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Meiosis and Postmeiotic Mitosis in *Boletus rubinellus*

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*Boletus rubinellus*에서 減數分裂 및 減數分裂後 有絲分裂

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ABSTRACT

Meiosis and postmeiotic mitosis in *Boletus rubinellus* were examined ultrastructurally. Meiosis occurred at the apex of the basidium. A sausage-shaped spindle pole body(SPB) was observed along with the presence of synaptonemal complexes during pachytene and a diglobular SPB was present on late pachytene or diplotene nuclei. During metaphase I, the monoglobular SPB at the spindle pole was surrounded by a membrane and the nuclear envelope was discontinuous. At anaphase I, the chromosomes became better defined and formed a central spindle. The nucleolus was extruded from the nucleus. During anaphase I, the SPB was excluded from the chromosomal region by a membrane and both poles were fully separated to opposite sides of the basidial wall. In meiosis II, the two nuclei divided synchronously and the spindles were parallel. The spindles were smaller than in meiosis I, while the SPB was approximately the same size as that of the similar stage in meiosis I. During anaphase-telophase II, the SPB was surrounded by a cap of endoplasmic reticulum (ER) that delimited it from the spindle. The postmeiotic interphase nuclei migrated to the mid-region of the basidium before migration to the spores. The SPB at this stage was diglobular. A postmeiotic mitosis occurred within the basidiospore, and the plane of the spindle was oblique to the long axis of the spore. The spindle and SPB were smaller than at meiosis I and there were fewer nonchromosomal microtubules. At anaphase, the nucleolus was present inside the nuclear envelope but lateral to the spindle.

INTRODUCTION

Two types of nuclear divisions are associated with basidiospore formation in basidiomycetes: meiosis in the basidium prior to spore formation and postmeiotic mitosis during spore development or spore maturation (Duncan and Galbraith, 1972; Wells, 1977; Thielke, 1982a, 1982b; O'Donnell and McLaughlin, 1981, 1984). Dividing nuclei of Homobasidiomycetes possess distinctive characteristics such as a morphologcally distinct SPB, a discontinuous nuclear envelope as well as the absence of a metaphase plate and asynchronous

chromosome segregation during anaphase (Wells, 1977; Heath, 1981). Cyclical variations in the morphology of SPBs during nuclear division have been documented in meiosis and mitosis. The diglobular SPB was observed in interphase nuclei, including prefusion nuclei and late prophase nuclei (McLaughlin, 1971; Wells, 1978); however, the morphological transition of SPBs between karyogamy and late prophase I is still obscure. Monoglobular SPBs have been invariably observed in major division phases (Lerbs and Thielke, 1969; McLaughlin, 1971; Raju and Lu, 1973; Thielke, 1974). Behavior of the nuclear envelope during nuclear division is also controversial in Homobasidiomycetes. Thielke (1974) reported that the nuclear envelope at metaphase was intact, while others observed that it appeared to be moderately (Raju and Lu, 1973; Gull and Newsam, 1976) to extensively (Peabody and Motta, 1979) disrupted. Girbardt (1978) mentioned that the disruption of the nuclear envelope during division might be the first evolutionary step of the process which ends in complete disintegration in higher organisms. Postmeiotic mitosis occurs in many species of basidiomycetes; both Homobasidiomycetes (Duncan, 1970; Duncan and Galbraith, 1972; Hoch and Setliff, 1976; Wells, 1978), and Heterobasidiomycetes (McLaughlin, 1981; Mims, 1981; O'Donnell and McLaughlin, 1984). This divison occurs in the basidium (Evans, 1959) or basidiospore (Duncan, 1970; Duncan and Galbraith, 1972; McLaughlin, 1981), or even in the sterigma (Duncan and Galbraith, 1972). In Homobasidiomycetes, the fine structure of this division was briefly examined only in *Poria latemarginata* (Hoch and Setliff, 1976), thus the understanding of postmeiotic mitosis in this group of fungi needs to be further clarified. In this paper, the behavior of the SPBs and nuclear envelope during meiosis and postmeiotic mitosis in *Boletus rubinellus* has been examined and the results are analyzed and compared with other members of the Homobasidiomycetes.

MATERIALS AND METHODS

Fruitbodies of *Boletus rubinellus* Peck were obtained by culturing from the stock culture on to modified Hagem's medium (Yoon and McLaughlin, 1979) at 22°C with an illumination of 12hr/day for 2-3 wks. The pilei of fully grown fruitbodies were sliced radially into several pieces and fixed in 4% glutaraldehyde in 0.1M cacodylate buffer, pH 7.2, for 1hr at room temperature (22-24C). Glutaraldehyde-fixed specimens were post-fixed in 1% osmic acid in the same buffer for 3hr at room temperature, dehydrated in a graded acetone series and embedded in Spurr's epoxy resin. Polymerized specimen blocks were cut at a right angle to the longitudinal axis of the basidia to obtain cross sections and some sections were cut at a right angle to the axis of the hymenial tubes to obtain longitudinal sections of basidia and spores. Sections were made with a Sorvall Porter-Blum MT-2B ultramicrotome using a diamond knife. After uranyl acetate and lead citrate staining, the sections were examined with a Hitachi Hu-11C electron microscope.

RESULTS

Meiosis. Pachytene nuclei with well developed synaptonemal complexes were located at

mid-region of the basidium (Figs. 1,2). The diameter of these nuclei was 3.5-4.0 μm and synaptonemal complexes with a narrow central component were common. Both central and lateral components frequently terminated at the nuclear envelope (Figs. 2, 6, 14) and a synaptonemal complex was seen associated with the nuclear envelope adjacent to the SPB (Fig. 6). During the prophase I, two types of SPBs were observed on or adjacent to the nuclei containing synaptonemal complexes; sausage-shaped and diglobular types (Figs. 3-12). The sausage-shaped form was seen on a nucleus which had relatively full length of synaptonemal complexes (Figs. 3-7, 13), while the diglobular type was found on a nucleus which seemed to have short segments of synaptonemal complexes (Figs. 8-12, 14). With both types of SPBs the perinuclear spaces of the nuclear envelope under the SPBs were clearly visible as an electron-light zone (Figs. 5, 9). Nuclei with both types of SPBs were situated at the mid-region of the basidium. At this stage numerous lipid droplets and presumed glycogen aggregates were present in the basidium and cytoplasmic microtubules were parallel to the lateral basidial wall (Figs. 1, 2). Small vacuoles were widely scattered in the cytoplasm (Figs. 1, 2).

The metaphase I spindle formed at the apex of the basidium (not illustrated), thus indicating that the late prophase nuclei probably move to the basidial apex at the time of metaphase I or immediately before this stage. Identifcation of metaphase I nucleus was difficlut; however, the following parameters were used to determine it: 1, nucleus with a wide central diameter (Sundberg, 1972); 2, chromosome masses only at the mid-region of the spindle and absence of parallel chromosomes. At late metaphase I (Fig. 15), the SPB was monoglobular, actually oval, and consisted of granular material with less electron density at the center. The SPBs were enlarged to approximatelly 0.4 μm in diameter and surrounded by ER with frequent fenestrations. This surrounding ER appeared to be discontinuous or overlapped the nuclear envelope at the SPB/spindle junction. The nuclear envelope had wide gaps or was fenestrated (Fig. 15). Occasionally, ER was connected to the nuclear envelope at the opening or fenestrations (Fig. 15). Numerous microbodies, ribosomes, membrane cisternae, vesicles and lipid droplets were seen in the nucleoplasm. Chromosomes were inconspicuous but clustered at the center of the spindle. Nonchromosomal microtubules were not prominent, but the chromosomal microtubules and a few kinetochore-like structure were seen. Some microtubules appeared to contact the granular periphery of the SPB. Astral microtubules around the SPB were not observed.

During anaphase I, the spindle was narrower (Figs. 16-20). The SPB was surrounded by ER with fenestrations or large openings (Figs. 16, 18, 19). The nuclear envelope was frequently open and connected to ER (Figs. 16, 17). The nucleoplasm contained numerous ribosomes, membrane cisternae, microbodies and, in one instance, a mitochondrion (Fig. 16). The chromosomes were better defined than at metaphase I and clearly segregating toward the poles. During mid-anaphase I, the nonchromosomal microtubules were most apparent and the chromosomes, aligned along the periphery of the nonchromosomal microtubules, thus formed a spindle characteristic of anaphase (Fig. 19, 20). A nucleolus was observed near the pole (Fig.

