plaques of the more mature cells duplicate, producing a stable side-by-side arrangement. Subsequently the plaques move apart to bracket a 0.8 μm spindle which immediately starts to elongate. When this meiosis I spindle reaches its maximum length of 3–5 μm, each of the plaques at the poles of the spindle duplicates and the resulting side-by-side plaques increase in size. The nucleus does not divide. The large side-by-side plaques separate and bracket a short spindle of about 1 μm which elongates gradually to 2 or 3 μm. Thus there are two spindles within one nucleus at meiosis II. To the side of each of the four plaques a bulge forms on the nucleus. The four bulges enlarge while the original nucleus shrinks. These four developing ascospore nuclei are partially surrounded by cytoplasm and by a prospore wall which originates from the cytoplasmic side of the spindle plaque. Eventually the spore nuclei pinch off and the spore wall closes. In some of the larger yeast cells this development is completed after 8 hr on sporulation medium.

INTRODUCTION

The dividing nucleus of fungi typically has an intranuclear spindle. The nuclear envelope remains intact during the division process in Phycomycetes and Ascomycetes. In the Ascomycetes there is an electron-opaque structure, the spindle plaque, associated with the nuclear envelope at the poles of the spindle. This arrangement has been reported for mitosis in *Aspergillus nidulans* (30), and in several strains of the yeast *Saccharomyces cerevisiae* (31). The presence of spindle plaques and the intranuclear spindle during the meiotic divisions has been reported in two species of *Ascobolus* and two species of *Podospora* (36, 37), and in *Neottiella* (35).

The sequence of events during nuclear division in fungi is best known from the extensive literature based on light- and phase-contrast microscope observations. Such information obtained from electron microscopy is limited, due, in part, to the loss of perspective in essentially two-dimensional

sections. This limitation can be troublesome where there is more than one structure of interest—in the case of yeast meiosis, four spindle plaques or four spores—because not all structures are likely to occur in the plane of section. The description of mitosis and meiosis in this report is based on the photographic record of complete serial sections of 150 yeast nuclei. The effectiveness of this method has been demonstrated in the model of nuclear division in a dinoflagellate by Kubai and Ris (14).

The morphological features of nuclei in mitosis and meiosis illustrated here are common in fungi. Three-dimensional reconstruction reveals an unusual feature, namely, the presence of a single nuclear mass with four spindle plaques during the second meiotic division. Division of nuclear material occurs when nucleoplasm flows into a bud which forms next to each of the spindle plaques. In this report, the interpretation of nuclear events during meiosis is restricted by the poor preservation of the nuclear membranes. In most cases, however, the boundary between nucleus and cytoplasm is quite clear so that the morphlogy of the nucleus as a whole can be described with confidence.

MATERIALS AND METHODS

Hansen strain CBS 5525 clone 10, isolated by Croes (5) for the study of meiosis was kindly supplied by Dr. Croes for these investigations. Stock cultures were maintained on yeast extract peptone medium (7). Sporulation was induced according to the method of Roth and Halvorson (33), which utilizes acetate as a carbon source in the presporulation medium as well as in the sporulation medium. This method gave nearly complete sporulation of the yeast cells at 24 hr.

Samples of yeast cells were fixed for electron microscopy immediately before placement in 1% potassium acetate sporulation medium, and at 4, 7, 8, 9, 10, and 12 hr thereafter. A sample of 15–20 ml was washed with sterile distilled water and suspended in a 3% glutaraldehyde solution in phosphate buffer (pH 7.2) at room temperature from 2 to 24 hr. Cells were washed with buffer, postfixed in buffered 2% osmium tetroxide solution (pH 7.2) for 1 hr, and collected by draining a pipette with the cell suspension slowly onto filter paper. The wet clumps of cells were placed on solid agar, and a drop of warm 2% agar was added to the clump. The solid agar blocks were trimmed and placed in 50% alcohol. After dehydration through an alcohol series and propylene oxide, the yeast was infiltrated with Epon 812, Luft's 1:1 mixture (17), without activator for several days in a 60°C oven, then rinsed with propylene oxide and reinfiltrated with Epon-containing activator. The yeast was then returned to the oven for another 2 days. Sectioning appeared to be improved through this thorough infiltration method. For comparison a part of each hour sample was fixed in $KMnO_4$.

Sections were cut on a Porter-Blum MT-2 microtome with a Dupont diamond knife. Serial sections were mounted on Formvar-coated single-hole grids, according to a modified Galey and Nilsson technique (9, 23), stained with saturated aqueous uranyl acetate solution, washed with water, and stained with Reynolds' lead citrate (29). Electron micrographs were made on 35 mm Kodak fine-grain positive film, or on Kodak electron image plates with a Philips EM 200. Three-dimensional models were made by tracing the nuclei from photographs onto plexiglas of appropriate thickness, then cutting the plexiglas and stacking the cuts. Glueing, filing, and polishing produced transparent models.

OBSERVATIONS AND COMMENTS

Mitosis: the Structure of Spindle Plaques

The disc shape of spindle plaques is demonstrated in Fig. 1 *b* for mitosis and in Fig. 6 *c* for meiosis. The spindle apparatus was sectioned perpendicular to its long axis. Some of the microtubules (Fig. 1 *c*) radiating upward from the plaque, therefore, appear as circles. In the section below the one shown in Fig. 1 *c*, plaque (*SP*) was sectioned (Fig. 1 *b*); it appears as amorphous electron-opaque material. Two sections farther down, the cytoplasmic (*CY*) protuberance in the nucleus, which supports this plaque, is visible (Fig. 1 *a*). Four plaques were sectioned in this manner and no structural differences were observed. The profile of mitotic spindle plaques in the section passing through the longitudinal axis of the spindle, is shown in Figs. 3, 4, and 5. There is an inner plaque (*IP*), an intermediate space (*IS*), and an outer plaque (*OP*) (Fig. 3 *a*). The inner plaque consists of two dense borders and a less dense central band. The intermediate space (*IS*) is divided by a narrow dense line, the intermediate line (*IL*) (Fig. 3 *a*). This line is better expressed in the meiotic plaques of Figs. 6 *b* and 9 *b*.

The outer plaque (*OP*) (Fig. 3 *a*) is lightly stained during mitosis, and meiosis I, but becomes large and dense during meiosis II. Not present during mitosis is a dense zone, which frequently occurs on the nuclear side of the plaques during meiosis (Figs. 6 *a* and 10 *a*, see arrow). The microtubules are usually present on both sides of

