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Ultrastructural Aspects of Nuclear Behaviors of *Pleurotus ostreatus*

- Behaviors of Astral Microtubules During Hyphal Development -

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느타리버섯균의 핵의 동태에 관한 미세구조적 연구

-균사분화중의 성상체 미세소관에 관한 연구-

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ABSTRACT: Premitotic, mitotic and postmitotic nuclei in the dikaryotic somatic hyphae of *Pleurotus ostreatus*, the oyster mushroom fungus were ultrastucturally examined using chemical fixation and freeze-substitution process, and the behaviors of astral microtubules associated with these nuclei were closely analyzed. Electron microscopic examinations revealed that astral microtubules are significantly abundant when the nuclei are in the stage of migration and at the stage of migration, the separation of spindle pole body occurs. Such an abundancy of astral microtubules in premitotic migrating nuclei is well contrasted with mitotic and postmitotic nuclei with much fewer astral microtubules and it should be noted that neither of these latter classes of nuclei exhibits the separation of the spindle pole body. It is remarkable that the postmitotic nuclei that are believed to migrate actively are associated with the astral microtubules that are less in numbers and length. During all the stages of nuclear division, astral microtubules are invariably radiating from the spindle pole bodies and nucleolus remains within the nuclear envelope of dividing nuclei throughout the division. The functions of astral microtubules developed during the nuclear division as well as the nuclear migration and separation of the spindle pole body were closely examined.

KEYWORDS: Astral microtubules, Clamp, Mitosis, Nuclear migration, *Pleurotus*, Ultrastructure

Microtubules are involved in the cell motility, intracellular motility, poistioning of cellular organelles and many other developmental processes such as cytokinesis in the eukaryotic cells. Microtubules are often categorized into three classes depending on their distributions or characteristic functions involved, or both: e.g. cytoplasmic-, astral- and spindle microtubules. The spindle microtubules can be subdivided into chromosomal microtubules (=kinetochore microtubules), and spindle microtubules (=polar microtubules). Many review papers dealing with the functions of these microtubules have been published (Bayley, 1990; Fosket, 1989; Lacey, 1988). Among many studies on the microtubules, behaviors of microtubules in relation with nuclear division have been one of the most intensively studied subjects (Aist and Bayles, 1991a, -b; Aist *et al.*, 1991; Bayles *et al.*, 1988; Heath and Heath, 1976; Nicklas, 1989; Nicklas *et al.*, 1989). Especially, the anaphase nuclei seem to be the focus of interest, because during this phase poleward chromosome movement as well as the extension of the poles resulting in telophase nuclei occurs almost at the same time. Astral microtubules recently have been sug-