19), but it was isolated from the spindle region. It was not clear whether it was completely membrane bound. The spindle microtubules terminated in an electron-dense region (30 nm thick) in the SPBs (Fig. 19). The SPB was circular in cross section and showed a somewhat less dense core surrounded by the outer dense layer (Fig. 18). Astral microtubules were not observed at this stage. The nuclear envelope and SPBs resembled those of early anaphase I. During anaphase-telophase I much of the polar region was occupied by a mass of chromosomes, the mid-region of the spindle was narrowed and only a few microtubles and occasional chromosomes were present in this region (Fig. 21). The SPB was partly extruded from the chromosomal region without a separating membrane between them and some ER was still visible around the SPB (Fig. 21).

Meiosis II nuclei were less commonly found than those of meosis I (Figs. 22, 23). Because of their rarity, only late anaphase and early telophase II were examined. In meosis II, the nuclei divided synchronously, spindle were parallel to each other and much shorter in pole to pole length than those of meiosis I. At anaphase-telophase II, the nuclear envelope at the mid-region of the spindle was considerably disrupted; however, a few microtubules were still present in this region (Fig. 22). The SPBs were separated from the chromosomal region by an incompletely formed membrane and they were bounded by fenestrated ER. During early telophase II, the nucleus was occupied by a mass of chromosomes and the nuclear envelope reformed extensively except at the opening where the nonchromosomal microtubules ran through. The SPBs were considerably extruded from the nuclear region and the presence of the ER cap around the SPBs became less discernible (Fg. 23). The size of the spindle (0.9 μm in diam) near telophase II is about 2/3 of that of telophase I (1.3 μm in diam).

The interphase II nuclei, prior to basipetal migration, stayed near the basidial apex (not illustrated). These nuclei contained a uniform nucleoplasm and a prominent nucleolus. Astral microtubules were not observed at this stage. After migration, the haploid nuclei were closely packed at the mid-region of the basidium and a diglobular SPB was observed (Fig. 24).

Postmeiotic Mitosis. A mitotic division occurred within the basidiospore after nuclear migration was completed (Fig. 25-27). During this division, the identification of the mitotic stage was more difficult because of the small size of the spindle.

Condensed chromatin was observed in the migrating nuclei (Figs. 2, 5, 6 of Yoon and McLaughlin, 1986) and after migration was completed. The SPBs were not observed immediately before the spindle formed; however, the diglobular SPBs were observed on the migrating nucleus(Yoon and McLaughlin, 1986). In the postmeiotic mitosis, the spindle was usually oblique to the long axis of the spore (Figs. 25, 26). Spindles measured at metaphase-anaphase were ca. 2.3-3.5 μm long. Nucleoli were often found at this and the later stages, thus it is assumed that they persist at least until later in this division. Nucleoli appeared to be slightly extruded from the spindle area (Figs. 25, 26). The nucleolus was distinguished from the chromosomes by its electon density and location. The SPBs were considerably smaller, ca.

0.18 μm wide by 0.22 μm long, than at meiosis and semicircular in sectional view unlike the spherical meiotic SPBs (Figs. 25, 26). The nuclear envelope during metaphase-anaphase or anaphase was frequently discontinuous and cytoplasmic organelles including ribosomes, small vesicles, membrane cisternae and small vacuoles were found within the nucleoplasm(Figs. 25, 26). The ER cap around the SPBs was frequently absent or extensively disorganized and astral microtubules were numerous (Figs. 25, 27). Several kinetochores were observed.

DISCUSSION

Meiosis. The morphology of the synaptonemal complex at pachytene in *B. rubinellus* appeared to be similar to that of other hymenomycetes (Lu, 1966, 1967; McLaughlin, 1971; Thielke, 1974, 1978, 1982b; Gull and Newsam, 1975). Several workers (Lu, 1967; Setliff *et al.*, 1974; Gull and Newsam, 1975) reported that the synaptonemal complexes often seemed to terminate at the nuclear envelope, and Lu(1967) claimed that only the lateral components terminate on the envelope; however, in *B. rubinellus* and also in *Psilocybe turficola* (Thielke, 1982b), the central component as well as the lateral components were sometimes associated with the envelope. Thus, Lu's suggestion (1967) that the pairing of the homologous chromosomes may not extend to the telomere region may not be correct.

The morphology of the SPB during prophase I is controversial. Raju and Lu (1973) reported that in *Coprinus lagopus* (=*C. cinereus*) the SPB was monoglobular during diplotene. However, later they found a diglobular type during late pachytene or early diplotene and assumed the diglobular type separates at mid-diplotene. McLaughlin (1971) demonstrated in *B. rubinellus* that during late prophase, perhaps late pachytene or diplotene, it was diglobular. Presence of a diglobular SPB during pachytene was also reported in *Coprinus atramentarius* (Gull and Newsam, 1976). Precise identification of pachytene and early diplotene nuclei is not easy in electron micrographs because reconstruction of the nuclei from serial sections is needed to obtain complete synaptonemal complexes. Thus it is not surprising that diglobular SPBs are repeatedly reported both in pachytene and diplotene nuclei of several Homobasidiomycetes. Several workers seem to be in agreement with the presence of the diglobular SPB in nuclei at late pachytene or early diplotene. However, Peabody and Motta (1979) reported that in *Armillaria mellea* the SPB at pachytene was discoid, and it appeared to have two foci with electron density. At a later stage, they found two monoglobular SPBs which had presumably arisen by separation of the discoid form. Indeed, what was called a discoid SPB in their picture appeared more like the sausage-shaped SPB with two electron dense foci within it. Unfortunately they did not provide serial sections on this particular SPB (Fig. 8 of Peabody and Motta, 1979), nor did they make any reference to the stage, thus it is possible that this elongate SPB may be the form undergoing an imminent change into the diglobular SPB. This organism may need to be reexamined using serial sections to determine the exact shape of the SPB. It is unusual to find discoid SPBs in the Homobasidiomycetes.

In *B. rubinellus,* the sausage-shaped SPB was found on a nucleus at early or mid-pachytene, whose stage was identifed by the well developed synaptonemal complexes and in another basidium a diglobular SPB was observed on a nucleus with synaptonemal complexes. This finding was rather puzzling; however, these synaptonemal complexes appeared to differ from those of the nucleus with a sausage-shaped SPB in two respects; 1, only short segments of the synaptonemal complexes were observed in serial section, and, 2, the lateral components seem to be more diffuse. Lu and Raju (1970) demonstrated that in *Coprinus spp.* the bivalents were separated and appeared to have diffuse edges at diplotene. The short segments of synaptonemal complexes in *B. rubinellus* may be chiasmata, which probably indicates that this stage is diplotene. It is still uncertain what form the SPB takes between karyogamy and pachytene. Girbardt and Hädrich (1975) reported that in the mycelium of *Coriolus versicolor* the diglobular SPB is generated form the monoglobular form through the formation of a sausage-shaped intermediate after mitosis; this report also indicates that the interphase nuclei have a diglobular SPB. Heath and Heath (1976) also showed in *Uromyces* that the reformation of interphase SPB was through the formation of sausage-shaped SPB. Lu (1978) claimed that the fusion of two monoglobular SPBs during karyogamy led to the oblong SPB. However, the diglobular SPB was observed in both prekaryogamy interphase nuclei and a nucleus undergoing fusion in *Panaeolus ater*(Gull and Newsam, 1975) and in the prekaryogamy nuclei of *Boletus rubinellus*(McLaughlin, 1971). It is noteworthy that in *Agaricus bisporus* (Gull and Newsam, 1975) the morphology of the SPB on the diploid nucleus prior to nucleolar fusion seems to be an elongated form, though it is not certain whether this SPB was identified using serial sections. Based upon numerous reports and our own interpretations of some electron micrographs published by other workers (Fig. 5 of Gull and Newsam, 1975; Fig. 8 of Peabody and Motta, 1979), we suggest that the morphology of the SPB from nuclear fusion through pachytene is sausage-shaped and the transition from the sausage shape to the diglobular form seems to occur during diplotene.