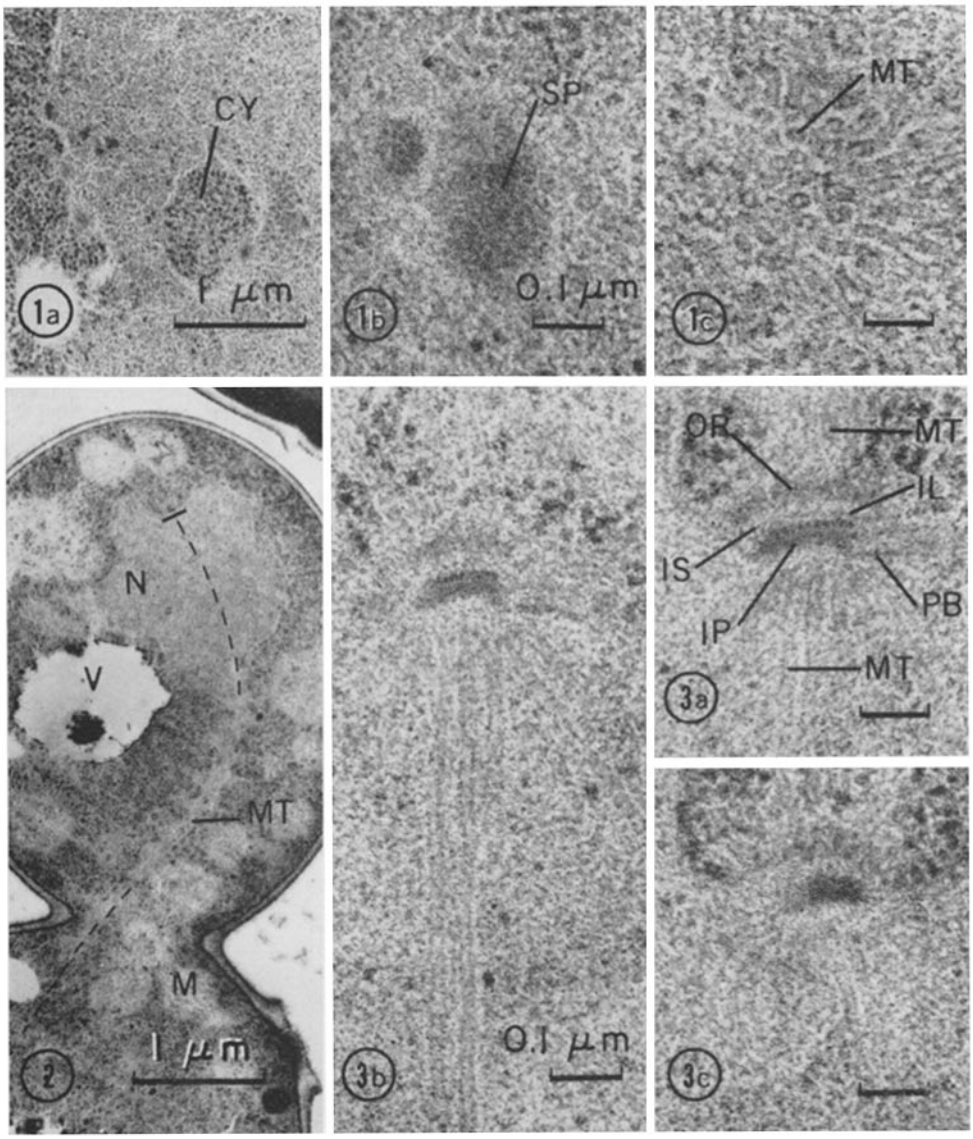
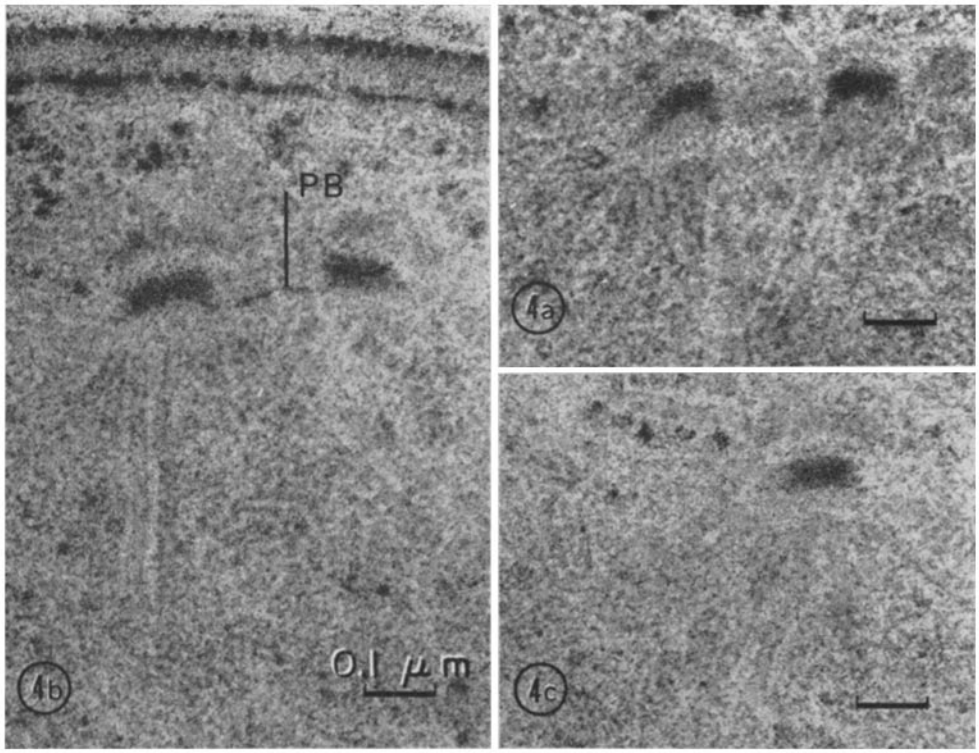


FIGURE 1 Budding yeast: serial sections of a spindle plaque cut perpendicular to the long axis of the spindle. In Fig. 1 *a*: underlying the plaque is a cytoplasmic invagination (*CY*) into the nucleus. × 17,000. Fig. 1 *b*: the spindle plaque (*SP*) is a disc-shaped structure, consisting of finely granular electron-opaque material. × 93,000. Fig. 1 *c*: radiating up from the plaque are the microtubules (*MT*). The scale line is 0.1 μm. × 93,000.

FIGURE 2 Budding yeast: the place of the spindle plaque (Fig. 3) is indicated by a solid bar. The continuous "long fiber" observed in a series of sections, is indicated by the broken line. The vacuole (*V*) is present in budding yeast but not in sporulating cells. Mitochondria, *M*; nucleus, *N*; microtubules, *MT*. × 18,000.

FIGURE 3 Budding yeast: serial sections of the spindle plaque from the cell in Fig. 2. Fig. 3 *a*: facing the nucleus is the inner plaque (*IP*); to one side is the plaque bridge (*PB*), and behind it is the intermediate space (*IS*). The dark line in the intermediate space is the intermediate line (*IL*). At the cytoplasmic side side is the outer plaque (*OP*). The microtubules (*MT*) radiate from the plaque into the cytoplasm as well as into the nucleus. × 93,000. Figs. 3 *b* and 3 *c*: subsequent sections of the same plaque are shown. All scale lines are 0.1 μm. × 93,000.



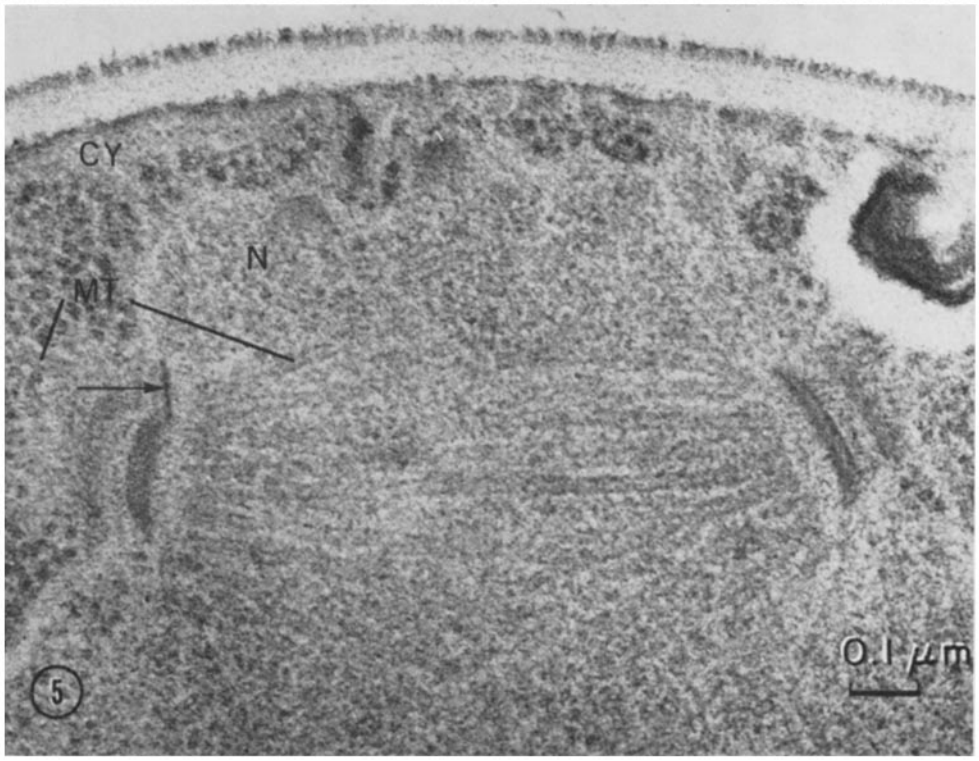


FIGURE 4 Budding yeast: serial sections of replicating plaques. In Figs. 4 *a*, 4 *b*, and 4 *c*, the two spindle plaques are connected by the plaque bridge (*PB*). Microtubules radiate from both plaques; some tubules intersect. The scale lines are 0.1 μm. × 93,000.

FIGURE 5 Budding yeast: the replicated plaques have separated and turned to face each other. The microtubules (*MT*) interlock and a "short spindle" of about 0.8 μm is formed. The plaque bridge (arrow) may be a remnant of the previous stage, or it may be newly formed. Nucleus, *N*; cytoplasm, *CY*. × 93,000.

the plaque; one set extending into the nucleus (Fig. 3 *b*), and the other extending into the cytoplasm (Figs. 3 *a* and 5). In sporulating yeast cells, tubules are not obvious in the cytoplasm.

Mitosis: the Replication of Spindle Plaques

Fig. 2 shows a nucleus of a recently divided cell, and Figs. 3 *a*, 3 *b*, and 3 *c* show the plaque. The long microtubules (*MT*) extend from the plaque, through the nucleus (*N*), to the parental cell (Fig. 2). The plaque has microtubules (*MT*) which radiate in several directions, and has a plaque bridge (*PB*) (Figs. 3 *a*, 3 *b*). In the next developmental stage, two spindle plaques are found close together, connected by the plaque bridge (*PB*) (Figs. 4 *a*, 4 *b*). The side-by-side arrangement of the plaques was recorded for three budding cells and for 30 cells in meiosis I (Fig. 7). The difference in numbers reflects, in part, our emphasis on meiotic processes. The replication of the plaques appears to occur at the time that the formation of a new bud is initiated by a bulge in the cell wall.