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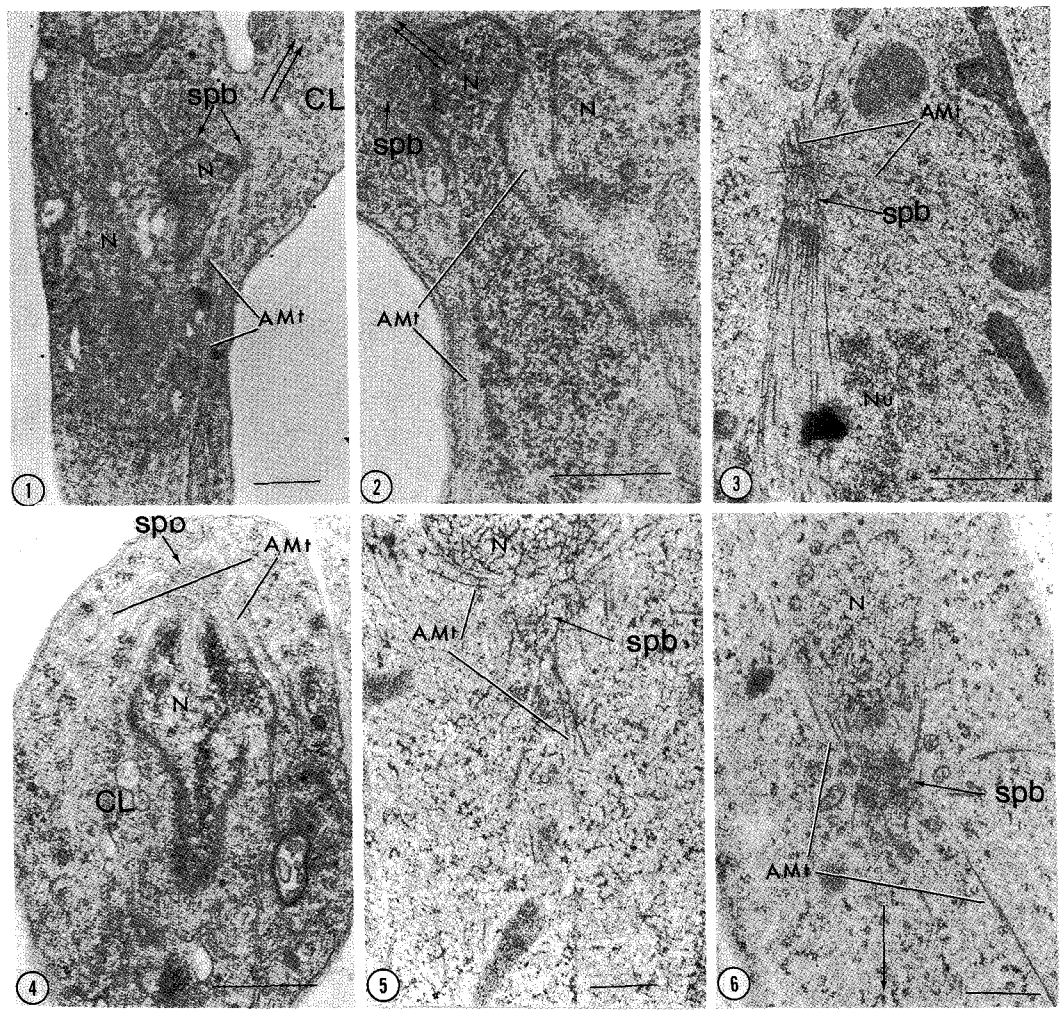
gested for the role in anaphase B nuclei of *Nectria haematococca* (Aist and Bayles, 1991a; Daub and Hauser, 1988; Kronebrush and Borisy, 1982). It was reported that in *Pleurotus ostreatus*, astral microtubules radiating from SPB of the postmitotic migrating nuclei to terminal and subterminal cells may not be directly involved in nuclear motility (Kaminskyj *et al.*, 1989). In dikaryotic somatic hyphae of clamp bearing basidomycete fungi, well developed arrays of astral microtubules associated with premitotic migrating nuclei which move into the developing clamp have been seen in *Pleurotus ostreatus* (Yoon and Kim, 1994). Also in *Lentinus edodes*, the migrating nuclei in basidium to the basidiospores prior to mitosis appeared to be associated with well developed astral microtubules (Nakai and Ushiyama, 1978). It is very interesting that nuclear migration and separation of biglobular spindle pole body into two monoglobular entities occur simultaneously. To understand the possible functions of astral microtubles, pre- and postmitotic nuclei in migration and also mitotic nuclei are examined using ultrastructural data and significances of astral microtubules in nuclear motility is discussed.

Materials and Methods

Somatic dikaryotic mycelium of *Pleurotus ostreatus* was used for the experiment. Dikaryon of this fungus was generated through mating process of two compatible strains, ATCC 42516 and 42517 on YMG agar (Difco yeast extract 4 g, Difco malt extract 10 g, glucose 4 g, Difco agar 15 g, water 1 L). Hyphae were grown on the strips of cellophane covered with 0.5% locust bean gum on YMG agar at 25°C. Portions of cellophane containing advancing hyphal margin were cut, and were further prepared for the electron microscopy. For the electron microscopy, hyphal samples were prepared according to the following different schedules. First, the pieces of cellophanes with hyphae were rapidly frozen in liquified propane chilled at −175°C in a copper well placed in liquid nitrogen (−190°C). Frozen hyphae were transfered to substitution medium (1% osmic acid, 0.5% uranyl acetate in dry acetone) kept at −85°C, and substituted for 72 hrs at this temperature. Substituted hyphae were transfered to −20°C, 4°C and finally room temperature, and embedded in Spurr's medium. The other batch of hyphae was fixed in 5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.5), post fixed in 2% osmic acid and block stained in 0.5% uranyl acetate in the same buffer. Hyphae were dehydrated through a graded series of acetone and embedded in Spurr's medium. Both batches of embedded hyphae on cellophane were sandwiched between 2 teflon coated microscope slides and polymerized at 60°C for 24 hrs. Teflon coating of glass slides were done using pressurized spray cans of dry film lubricant (Fischer). Thin films of plastic impregnated hyphal samples were detached from sandwiched slides and examined with microscope under the 100X objective lense with oil immersion. Adquate hyphae at the proper stages were selected and marked with diamond scriber, then cut out and mounted on empty Beem blocks and used for thin sectioning. Thin sections were stained with lead citrate and uranyl acetate to enhance the contrast of electron image and examined with Zeiss 109 EM.