During metaphase I in *B. rubinellus* the nucleus was broadly spherical except at the poles. The spindle was perpendicular to the long axis of the basidium. The final orientation of the spindle relative to the axis of the basidium is not uniform among the holobasidiate fungi. In some species of the Aphyllophorales the spindle is perpendicular to the long axis of the basidium (chiastobasidia) like most species of Agaricales, while some of these species form the spindle parallel to the longitudinal axis (Kühner, 1977; Wells, 1978). Two types of spindles have been known for a longtime and it was suggested that they might have taxonomic sgnificance, but this suggestion has been doubted by some workers because of reports of variaions in spindle orientation (Wells, 1977). The monoglobular SPB during metaphase, anaphase and telophase of meiosis I and II in Homobasidiomycetes has been well documented(Lerbs, 1971; McLaughlin, 1971; Thielke, 1974, 1978, 1982a, 1982b; Wells, 1978), and it is believed that it originates from the diglobular form during nuclear division, probably at prometaphase (Gull. and Newsam, 1976; Wells, 1977; Thielke, 1982a, 1982b); however, the

details of the separation of the diglobular form to give the monoglobular form are not well understood. Wells(1978) reported that during meiosis I half of the middle piece remained with the monoglobular SPB, thus suggesting the equal halving of the diglobular form into two monoglobular forms. During early metaphase I chromosomes are clustered in a large mass and at late metaphase the chromosomes are diffused towared the poles. The absence of a metaphase plate seems to be the prevailing phenomenon in most fungal groups, holobasidiate fungi (Motta, 1969; Setliff, 1977; Setlff *et al.*, 1974; Wells, 1978; Heath, 1981; McLaughlin, 1981) as well as phragmobasidate fungi (O'Donnell and McLaughlin, 1981; Bourett and McLaughlin, 1986) and also in many species of ascomycetes (Heath, 1978). The reason for the absence of a metaphase plate is not understood.

It is remarkable that in nuclear division in most fungi the nuclear envelope does not completely disappear unlike that in animal or most green plant cells. Instead it remains intact or disrupted to varying degrees (Heath, 1978). In *B. rubinellus* the nuclear envelope during metaphase I was disorganized, but the disorganization was localized. This result has been repeatedly confirmed in this research using serial sections. Thus reports on the condition of the nuclear envelope during division in fungi must be based upon the examination of serial sections. The presence of ribosomes within the nuclear envelope was reported by Thielke (1974), while she maintained that the nuclear division was intranuclear and atributed the earlier observation of disrupted nuclear envelope (Lerbs and Thielke, 1969) to a fixation artifact. Several other workers (Lu, 1967; Lerbs, 1971; McLaughlin, 1971; Raju and Lu, 1973; Wells, 1978) also reported disrupted nuclear envelope during metaphase I. However, Setliff *et al.* (1974) reported that the envelope was intact except in the vicinity of the SPB. In *B. rubinellus,* disruption is not extensive, but numerous ribosomes and other organelles were present within the nucleus. This observation supports Wells'view (1977) that the nuclear envelope does not function as barrier between the nucleoplasm and cytoplasm. However, it would be helpful to check the nature of nuclear envelope during the nuclear division with freeze-ubstitution.

The cylindrical-shaped spindle at anaphase I in *B. rubinellus* is similar to that reported in meiosis in *Pholiota terrestris* (Wells, 1978) and *Coprinus spp* (Lerbs and Thielke, 1969; Lerbs, 1971; Thielke, 1974, 1978, 1982b). The largest number of nonchromosomal microtubules were seen during this stage most of which seemed to connect to the poles. The presence of chromosomes around a central spindle during anaphese I is common in many fungal groups. This unusual configuration has been attributed to the asynchronous disjunction of the chromosomes (Lu,1967; Setliff *et al,* 1974; Thielke, 1982b) and the movement of the chromosomes along the periphery of the central spindle(Setliff *et al.*, 1974; Wells, 1978). During anaphase I the prominently elongated nonchromosomal microtubules and shortened chromosomal microtubule seem to be a consistent observation in higher fungi (Setliff *et al.*, 1974; Wells, 1978; O'Donnell and McLaughlin, 1981). Heath (1974) described five types of microtubules after extensive analysis of serial sections of *Thraustotheca clavata.* an oomycete, However, it is

uncertain whether such a variety of microtubules are present in the spindles of *B. rubinellus*. In *B. rubinellus* the nuclear envelope during anaphase I is similar to that of metaphase I; however, among the hymenomycetes the results are variable (Wells, 1977).

In the hymenomycetes, the presence of the nucleolus during metaphase or anaphase is rare (Heath, 1978). In *Fomes annosus,* Wilson *et al.* (1967) reported that the nucleolus persists until anaphase I as a separate entity in the distal end of the basidium. In *B. rubinellus* the nucleolus during anaphase I appeared to be present next to the nucleus, but separated by a membrane; however, it is assumed to be an extension of the nuclear envelope. Its subsequent behavior during telophase is uncertain. The retention of the nucleolus until late in mitosis has been observed in heterobasidiomycetes (Heath and Heath, 1976; Bourett and McLaughlin, 1986). The SPB during anaphase I seems to be similar to that reported by others (Setliff *et al.*,1974; Thielke, 1974, 1982a; Wells, 1978). Wells (1978) suggested that the remnant of the middle piece of the SPB might persist as an electron-dense region within the SPB during metaphase, anaphase, and telophase; however, the presence of this structure in *B. rubinellus* is uncertain. At anaphase-telophase I a few chromosomes were present in the narrow interpolar region which contained the central spindle with fewer microtubules. This indicates that the chromosomal microtubules might start to disappear prior to completion of chromosome movement as was also observed in *Helicobasidium mompa* (Bourett and McLaughlin, 1986). However, the mechanism of chromosome movement is not understood. It should be noted that absence of astral microtubules during meiosis in this study of *B. rubinellus* may be a preservation artifact, During anaphase-telophase I, the nuclear envelope around the daughter nuclei reformed and interpolar nuclear envelope became inconspicuous.

It is believed that prophase II is short. In *Coprinus lagopus* (=*C. cinereus*), Lu (1970) reported that the division II may take only 1/15 of the time required for completion of the whole meiotic division. Thus as expected, meiosis I nuclei were rare and only two basidia at late anaphase II have been examined. It appears that the two nuclei in a basidium divide synchronously and the division spindles are parallel. Similar results have been reported in several hymenomycetes (Wilson *et al.*, 1967; Wells, 1978; Thielke, 1982a and 1982b); however, in some organisms the planes of division varied (Lerbs, 1971; Setliff, 1977). At this stage, the SPBs appear to be extruded from the daughter nuclei with newly formed membranes, while the interpolar nuclear envelope is extensiively broken down leaving vesiculated membrane as Sundberg (1972, 1978)reported.

Interphase I nuclei migrated to the mid-region of the basidium. The nuclei after this migration appear to have a diglobular type SPB, and in *Pholiota terrestris*. Wells (1978) reported similar results. It is suggested that the reversion of the monoglobular SPB to the diglobular type may occur after this migration. It is believed that the nuclear movement probably is associated with some sort of interaction between cytoplasmic (or astral) microtubules and the SPB based upon the results from various organisms (Heath, 1978). However, in *B. rubinellus* haploid nuclei prior to migration do not apper to have cytoplasmic microtubules associated

with or in the vicinty of the nuclei. It is known that cytoplasmic microtubules are fixation labile. Thus the absence of cytoplasmic microtubules at this stage might be due to inadequate fixation. Perinuclear ER was not observed around the haploid nuclei of *B. rubinellus* unlike nuclei of *Pholiota terrestris* (Wells, 1978) and *Poria latemarginata* (Setliff *et al.*, 1974). The significance of the perinuclear ER has not been elucidated.

Postmeiotic Mitosis. In many homobasidiomycetes a mitotic division regularly occurs after meiosis in various sites from the basidium to the basidiospore (Sequeira, 1954; Evans, 1959; Duncan, 1970; Duncan and Galbraith, 1972; Setliff *et al.*, 1974; Hoch and Setliff, 1976; Kühner, 1977). Duncan and Galbraith (1972) examined a number of hymenomycetes and categorized them into three types on the basis of the site of the postmeiotic mitotic division and the fate of the resulting nuclei. Also, occurrence of this division has been reported in Heterobasidiomycetes(McLaughlin, 1981; O'Donnell and McLaughlin,1984).

Postmeiotic mitosis occurs in the basidiospore in *B. rubinellus*. It was observed that nuclei migrating from the basidium into the spore had condensed chromatin which seems to support Ehrlich and McDonough's (1949) suggestion that the nuclei entering the spore were already in an early stage of division (Yoon and McLaughilin, 1986). In *B. rubinellus* the spindle was usually oblique to the long axis of the basidiospore and the onest of the divison in the spore from a particular basidium seemed to be synchronous. In *Poria latemarginata* Hoch and Setliff (1976) reported similar results on spindle orientation and timing in the basidiospore. However, Sequeira (1954) and Duncan and Galbraith (1972) reported that many organisms completed this division within the basidium or sterigma and the division was frequently asynchronous.