Mitosis: the Movement of Spindle Plaques

From their side-by-side position (Fig. 4), the plaques move apart until they bracket a spindle of 0.8 μm in length (Fig. 5). This arrangement is well documented and summarized by Matile, Moor, and Robinow, their Figs. 28, 30, and 31, and diagrams in Fig. 2 (19). In 11 cases where the two opposite plaques were close together, more or less in the plane of section, six were at a distance of 0.8 μm, one at 0.9 μm (Fig. 5), two at 1.0 μm, one at 1.1 μm, and one at 1.3 μm. Neither in mitosis nor in meiosis did we find plaques facing one another at a distance of less than 0.7 μm. In other words, no spindle smaller than 0.7 μm was observed. It appears that the separation of the plaques is rapid and that the 0.8 μm "short fiber" is again a more stable state. In 10 of these 11 cases, at least one of each pair of plaques was situated on the tip of a long cytoplasmic invagination into the nucleus. Fig. 1 *a* represents a cross-section of the invagination. The impression is gleaned that the nucleus expands or undergoes ameboid movements while the spindle remains fixed, thereby producing the finger-like invaginations. We suspect that separation of plaques is also accomplished through nuclear movement. It should be noted that numerous cytoplasmic invaginations *without* plaques were observed.

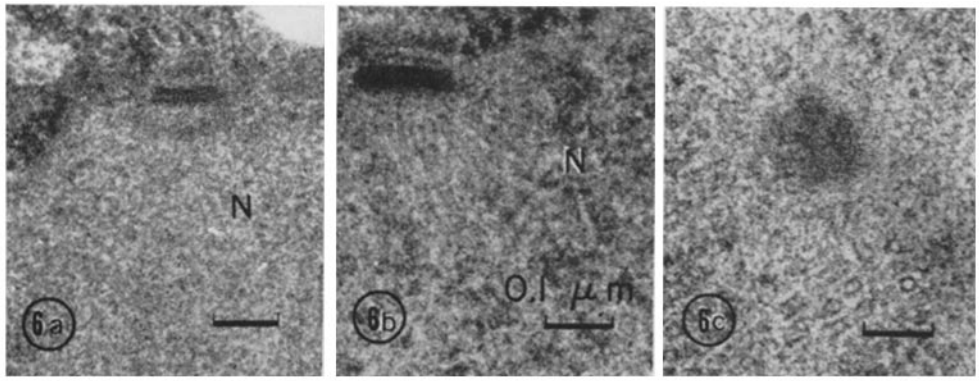
In the next stages of development, the cell forms a bud, part of the nucleus moves in, one of the plaques moves with the nucleus into the bud, and the microtubules become much elongated (Figs. 2, 3 *b*) (Robinow and Marak's "long fiber") (31). Whereas the short fiber is straight and appears quite rigid, the long fiber appears sinusoid in electron micrographs (Fig. 2). Light microscopy, on the other hand, gives the impression that the long fibers are perfectly straight (C. F. Robinow, personal communication).

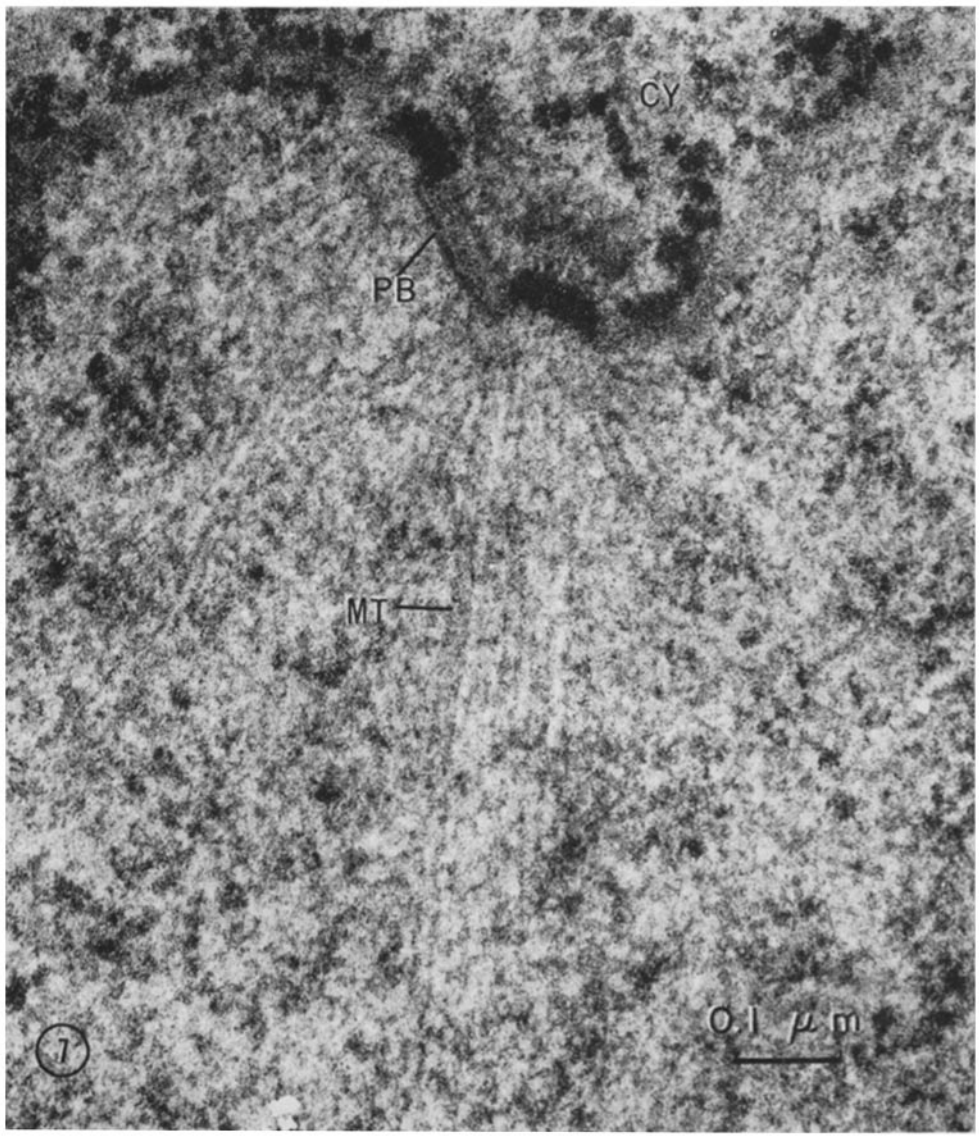
Meiosis I: Structure of the Spindle Plaque

During the first 4–5 hr that the yeast cells are on sporulation medium, they have small, indistinct spindle plaques. Often there is a dense zone at the nuclear side of the plaque (Fig. 6 *a*). There are few microtubules but, as the first meiotic division approaches, the number of tubules increases and the plaque becomes more distinct (Fig. 6 *b*). The tubules are obvious only on the nuclear side of the plaque. This is unlike budding yeast, where distinct tubules are present on the

FIGURE 6 Sporulating yeast: meiotic prophase. In Fig. 6 *a*, a small single plaque is present in cells which have been on sporulation medium for less than 4 hr. There is a dense zone on the nuclear side (*N*) of the plaque. In Fig. 6 *b*, the plaque is more distinct in the larger cells which have been on sporulation medium for more than 4 hr. The microtubules associated with the plaque also become more distinct. In Fig. 6 *c*, a cross-section of a meiotic spindle plaque. The structure is similar to that of the mitotic disc in Fig. 1 *b*. All scale lines are 0.1 μm. × 93,000.

FIGURE 7 Sporulating yeast: a meiotic prophase nucleus with replicated side-by-side spindle plaques. The two plaques are connected by a plaque bridge (*PB*), and microtubules (*MT*) radiate from each plaque into the nucleus. Cytoplasm, *CY*. × 140,000.


N
6a
N
0.1 μm
6b
6c


CY
PB
MT
0.1 μm
7

cytoplasmic as well as on the nuclear side of the plaque. The structure of the plaque at meiosis I is similar to the mitotic plaque described in the section on the structure of spindle plaques.

Meiosis I: the Replication of Spindle Plaques

After about 8 hr on sporulation medium many of the larger yeast cells have two plaques, side-by-side, connected by a plaque bridge (*PB*) (Fig. 7). Some 30 cells with two plaques side-by-side, connected by a bridge, were recorded. Microtubules project from the plaques into the nucleus. Serial sections, also through the spindle axis, but perpendicular to the plane of Fig. 7 show that the connecting bridge is nearly as wide as the spindle plaque itself. Without exception, the two plaques occur side-by-side but the distance between the two varies. The mode of replication may be through growth of the new plaque at the end of the bridge, or through a division of the old plaque. Occasionally, the undivided plaque appears to consist of two segments next to each other, separated by a narrow, pale zone in the inner and outer plaques. Widening of the zone could lead to the side-by-side plaques. In addition, in one case, two short bars were observed to project from the inner plaque into the nucleus. These may be the bridge attachments. In the side-by-side plaques of Fig. 7, the attachments are also perpendicular to the plaque discs but they are now widely separated by the bridge.