Results

While the clamp formation is continuing, one of two compatible nuclei in the terminal cell of hypha migrates into the clamp initial



Figs. 1, 2. *Pleurotus ostreatus*. Premitotic migrating nuclei during clamp development. 1. Premitotic nucleus with separating spindle pole bodies (SPBs) migrates into the developing clamp. In this section, one of two monoglobular SPBs is not shown. Long strands of astral microtubules are prominent. A double arrow indicates the direction of migration. Bar=1 μm. 2. Approximately the same stage as that of Fig. 1. Based upon the serial sections, two separating monoglobular SPBs (only one of them shown) are located at the leading apex of migrating nucleus. Note that well developed astral microtubules are radiating from separating monoglobular SPB. Bar=0.5 μm. Key to Abbreviations: AMt, astral microtubules; CL, clamp; N, nucleus; Nu, nucleolus; SPB, spindle pole body.

Figs. 3, 4. Mitotic anaphase nuclei in hypha and clamp. 3. A late anaphase nucleus in hypha shows radiating astral microtubules from the spindle pole. At this stage, astral microtubules are associated with a SPB; however, they are present in much fewer numbers. During the anaphase, it is remarkable that nucleolus remains within the nuclear envelope. Bar=1 μm. 4. An anaphase nucleus in the clamp shows a SPB associated with astral microtubules. Bar=0.5 μm.

Fig. 5. A mitotic telophase nucleus shows a SPB with radiating astral microtubules. Bar=0.5 μm.

Fig. 6. A postmitotic nucleus is migrating in the direction of the arrow. Note that the median section of SPB is associated with astral microtubules. Density of the array of astral microtubules at this stage appears to be similar to those at the telophase and anaphase. Bar=0.5 μm.

(Fig. 1, 2). Migrating nucleus seems to be led by a spindle pole body (SPB) at the leading apex toward the clamp. At this stage, SPB is apparently separating and two arrays of astral microtubules are radiating from two monoglobular SPB (one of two monoglobular SPBs not shown in figures). A combined process of nuclear migration and initiation of SPB separation appear to be simultaneous. Separation of SPB is associated with enormous numbers of astral microtubules (Fig. 1, 2), and its trailing astral microtubules are extended long. These microtubules are largely arranged in proximal and distal directions. Migration of a nucleus into the devloping clamp initial occurs before the clamp reaches its full size (Fig. 1). Though the duplication of SPB is underway, there is no visible sign of the imminent nuclear division in both nuclei (Fig. 2). At this stage, the size of the trailing nucleoplasm seems to be similar to that of interphase nucleus.

In each stage of mitotic division, astral microtubules are always associated with mitotic nucleus at the SPB; however, they are not as much numerous as those of premitotic migrating nuclei (Fig. 3, 4, 5). During the anaphase, astral microtubules are associated with anaphase B spindle, though its numbers seem to be much fewer than those at the premitotic migrating nucleus (Fig. 3, 4). During the anaphase, nucleolus is present inside the nuclear envelope. At telophase, astral microtubules are well developed and polar cap is not observed (Fig. 5). At the early telophase, there are always prominently developed spindle microtubules connecting two sibling nuclei (not shown in Fig. 5) and also astral microtubules are associated with the spindle poles; however, these microtubules are apparently reduced in numbers and length compared with the astral microtubules of premitotic migrating nucleus as shown in Fig. 1 (Fig. 5).

In hyphal development of clamp bearing fungus, there are two distinctive types of nuclear migration: the premitotic migration of a nucleus into the developing clamp, and the postmitotic nuclear migration of progeny nuclei when the mitoses are complete. The postmitotic migrating nuclei are associated with astral microtubules radiating from the SPBs (Fig. 6). Similar to the telophase nuclei, astral microtubules associated with these nuclei are notably fewer in numbers and many multivesicular bodies are located around the SPB.