Compared with meiosis I the nuclei during this mitotic division show several distinctive characteritics. 1, the spindle is considerably shorter, i.e., pole to pole distance, than at meiosis I. 2, the number of nonchromosomal microtubules during anaphase is fewer than that in meiosis I. 3, the SPB is smaller than that of meiosis I and semicircular. 4, abundant astral micrtubules, unlike in meiosis, are associated with the SPB.

It seems that the smaller size of the spindle and SPB may be correlated with the presence of a reduced number of chromosomes. The close association of the cytoplasmic (or astral) microtubules with the SPB has been suggested to be associated with nuclear movement (Heath, 1981). Duncan (1970) and Duncan and Galbraith (1972) reported that in *Boletus spp.* after mitosis one of the sibling nuclei migrates back to the basidium and subsequently degenerates. The mechaniism of nuclear movement is obscure; thus, it is uncertain whether the presence of astral microtubules during this division is related to the basipetal migration of the sibling nucleus. The nuclear envelope during this division shows a moderate disruption similar to that in meiosis I; however, in *Poria latemarginata* Hoch and Setliff (1976)reported an intact nuclear envelope in postmeiotic mitosis. Discontnuity of the nuclear envelope during mitosis has been well documented in several holobasidiate fungi and such a disrupted condition of the nuclear envelope during nuclear division seems be a widespread phenomenon in higher fungal groups-(Wells, 1977; Heath, 1978). During this division, *B. rubinellus* showed a completely open

spindle cap. It is not clear whether this opening is caused by the astral microtubules; however, it might be expected that the absence of the nuclear envelope at the pole might be associated with a similar opening of the SPB cap late in mitosis as occurs in *Helicobasidium mompa* (Bourett and McLaughlin, 1986). In *B. rubinellus,* during postmeiotic mitosis a nucleolus was contained inside the spindle without any delineation from the nucleoplasm. The presence of a nucleolus during mitiotic telophase was also reported in other Homobasidiomycetes (Setliff *et al.,* 1974). Thus the presence of nucleolus during nuclear division in *B. rubinellus* indicates that it may persist in other Homobasidiomycetes; however, because of insufficient data, a conclusion can not be made.

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Explanation of Figures

Key to labelling: Asmt, astral microtubules; B, basidium; Ch, chromosomes; Chmt, chromosomal microtubules; Comt, nonchromosomal microtubules, ER, endoplasmic reticulum; G, glycogen; Kc, kinetochore; L, lipid droplet; M; mitochondria; Mb, microbody; Mc, membrane cisternae; Mt, microtubule; N, nucleus; Ne, nuclear envelope; Nu, nucleolus; SPB, spindle pole body; SNC, synaptonemal complex; Vc, vacuole.

Figs. 1-2. Basidia with prophase I nuclei. 1, Longitudinal section of basidia, one with a pachytene nucleus with synaptonemal complex(bottom)and the other nucleus at a later stage(top). 2, Same nucleus as that of bottom cell in Fig. 1 showing several synaptonemal complexes with central and lateral components.

Figs. 3-7. Serial sections parallel to the longitudinal axis of a sausage-shaped SPB on a prophase I nucleus. 6, A synaptonemal complex terminated at the nuclear envelope adjacent to the SPB.

Figs. 8-12. Serial sections at a right angle to the longitudinal axis of a diglobular SPB a prophase nucleus.

Fig. 13. Nucleus with a sausage-shaped SPB. Same section as that in Fig. 5. A relatively complete synaptonemal complex is visible, though its lateral components are somewhat diffused.

Fig. 14. Nucleus with a diglobular SPB. Only a globular element is shown. Note the rather short segments of synaptonemal complexes. This micrograph is one of eight examined serial sections (same section as that in Fig. 8)

Fig. 15. Late metaphase I nucleus with a SPB at the pole. Note the fenestrated membrane surrounding the SPB and a large gap in the nuclear envelope(asterisk).

Fig. 16. Aanaphase I nucleus with an elongate spindle. Envelope over the SPB has a wide opening (arrowhead) and fenestrations. A mitochondrion is present within the nuclear envelope.

Fig. 17. Cross section of a presumed late anaphase I nucleus. Chromosomes are located only at the periphery of the prominent nonchromosomal microtubules. Note an opening in the nuclear envlope (double arrow) and ER in continuity with the nuclear envelope.

Fig. 18. SPB of the same spindle as that in Fig. 17. Central part of the SPB is slightly electron-light. The envelope around the SPB is considerably fenestrated.

Fig. 19. Late anaphase I. Chromosomes surrounding well developed nonchromosomal microtubules. A nucleolus is displaced from the major part of the spindle.

Fig. 20. Near median longitudinal section of a basidium with an anaphase I nucleus at the apex.

Fig. 21. Anaphase-telophase I nucleus with considerably narrowed interpolar region and polar region packed with chromosomes. Fewer nonchromosomal microtubules are present in the interpolar region, some chromosomes lag behind the major portion of the chromosomes.

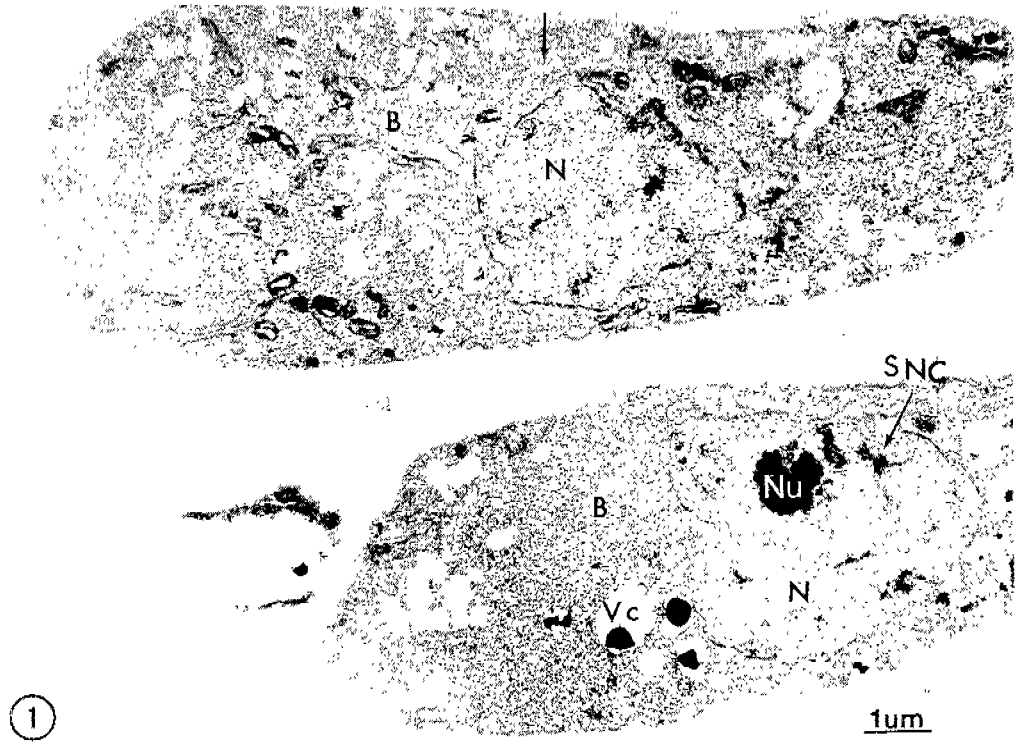
Fig. 22. Anaphase-telophase II. Two division figures with one out of median section. SPBs are separated from the sibling nuclei by the incompletely formed membrane. Nuclear envelope in the interpolar region is disrupted. Compare the size of the spindle with that in Fig. 19.

Fig. 23. Anaphase-telophase I. Near median section through the spindles, each of the sibling nuclei are partly shown. Poles are packed with chromosomes and the SPB is slightly extruded.

Fig. 24. Interphase II nuclei after migration to the mid-region of the basidium. A diglobular SPB is located in a slight invagination of the nuclear envelope. Note the prominent nucleolus in one nucleus.

Figs. 25-26. Postmeiotic mitosis (metaphase-anaphase) in the basidiospore. Note the oblique spindle with a nucleolus, astral microtubules and some cytoplasmic organelles inside the nuclear envelope. The nuclear envelope is fenestrated and the SPB are semicircular. 25, Postmeiotic mitosis at metaphase-anaphase. Note the prominent nonchromosomal microtubules, absence of envelope around the SPB (double arrow) and a few vesicles inside the nuclear envelope. The nuclear envelope has a discontinuity near the polar region. 26, Aanaphase nucleus. The nucleolus is slightly extruded from the major portion of the spindle.