Meiosis I: the Movement of Spindle Plaques

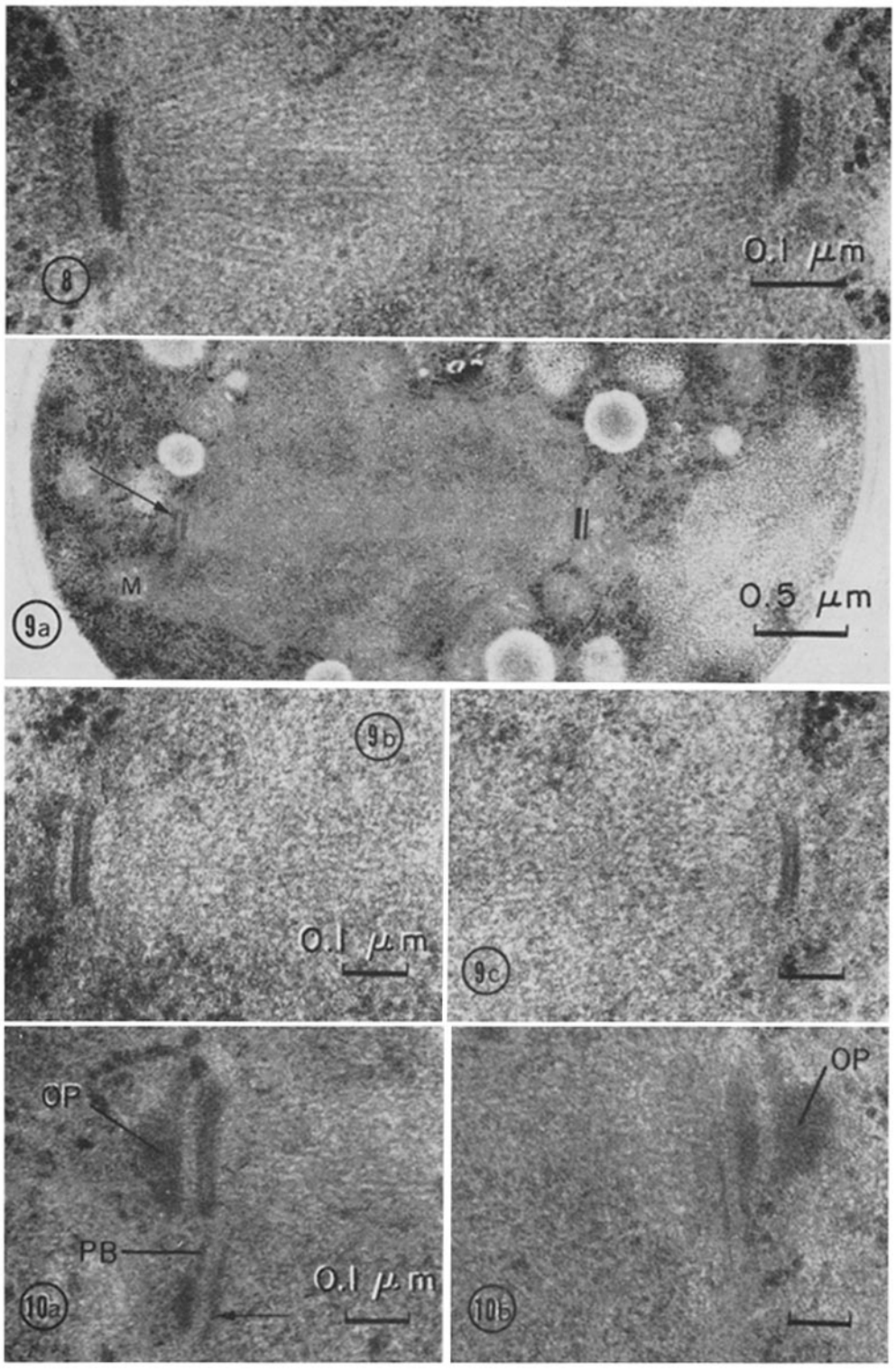
The spindle plaques are found to face each other at a subsequent developmental stage (Fig. 16 *c*). In 10 cells which had both plaques in the plane of section, the following distances between the plaques were recorded in μm: 0.7 (Fig. 8), 1.4, 1.6, 1.8, 2.0, 2.1 (Figs. 9 *a*, 9 *b*, 9 *c*), 2.4, 2.5, 2.8, and 3.3 (Figs. 10 *a*, 10 *b*). This distribution is different from the one observed in mitosis. There is no indication that during meiosis I the plaques remain locked at 0.8 μm for any prolonged period. Furthermore, meiosis I plaques and tubules remain rigidly in one plane as the spindle extends; while in mitosis we did not see straight spindles longer than 1.5 μm.

The meiosis I plaques have a normal morphology while they are in the process of moving apart (Figs. 8, 9, 16 *d*). When they reach their maximum separation, each plaque duplicates and they undergo marked structural changes. When the plaques move apart, the nucleus becomes elongate. The nuclear mass does not divide in two parts but instead remains a single, irregularly shaped structure (Fig. 9 and later Figs. 11 *b*, 12 *a*, 14 and 15). Whether or not the nuclear membrane is intact during the latter part of this stage is difficult to ascertain in our glutaraldehyde-fixed preparations. In $KMnO_4$-fixed material, the nuclear envelope is more obvious (18) but identification of this stage is uncertain because the plaques and tubules are not preserved. A slightly later stage, as in Figs. 12 *a*, 12 *b*, and 12 *c*, is easily recognizable in $KMnO_4$-fixed material and it was found to have an intact nuclear envelope (Fig. 14, description in the section on meiosis II: movement of spindle plaques; ascospore development).

FIGURE 8 Sporulating yeast: The plaques have separated and now they bracket a short 0.7 μm spindle at meiosis I. Only intranuclear microtubules are clearly defined. × 135,000.

FIGURE 9 Sporulating yeast: the separation of spindle plaques. In Fig. 9 *a*, one plaque is marked by an arrow, the position of the other by a solid bar. The distance between the plaques is 2.1 μm. At this stage, the mitochondria (*M*) aggregate around the nucleus and the two are difficult to differentiate. × 27,000. Figs. 9 *b* and 9 *c* are details of the plaques in Fig. 9 *a*. The microtubules seen at the cytoplasmic side of mitotic plaques (Fig. 5) do not appear to be associated with meiotic plaques. The scale line is 0.1 μm. × 93,000.

FIGURE 10 Sporulating yeast: replicating meiotic plaques of one cell. In Figs. 10 *a* and 10 *b* the distance between the plaques is 3.3 μm. The outer plaques (*OP*) are much enlarged, possibly expanding to their maximum size at the next stage (Figs. 11 and 13). At the lower side of the plaque bridge (*PB*) is the new plaque. The arrow points at a dense line frequently seen at the nuclear side of meiotic plaques (Fig. 6 *a*). The scale lines are 0.1 μm. × 93,000.


8
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9a
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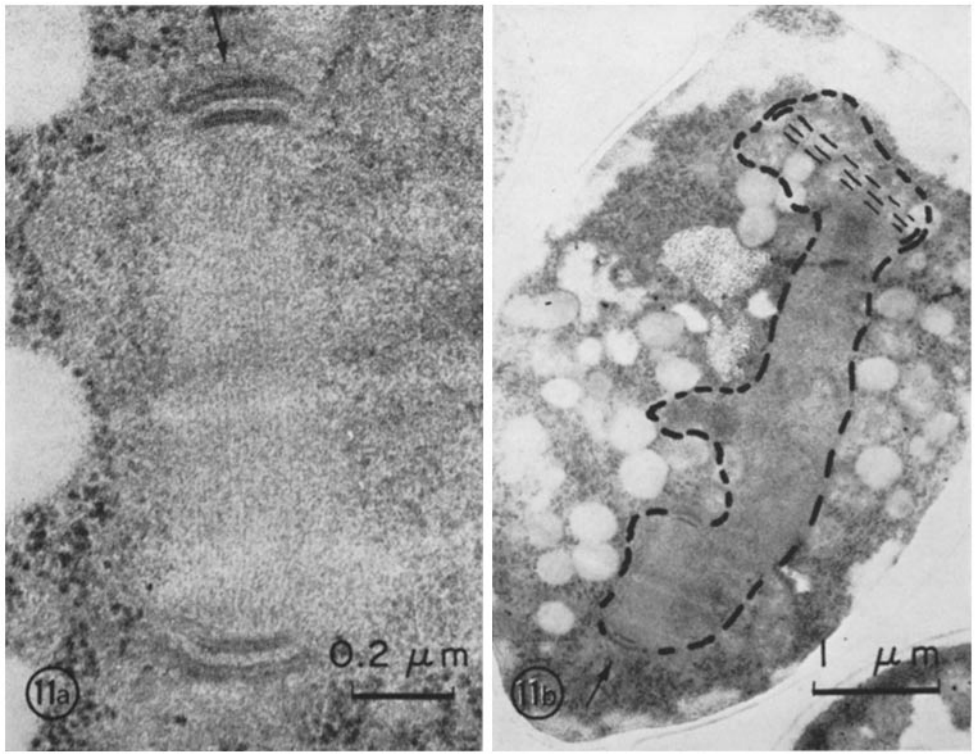
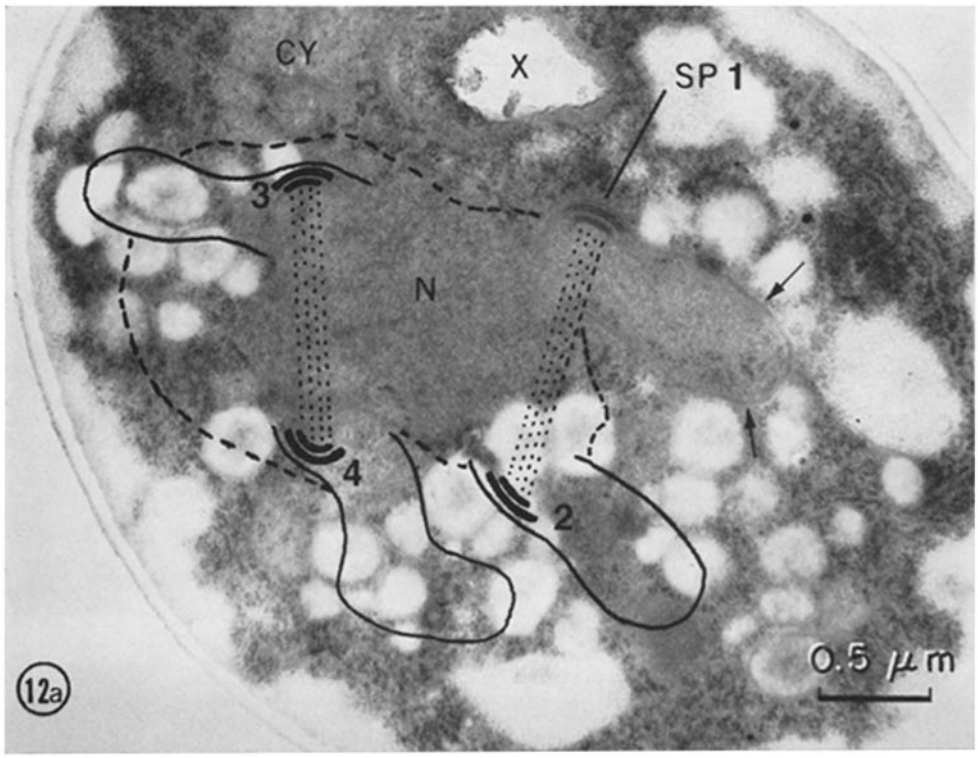
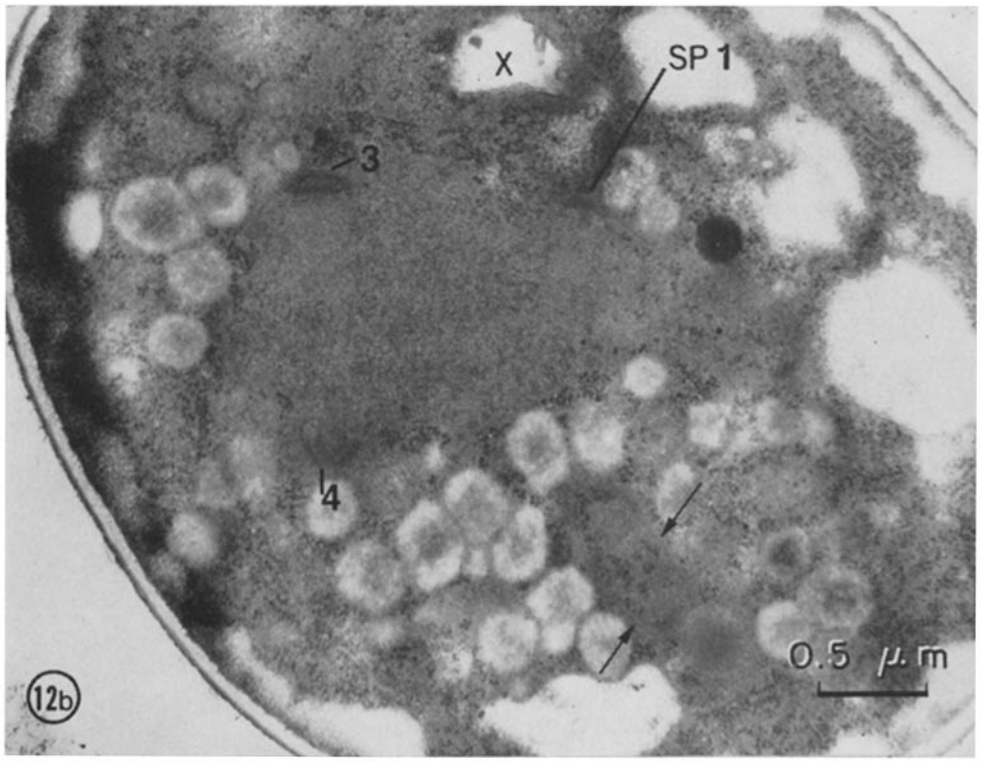
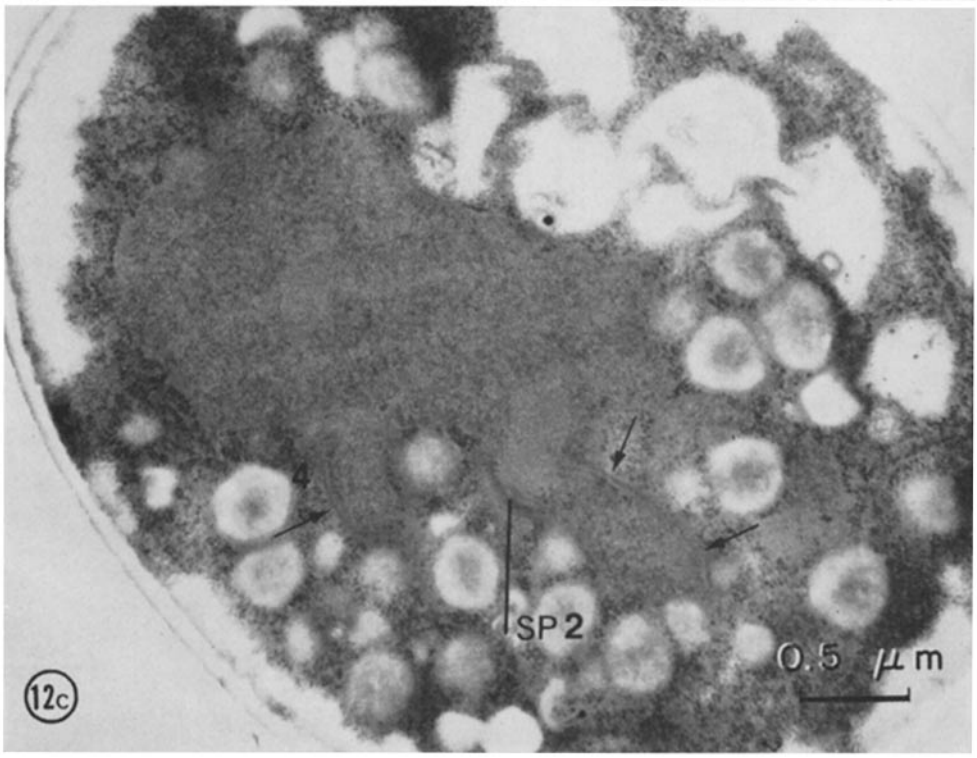


FIGURE 11 Sporulating yeast: meiosis II. In fig. 11 *b* one spindle is 1.0 μm, the other is 1.3 μm long. The distance between centers of the two spindles is 3.3 μm. The nucleus, as it appeared in consecutive sections, is outlined by a heavy broken line. One spindle is drawn with fine broken lines. The other spindle is marked by an arrow. × 17,000. Fig. 11 *a*: details of spindle in Fig. 11 *b*. The outer plaque is large and stains densely. The prospore wall has just started to form next to the upper plaque (arrow) and it was more advanced in the two other plaques of this cell. × 70,000.