Fig. 27. Metaphase-anaphase nucleus with a relatively intact nuclear envelope except at or near the spindle poles (double arrow). The SPB is partly exposed to the cytoplasm with limited surrounding membrane, some astral microtubules are visible.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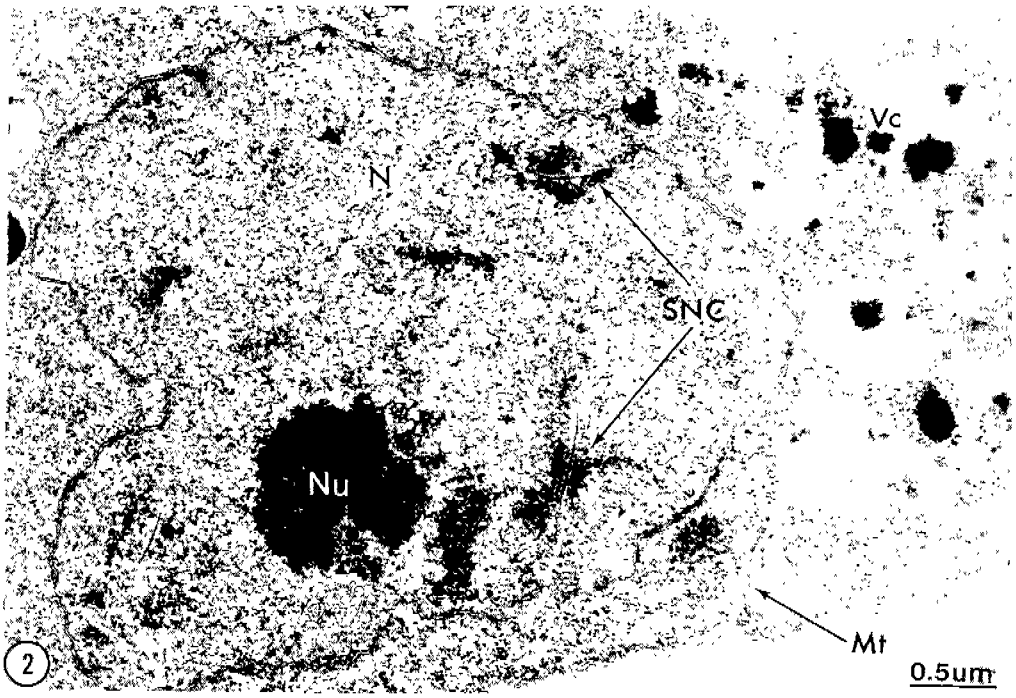
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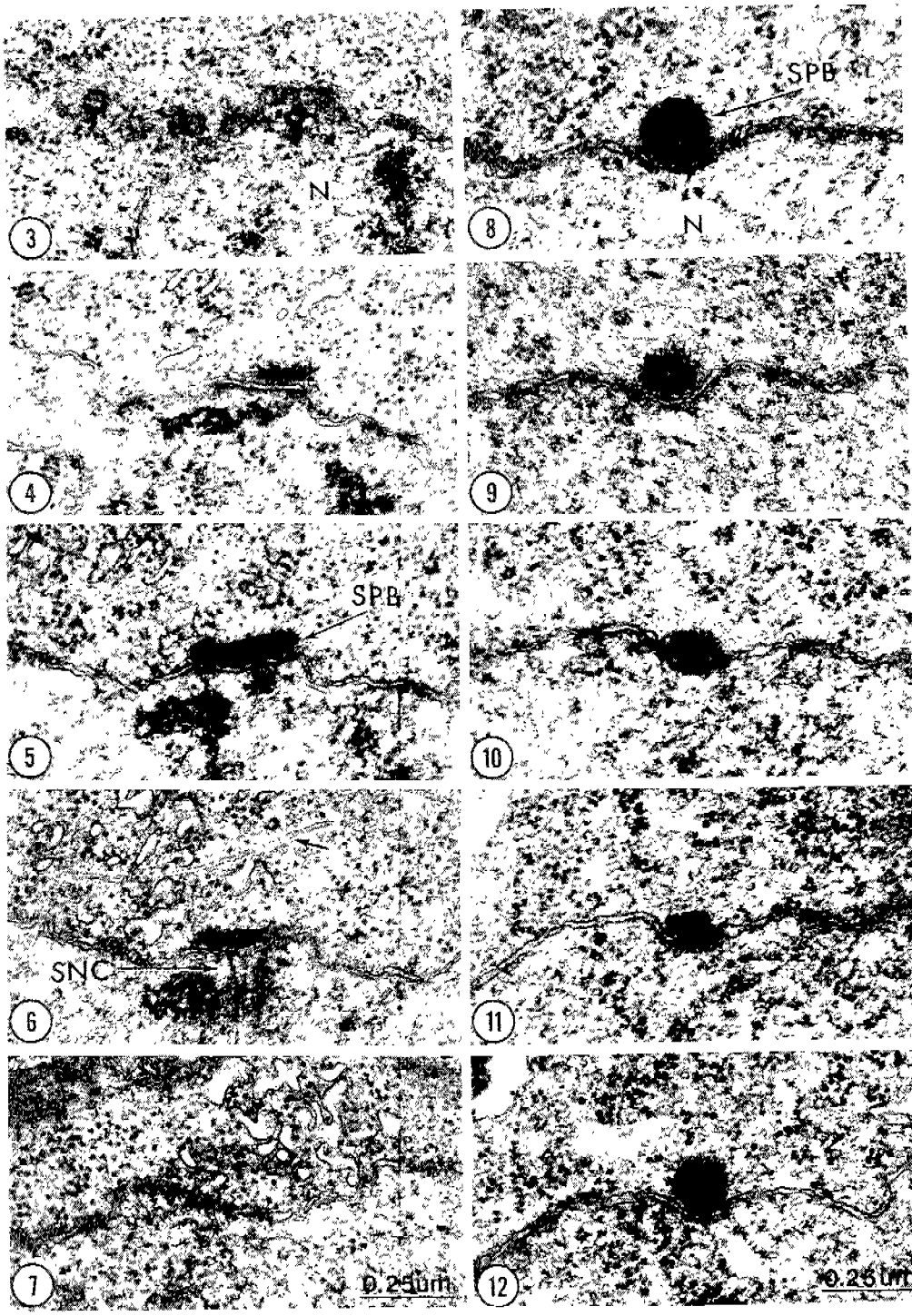
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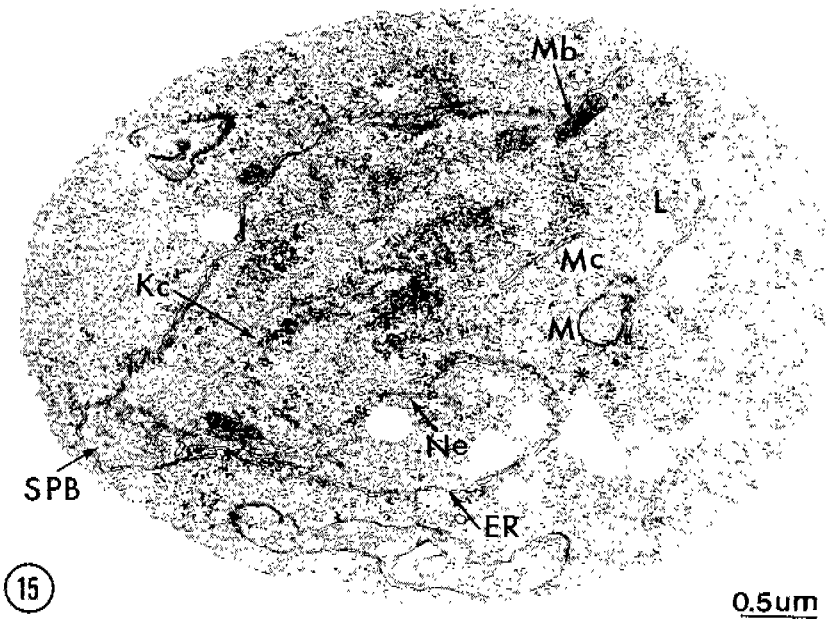