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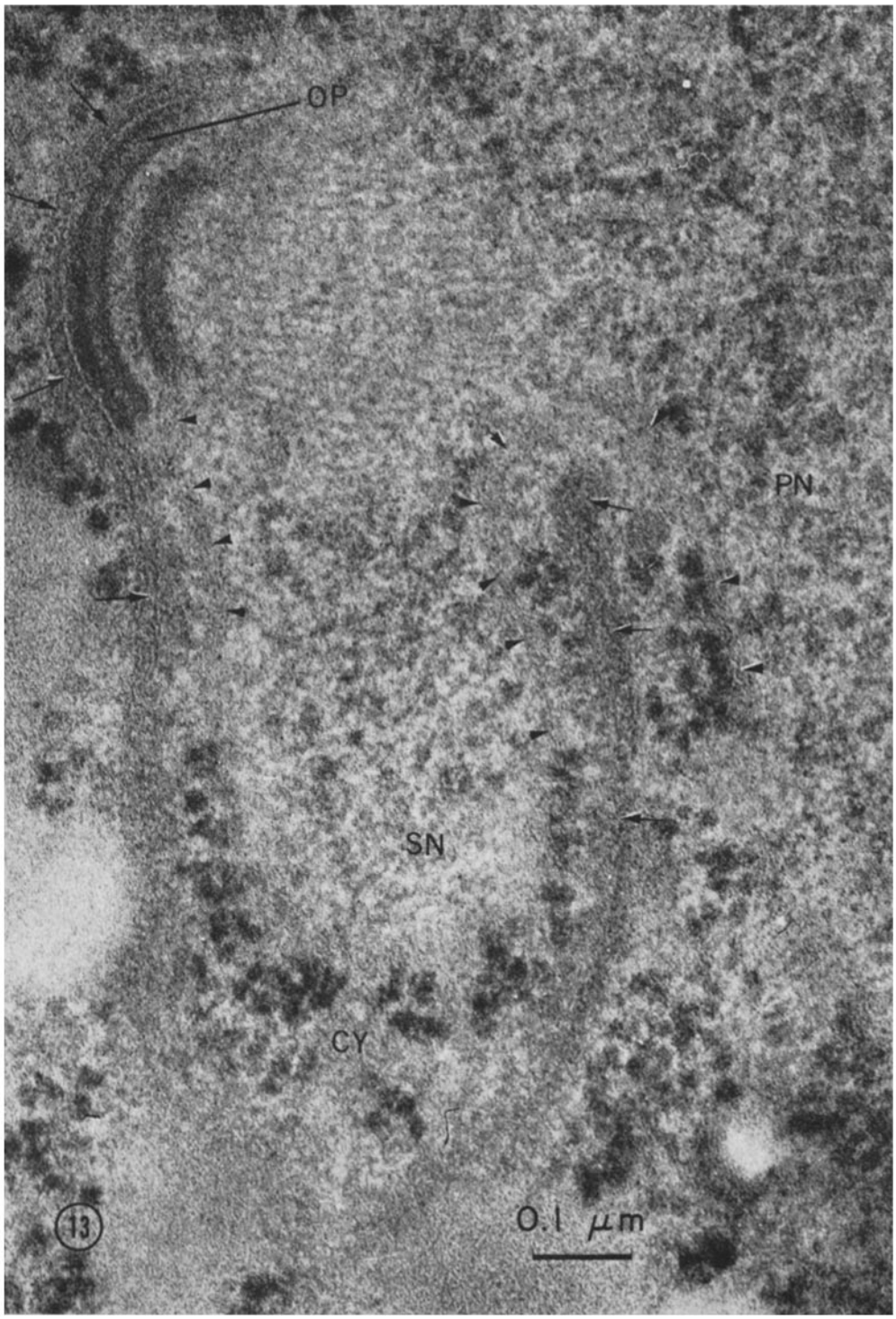

FIGURE 13 Sporulating yeast: meiosis II. Higher magnification of the early ascospore development is shown. The dark-staining structure is the spindle plaque which, at this stage, is at its maximum size and, which has a large outer plaque (*OP*) and a smaller inner plaque. Immediately adjacent to the outer plaque at the cytoplasmic side, is the prospore wall marked by arrows. The prospore wall has the shape of a shoe, which encloses nuclear material (*SN*) and cytoplasm (*CY*) somewhat like a foot surrounded by a sock. The prospore wall does not actually end on the nuclear envelope, which is marked with arrowheads. Thus, the cytoplasm of the ascus is continuous with the cytoplasm of the ascospore. The inner plaque appears to lie at the level of the nuclear membranes. The ascospore nucleus (*SN*) is continuous with the parent nucleus (*PN*), but the latter has no wall around it. × 145,000.

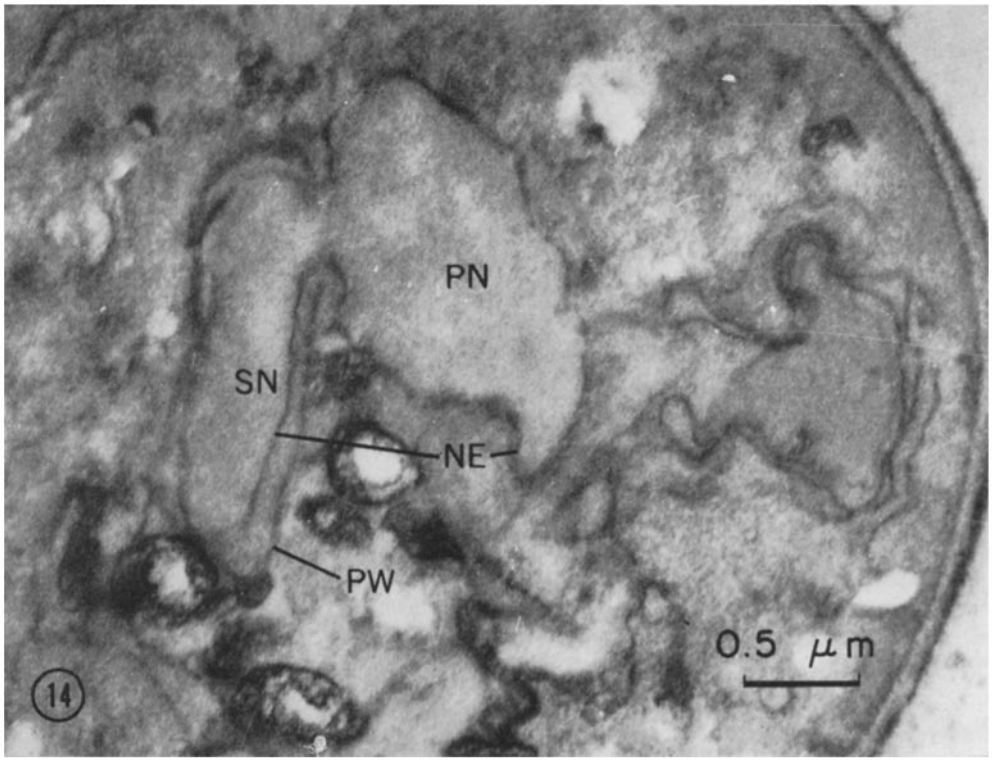

FIGURE 14 Sporulating yeast: meiosis II. A section of a $KMnO_4$-fixed cell is shown, which should be compared with Fig. 13 for identification of parts. The complete nucleus is shown in the model of Fig. 15. The parent nucleus (*PN*) and spore nucleus (*SN*) are connected and they are surrounded by a single envelope (*NE*). The spore nucleus (*SN*) is surrounded by a second envelope, the prospore wall (*PW*), analogous in orientation to the prospore wall of Fig. 13. The parent nucleus (*PN*) has no second envelope. × 30,000.

Meiosis II: the Structure of Spindle Plaques

The replication of two meiosis I plaques into the four meiosis II plaques (Fig. 16 *e*) was only observed in three cells. The distance between one set of replicating plaques was 3.2 μm, between another set it was 3.3 μm (Figs. 10 *a*, 10 *b*), and it was 5.4 μm between plaques which had completed replication. In these six pairs of plaques, the plaque bridge (*PB*) (Figs. 10 *a*, 10 *b*) was present. On the nuclear side of the bridge there is an additional dense line which seems to connect with a dense element on the nuclear side of the plaque (arrow in Fig. 10 *a*). The replicating

FIGURE 12 (pages 352–353) Sporulating yeast: meiosis II. In Fig. 12 *a* the two spindles are about 1 μm long and the centers of the spindles have come as close together. To one side of each plaque, an ascospore is developing. The arrows indicate the prospore wall surrounding the bud. It contains cytoplasm and nuclear material. One of the spindle plaques, situated at the neck of the prospore, is visible, (*SP*1). The two spindles, three plaques, three prospores, and the nuclear boundary have been drawn on the photograph according to their position in serial sections. × 30,000. Fig. 12 *b* is an electronmicrograph of the section next to the one shown in Fig. 12 *a*. One of the recognition points is marked with an *X* in both figures. The plaques (*SP*3) and (*SP*4) of the second spindle are to the left of the first spindle with plaque (*SP*1). The arrows mark the prospore wall associated with plaque (*SP*2) in Fig. 12 *c*. × 30,000. Fig. 12 *c* comes three sections after Fig. 12 *b*. The second plaque (*SP*2) of the right side spindle lies at the entrance of its developing ascospore (arrows). Part of the prospore wall associated with plaque (*SP*4) is also marked with an arrow. × 30,000.

plaques usually had large amounts of dense material associated with the outer plaques (*OP*) (Figs. 10 *a*, 10 *b*). This may in part be due to the plane of section. It may also be related to the marked enlargement of the plaques at the next stage. The spindle plaques at meiosis II are much more prominent than they are at earlier stages. The outer plaque (*OP*) (Figs. 11 *a*, 13) is no longer an indistinct band but has transformed to a well-defined structure, resembling the inner plaque. The faintly visible nuclear envelope in Fig. 13 (arrowheads) appears to be confluent with the inner plaque. As the formation of the spores starts, a prospore wall (Figs. 11 *a*, 13, arrows) appears at the cytoplasmic side of the outer plaque (Fig. 16 *g*). The plaques remain associated with the prospore wall until the entire spore is enclosed in the wall (Fig. 16 *i*). Sometime during maturation of the spore, the nucleus, and hence the plaque, becomes dissociated from the wall. Simultaneously the plaque gradually loses its distinctive meiosis II characteristics until it has the characteristics of plaques in mitosis as in Fig. 3 *b* but lacking a plaque bridge. First the outer plaque becomes less deeply stained, and then the remaining inner plaque becomes smaller. A few microtubules remain associated with the plaque.

Meiosis II: Movement of Spindle Plaques; Ascospore Development

The two plaques at each pole of the meiosis I spindle separate, turn to face one another, and bracket a spindle between them, while the meiosis I spindle tubules disappear (Figs. 11 *a*, 11 *b*, 16 *f*). The length of each of the two spindles in Fig. 11 *b* is 1.0 μm and 1.3 μm, respectively. The centers of the two spindles are about 3.3 μm apart. During meiosis II, the two spindles move back to a more central position in the cell while they continue to elongate (Figs. 16 *g*, 16 *h*). The two spindles in Fig. 12 *a* are still about 1 μm long, but their centers are only 1 μm apart. At this stage, the internal formation of the future ascospore has started. On the parent nucleus four buds develop, one beside each of the four plaques (Figs. 12 *a*, 12 *b*, 12 *c*, 14, 15, 16 *g*). Each of the four buds is capped by a precursor of the eventual spore wall, the prospore wall. The prospore wall does not quite end on the nuclear envelope (Figs. 13, 14), thus allowing both cytoplasmic and nuclear material to flow into the developing ascospore. The prospore wall can be compared with a shoe, the nucleus with the foot in the shoe, and the cytoplasm with a sock around the foot. During the formation of the buds there is still only a single nuclear mass (Fig. 15). Observations by others (18) and by us on $KMnO_4$-fixed material indicate that the nuclear substance is surrounded by an envelope during bud formation. In Fig. 14, the nuclear envelope (*NE*) of the spore nucleus (*SN*) is continuous with the envelope of the parent nucleus (*PN*). The prospore wall is not differentiated as it is in glutaraldehyde-fixed material (Fig. 13). The structural similarity of Figs. 13 and 14 leaves little doubt that the second envelope surrounding the spore nucleus (*SN*) in Fig. 14 is the prospore wall (*PW*), the spore plasmalemma referred to by Lynn and Magee (18). The fact that there is no second envelope around the parent nucleus (*PN*) further supports the identification of the prospore wall in Fig. 14. The unity of the budding nucleus is demonstrated in a model (Fig. 15). The model represents the complete nuclear mass of the $KMnO_4$-fixed cell shown in Fig. 14. The nuclear envelope (*NE*) of each electron micrograph was traced onto the plexiglas sheets. Such three-dimensional models were built from four nuclei in successively later developmental stages. They showed that the prospores and their nuclei gradually increase in size, while the original nucleus decreases in size. The developing ascospores become large, elongate structures. Eventually the wall closes. During ascospore maturation, the spore becomes round and the ascospore wall thickens (24).

DISCUSSION

Spindle Plaques

The dense body at the poles of the spindle and attached to the nuclear envelope has been referred to as centriolar plaque by Robinow and Marak (31), as spindle pole by Westergaard and Wettstein (35), as centrosomal plaque by Zickler (37), and as kinetochore equivalent by Girbardt (10). The term "centriolar plaque" has priority in reported electron microscope studies of this structure, and it fits best the revised definition of a centriole by Boveri (3), where the centrosome is the hyaline body which surrounds the small, deeply staining centriole. Later, in many organisms, the centriole proved to be a specific organelle, consisting of nine sets of tubules arranged in a

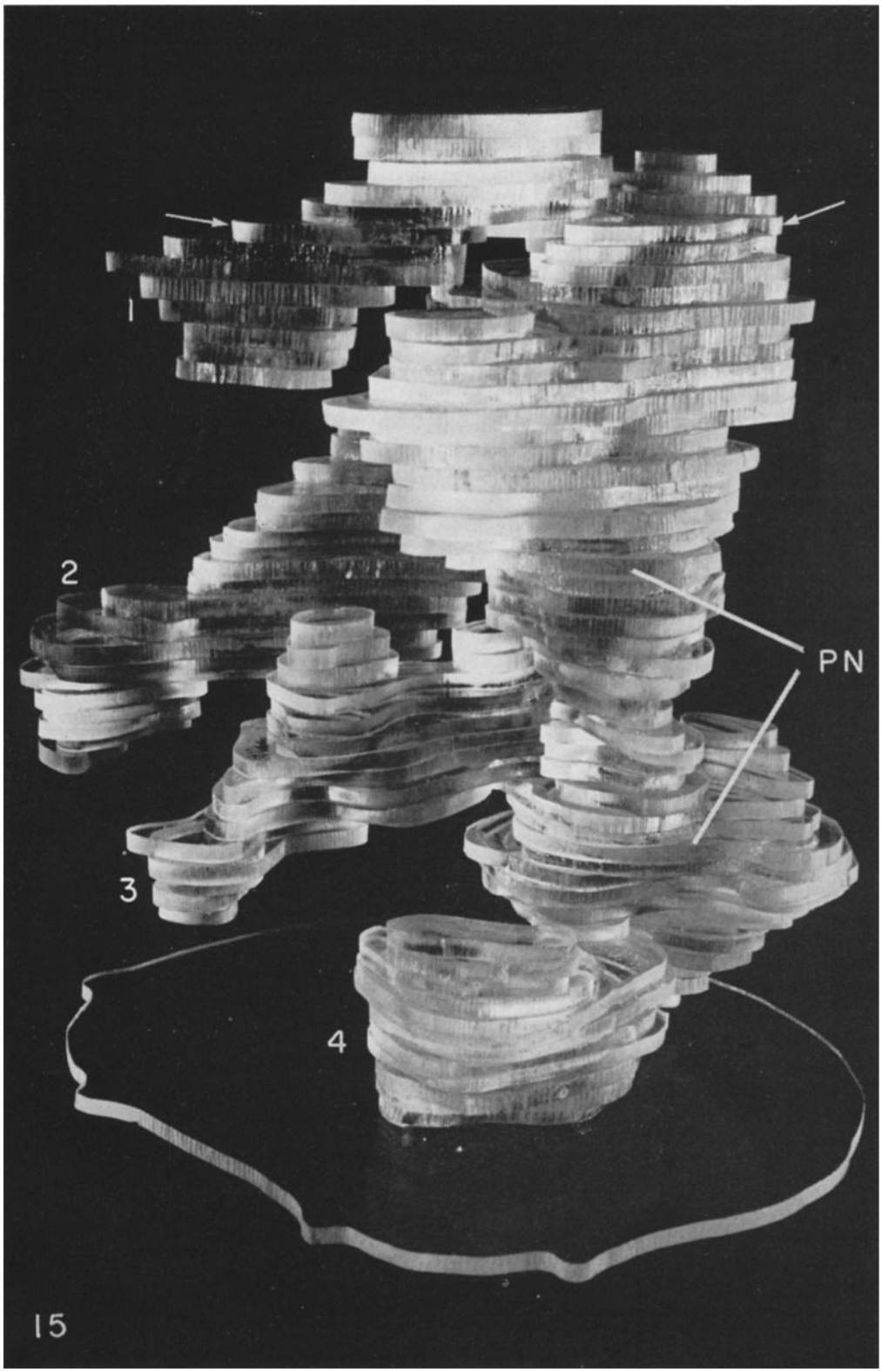

FIGURE 15 Sporulating yeast: meiosis II. A model based on a $KMnO_4$-fixed cell (Fig. 13) made by tracing the nuclear envelope of each consecutive electron micrograph is shown. There is a large parent nucleus (*PN*), and four developing ascospore nuclei (Nos. 1–4) are budding from it. The arrows mark the position of the section shown in Fig. 14. The base plate shows the outline of the yeast ascus with two bud scars.

cylinder. It has been argued, however, that it is not necessary to reserve the term centriole exclusively for this organelle. The centriole may exist in a variety of forms, including a bar-like structure (4), and the centrosome is frequently absent. Meanwhile, it would seem that basal bodies and tubular centrioles are organelles for flagellum formation, but they do not participate in spindle formation (11, 27). Under these circumstances, the term centriolar plaque to describe a part of the spindle apparatus is ambiguous. Pickett-Heaps (27) proposes the term "microtubule-organizing-center, (MTOC)." The MTOC includes the plaques and the closely related kinetochores of the chromosomes. To designate one component of the MTOC we use the term "spindle plaque," which we feel is descriptive and free from unwarranted functional or structural implications.