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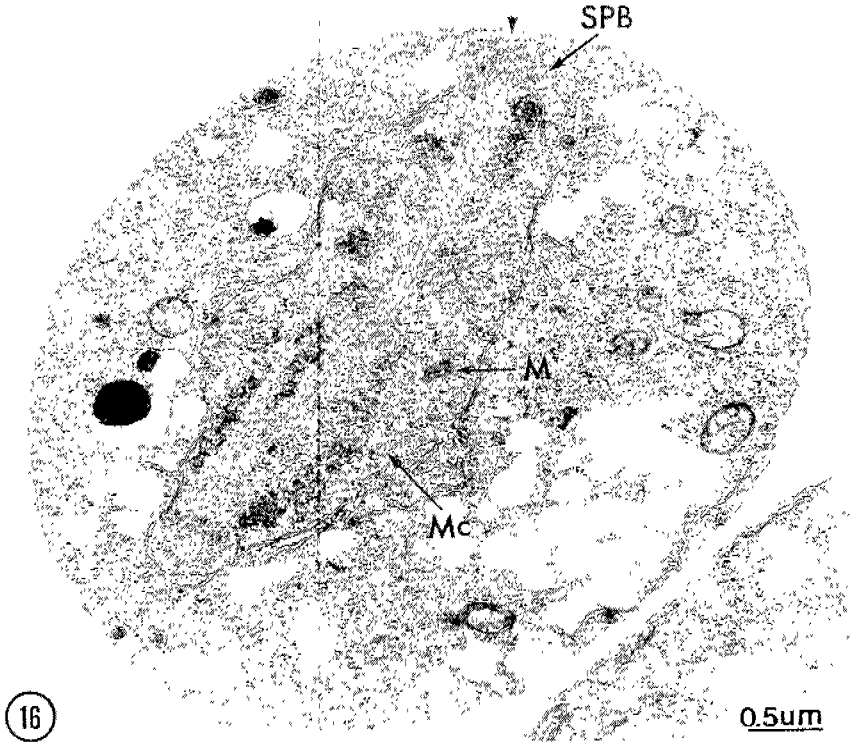

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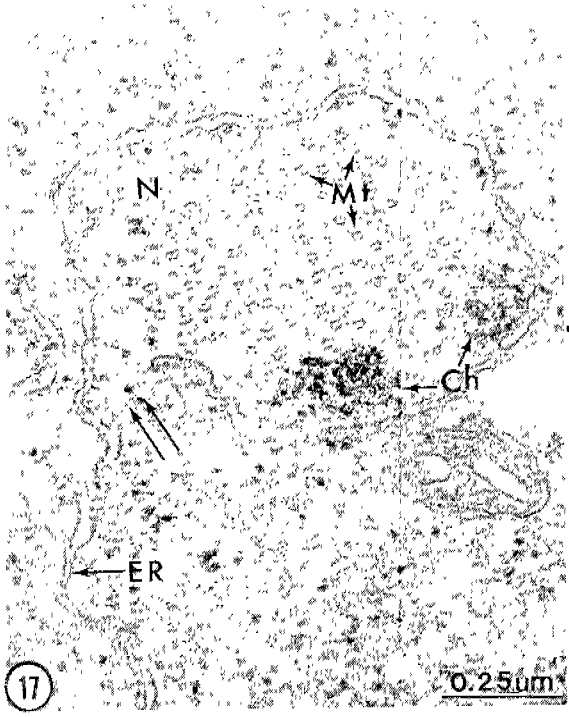

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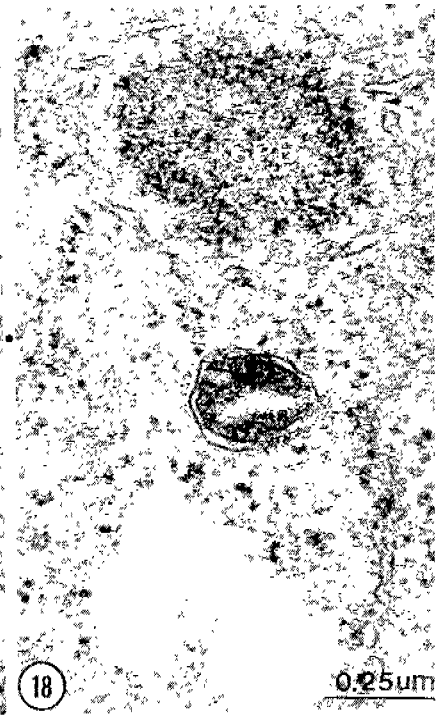

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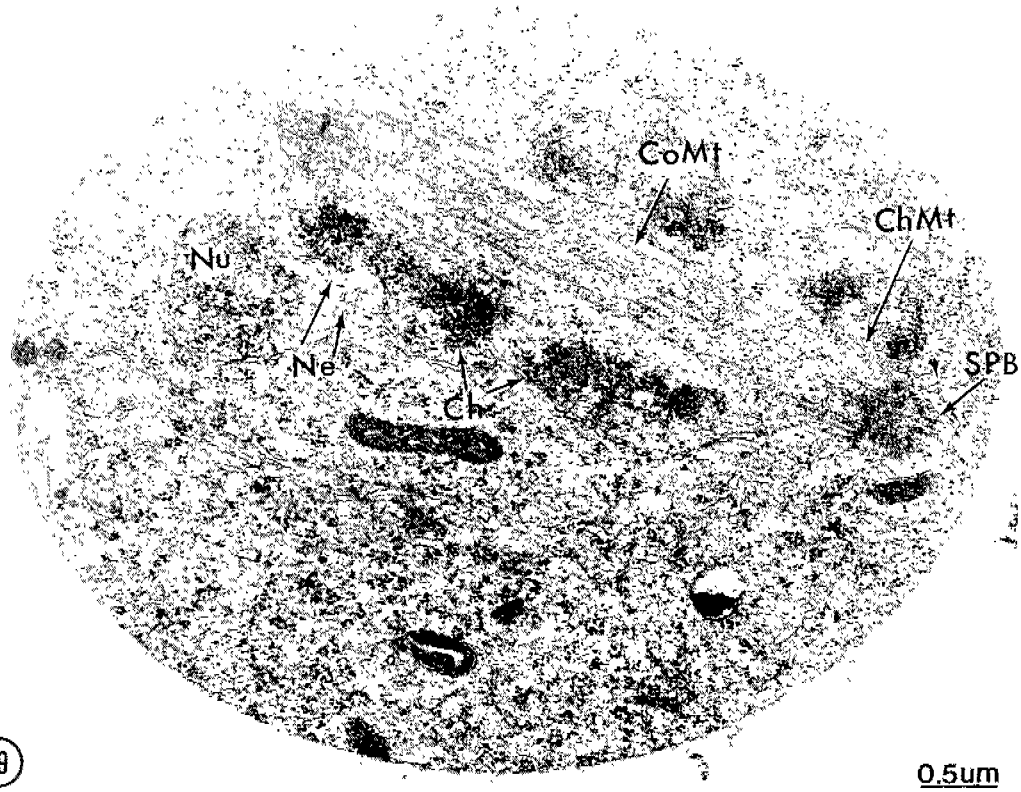

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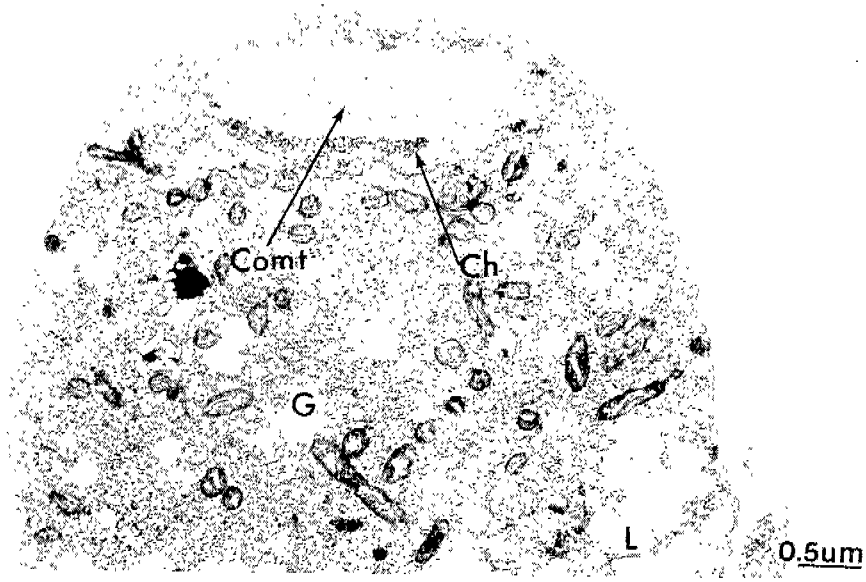

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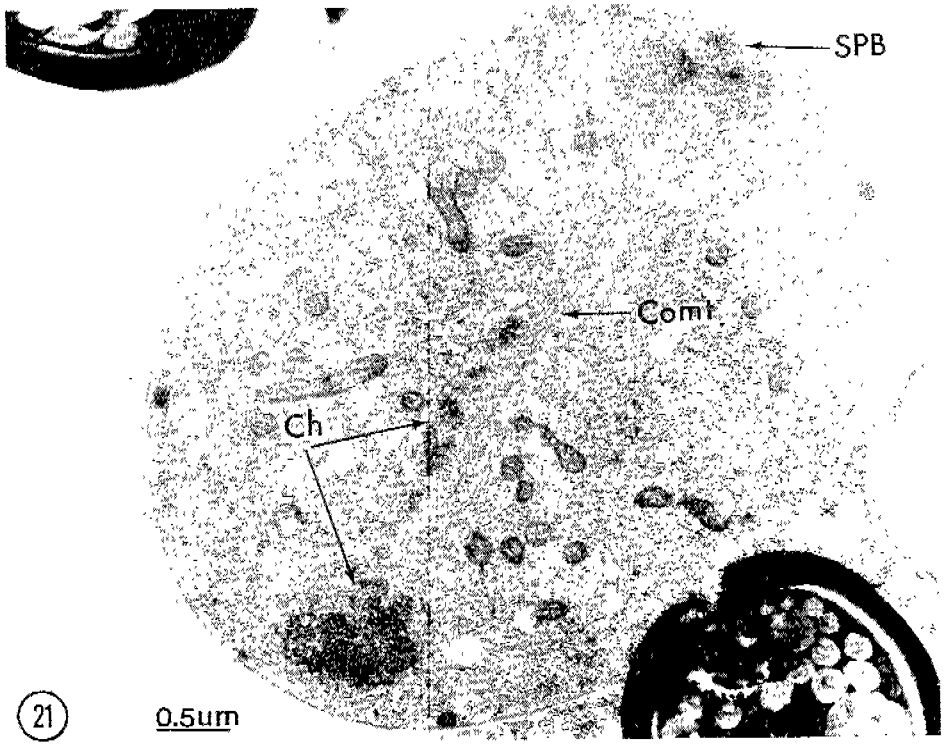

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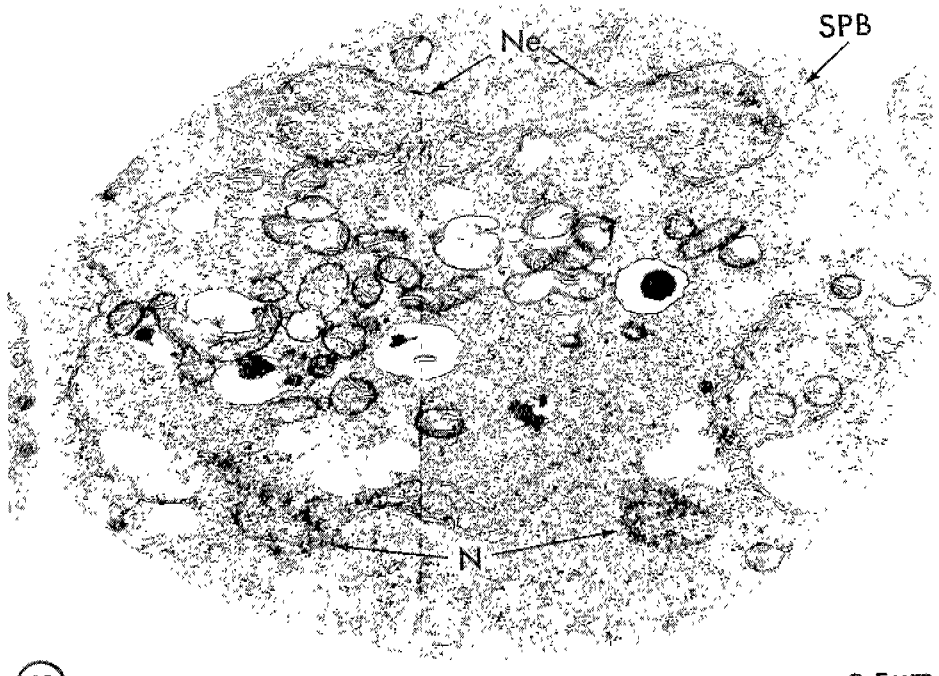

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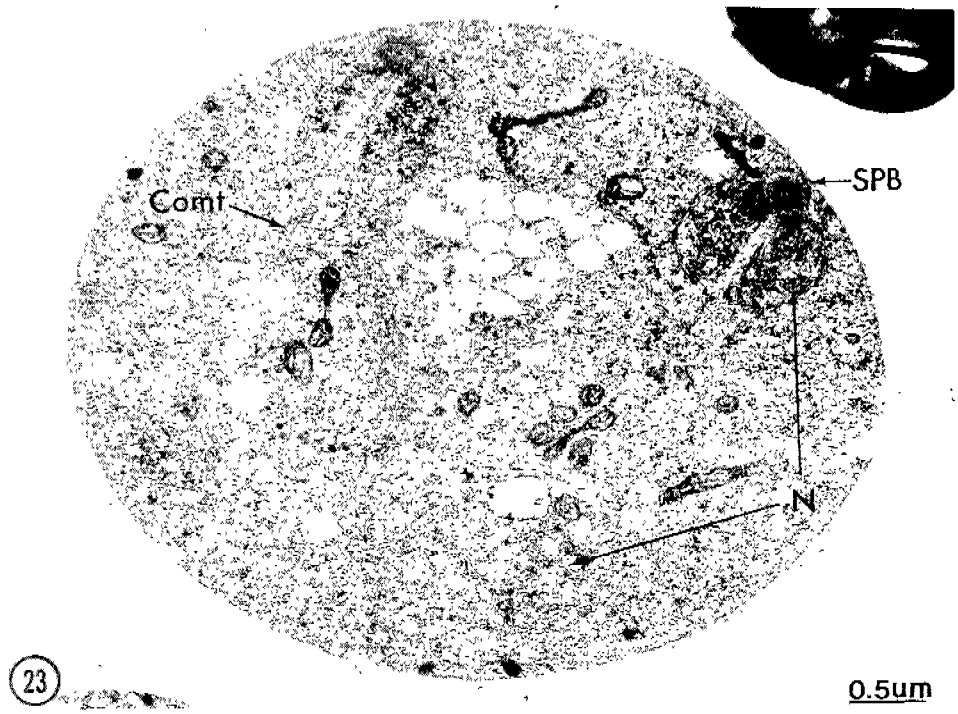

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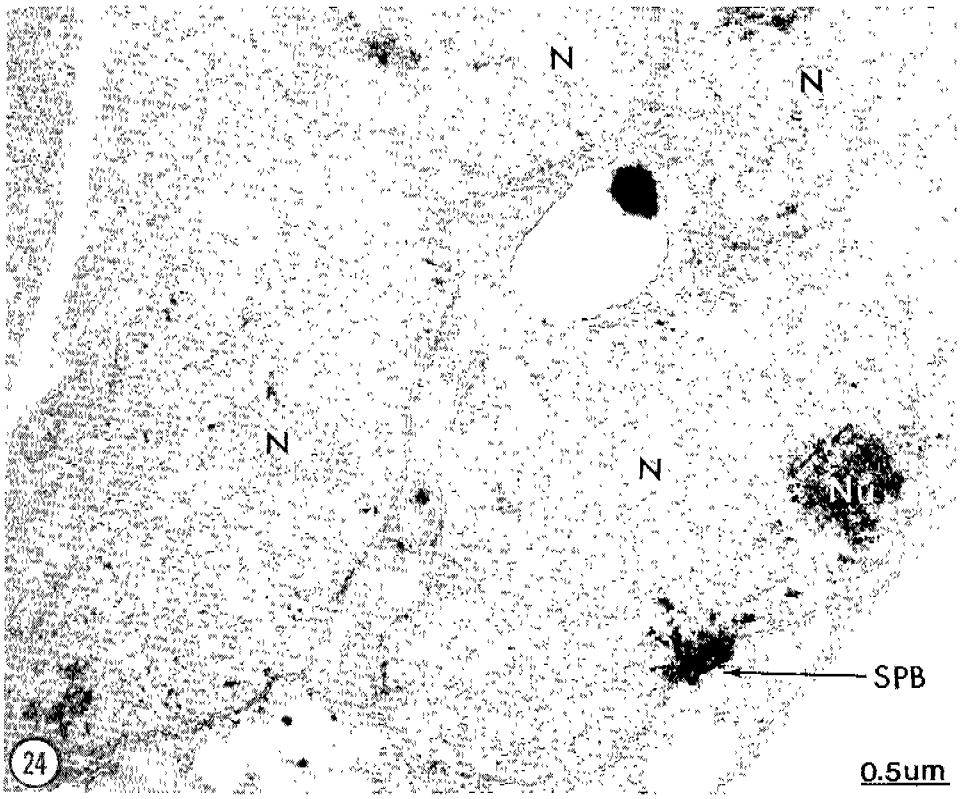