Uninuclear Meiosis

Meiosis consists of two consecutive divisions of the nuclear material, one reductional division, and one division of the haploid products. Normally, each division results in two nuclei, so that there are four nuclei at the completion of meiosis, Zickler (37) has shown that this system also holds in four species of Ascomycetes, with the elaboration that the nuclear membranes stay intact during each of the divisions. Meiosis in the yeast reported here is unusual in that both meiotic divisions of the genetic material appear to take place within the confines of a single nuclear mass. The term uninuclear meiosis may be useful to describe this system. We anticipate that it may be of phylogenetic significance whether the type of meiosis described by Zickler, or the type of meiosis described in this paper occurs.

Meiotic Stages

In the absence of electron-opaque chromosomes, the meiotic stages of *S. cerevisiae* cannot be defined in terms of chromosome morphology and movement. The organization of nuclear substance in light microscopy shown by Pontefract and Miller (28), and the comparable configurations in electron microscopy may serve as a guide. Meiotic prophase can be taken to last up to the time when the two plaques take up a position facing one another with connecting microtubules (Figs. 8 and 16 *c*). The first meiotic division is completed when the plaques have drawn apart to their maximum separation (Fig. 10).

Each plaque divides (Figs. 10 and 16 *e*) and the members of each pair move apart and bracket a spindle. There are now two short spindles in the cell (Figs. 11 *b* and 16 *f*). The second meiotic division, probably, commences at this point. Subsequently, light microscope observation shows two long chromatinic cords between separating nuclei, either parallel or crossed, depending on the point of view (Pontefract and Miller's Figs. 24 and 22 respectively). Electron microscopy shows that a sequence of elaborate developmental processes take place at this time. While the plaques of each spindle move apart, ascospore formation has already been initiated. Beside each plaque, a bud is formed, which is covered with a prospore wall. In the center of the bud is nuclear material, which is contiguous with the parent nucleus, and surrounding the nuclear material is cytoplasm which is contiguous with the cytoplasm of the cell (Figs. 12 and 13, 14, 15, and 16 *g*). In cells where the prospore wall is just being formed, it is usually first seen at the cytoplasmic site of the outer plaque, and it would appear that the plaque is instrumental in the organization of the membrane (24). As the spindle elongates, the four buds become large, elongate structures, while the original nuclear mass becomes much reduced (Fig. 16 *h*). It seems reasonable to assume that the genome segregation is completed at this time and that this stage is the end of the second meiotic division in this yeast. Eventually, the prospore wall closes around each young ascospore (Fig. 16 *i*). The spindle plaques persist in the ascospores, and they have some poorly defined microtubules associated with them. Upon fusion of two haploid ascospores, the nucleus would presumably contain two plaques, and some form of reduction probably occurs.

Intranuclear Spindles

The record of fine structure of meiosis in fungi is incomplete and, consequently, generalizations based on few comparisons lack validity. Among the Ascomycetes, the intranuclear spindle and the spindle plaque appear to be the rule. Certainly, in some Basidiomycetes, the nuclear membrane is less persistent. During mitosis in *Polystictus versicolor* (10) and *Candida scotti*, formerly a "yeast" (22), electron micrographs show a breakdown of

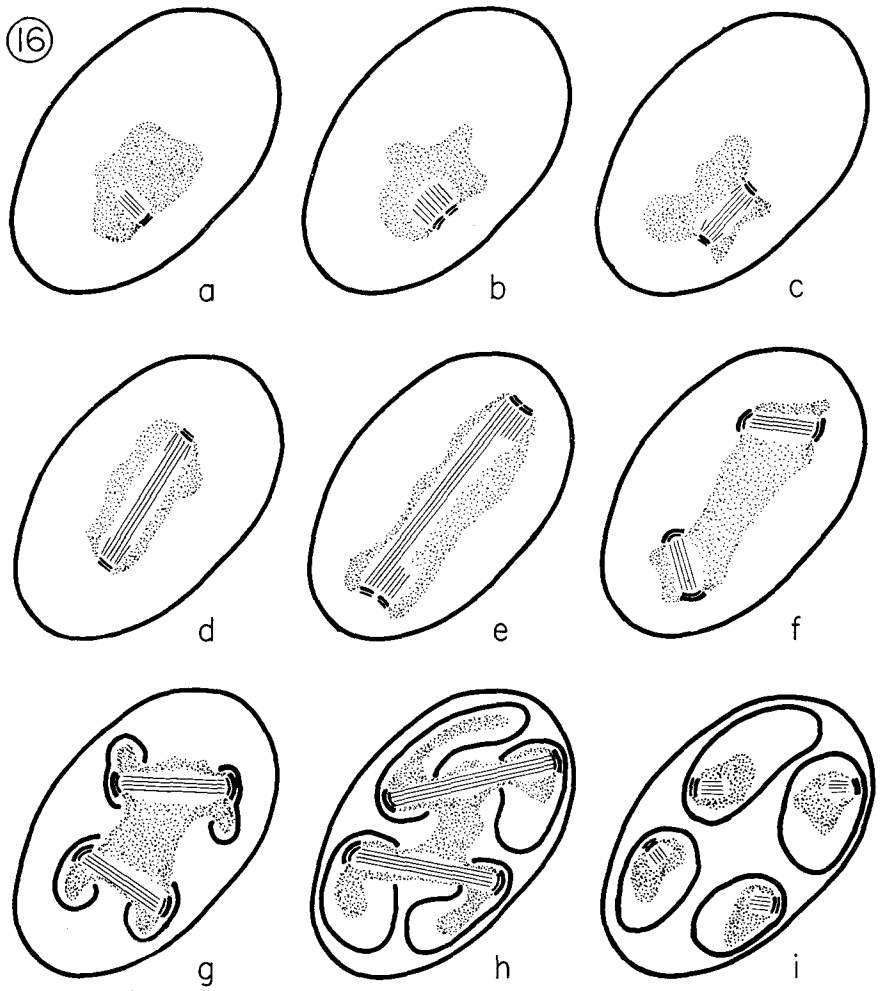

FIGURE 16 Sporulating yeast: summary. Figs. 16 *a*–16 *i* are sketches showing the development of spindles, spindle plaques, and ascospores.

the nuclear membranes. Lu (16) has shown that in metaphase I of meiosis in the Basidiomycete *Corpinus lagopus*, the nuclear membrane is entirely dissolved. The more primitive Phycomycetes, on the other hand, all appear to have a stable nuclear membrane during mitosis (8, 12, and 15). The functional significance of the persistent nuclear membrane may emerge from observations on several species of the true slime molds, where the state of the nuclear membrane depends on the stage in the life cycle of the organism (1, 2, 13, and 32).

In the Ascomycetes with a fruiting body and elongate asci, in which four or eight ordered asci are formed, it seems that products of each meiotic division and the postmeiotic mitosis are separate and have individual nuclear membranes, this is suggested by light microscope observations (20, 21, and 34), and is shown to be so through electron microscope observations of four species of Ascomycetes (36, 37). In bakers' yeast, clearly a different nuclear organization exists during meiosis, a single nuclear mass contains the two spindles of meiosis II. Spore delineation also is different. It remains to be seen whether this form of sporulation is confined to this yeast or to yeasts in general (Saccharomycetaceae), or whether it occurs in fungi of the subdivision of the Ascomycetes with individually formed asci (Hemiascomycetes).

Synaptonemal Complexes

Meiotic chromosome-pairing is characterized by the formation of synaptonemal complexes (26). The report of synaptonemal complexes in *S. cerevisiae* by Engels and Croes (6) suggested to us that the number of chromosomes of this yeast might be determined in the manner used in the marine protist *Labyrinthula* sp. (25). It was found, however, that the meiotic prophase nucleus usually has one finely granular dense body which contains one to several branching synaptonemal complex-like structures. In the rest of the nucleus, faint complexes were encountered only very rarely. No count of bivalents was therefore possible.

We thank Dr. A. F. Croes for providing us with the yeast. Dr. C. F. Robinow discussed the work with us while it was in progress and gave us helpful suggestions on methods and interpretation.

The research was supported by a grant from the National Research Council of Canada.

Received for publication 30 September 1970, and in revised form 24 November 1970.

Note Added in Proof: One other instance of uninuclear meiosis has been reported. K. L. Howard and R. T. Moore (1970. *Bot. Gaz.* **131**:311.) show that in the Oomycete *Saprolegnia terrestris* (thought to be less advanced than the Ascomycetes) the nucleus forms a clover leaf configuration during meiosis II. A centriole lies at the base of each of the four nuclear buds.

The side-by-side arrangement of spindle plaques has been observed in at least one other yeast, *Saccharomyces fragilis* (Dr. E. Unger, personal communication).

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