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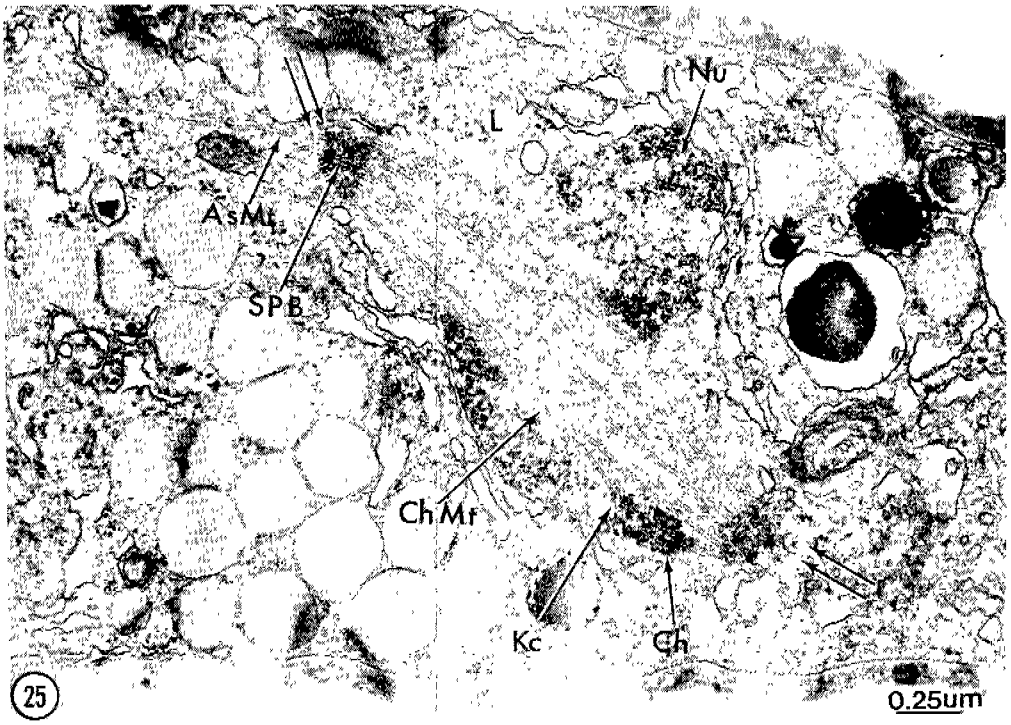
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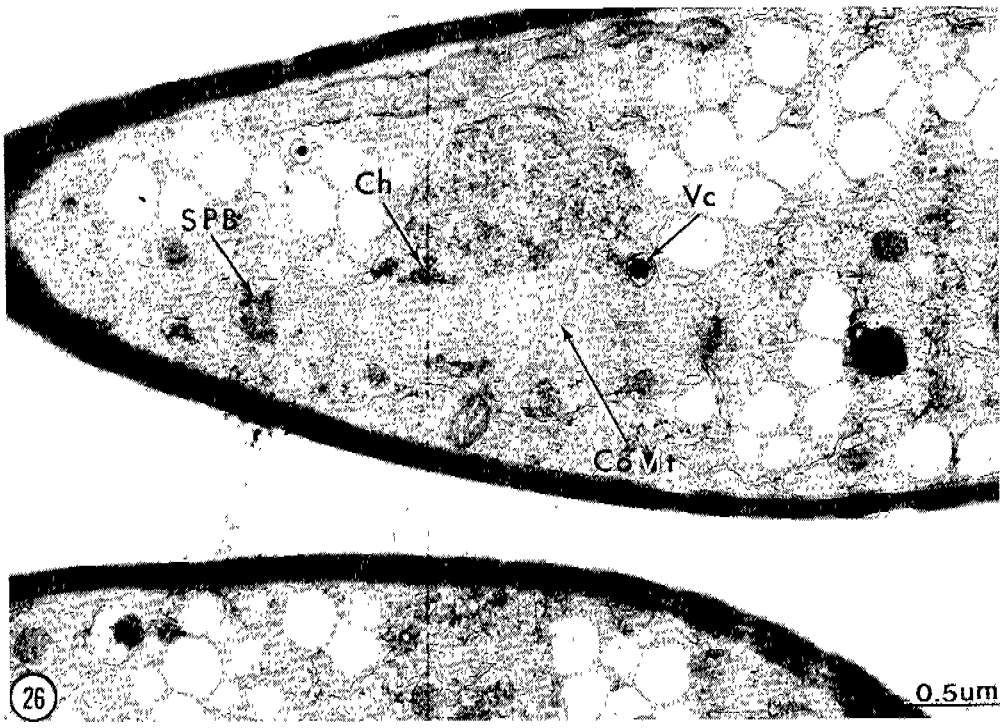
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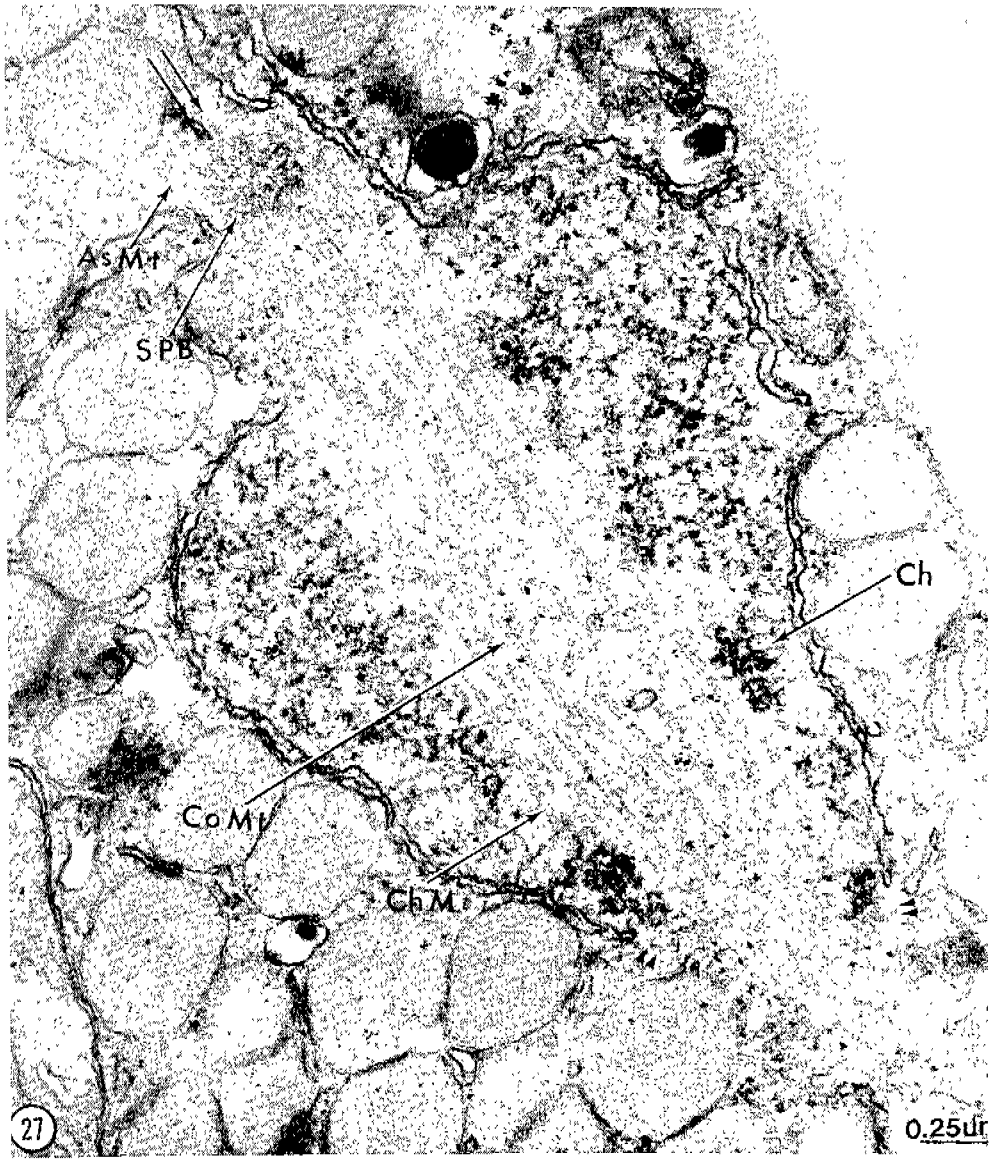
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