Discussion

Astral Microtubules and Premitotic Nuclei

In *Pleurotus ostreatus*, migration of premitotic nucleus and separation of biglobular SPB on this nulcleus seem to be a simultaneous process. This process has been well documented (Yoon and Kim, 1994). Occurrence and function of astral microtubules are extensively examined in many organisms (Aist and Bayles, 1991a; Aist and Bayles, 1991b; Aist *et al.*, 1991; Bayles *et al.*, 1988; Daub and Hauser, 1986; Snyder and Mullins, 1993; Sullivan and Huffaker, 1992; Tanabe and Kamada, 1994). However, many studies directed to the astral microtubules have been aimed to reveal the mechanism of mitotic or meiotic spindle behaviors. In this study, it is especially noteworthy that profusely developed astral microtubules are associated with premitotic, interphase nucleus that is still away from the prophase. However, the SPB on this nucleus is apparently separating in preparation for the oncoming mitotic division. In a recent study of *Nectria haematococca*, Aist and Bayles (1991a) suggested that astrers are responsible for the separation of SPBs during the nuclear division and

accordingly showed that when MBC (methybenzimidazole-2-yl carbamate, as an antimicrotubular agent) was treated to the dividing nuclei, elongation of spindle at anaphase B almost stopped. Presence of well developed astral microtubules in premitotic nuclei with separating SPB in *Pleurotus ostreatus* seems to be consistent with its role in anaphase B in *Nectria* in terms of widening of two spindle poles. But in *Pleurotus ostreatus*, this premitotic nucleus actively moves into the developing clamp. At this stage, it is still uncertain whether the astral microtubules associated with this nucleus is related with force generation for nuclear motility. Also, in this case, it should be carefully accounted that in *Pleurotus ostreatus*, only small portion of the nucleoplasm actually moves into the clamp leaving most of the nucleoplasm behind, then sheared away later (Yoon and Kim, 1994).

Astral Microtubules and Mitotic Nuclei

Practically the astral microtubules are present in all classes of mitotic nuclei, not confined only in certain stages, e.g. anaphase nuclei. Astral microtubules are known to be responsible for the separation of two poles to the opposite direction at the anaphase (Aist and Bayles, 1991a; Bayles *et al.*, 1988; Kronebush and Borisy, 1982).

It is also interesting what these microtubules are really doing in prophase and metaphase that are nothing to do with separation of the SPB, if astral microtubules are only accounted for the separation of SPB (or elongation of spindle). In the study using tubulin mutant of *Coprinus cinereus*, it was shown that the abnormally formed asters or complete lack of aster at the dividing nuclei did not interfere either with the positionig of nuclei or elongation of mitotic spindles (Tanabe and Kamada, 1994). These results indicate that the true function of the astral microtubules during the mitoses should be reconsidered.

Astral Microtubules and Postmiotic Migrating Nuclei

Mechanism of intracellular motility of organelles including nuclei has been examined in many classes of organisms: sea urchin (Cohn *et al.*, 1989), alga (Menzel, 1987; Weissenfels *et al.*, 1990), insect (Stebbing and Hunt, 1987), fungi (Stenlid and Rayner, 1991) and plant (Heslop-Harrison and Heslop-Harrison, 1989a, -b, -c, 1992). In numerous studies, microtubules are known to be responsible for the intracellular movement of organelles; however, in other studies on plants, myosin was found to be associated with migrating nuclei (Heslop-Harrison and Heslop-Harrison, 1989c), thus, the operation of actomyosin system in organelle motility was suggested (Heslop-Harrison and Heslop-Harris on, 1989b). Ultrastrucrual study on migrating post-meiotic nucleus in to developing basidiospores of *Lentinus edodes* showed that a long bundle of astral microtubules extended into the cytoplasm of basidiospore was closely associated with SPB at the leading apex of nucleus (Nakai and Ushiyama, 1978). Other study of migrating nucleus of *Boletus rubinellus* at the apporoximately similar stage revealed that SPB was probably separating (Yoon and McLaughlin, 1986). In both fungi, when the nuclei reach the center of the basidiospore, a mitosis ensues rapidly. Presence of astral microtubules associated with the migrating nuclei and the concurrent separation of SPB in *Lentinus* and *Boletus* may indicate that astral microtubules associated with this nucleus must be involved in the movement of nucleus or the separation of SPB or both. In *P. ostreatus*, dynamics of postmitotic migrating nuclei in somatic hy-

phae was examined in detail by Kaminskyj *et al.* (1989) in relation with the mechanism of nuclear motility. In this study, movement of nuclei was not affected by the disruption of the microtubules after treatment with MBC, a microtubule inhibitor (Kaminskyj *et al.*, 1989). However, in movement of chloroplast, functions of actin has been suggested for the force generating system (Chen and Li, 1991; McLean and Juniper, 1993; Schonbohm and Meyer-Wegener, 1989). Presence of actin on migrating nuclei of *Pleurotus ostreatus* was examined intensively using immunofluorescence cytochemistry in our laboratory without success.

적 요

느타리버섯균의 이핵체 체세포 균사에서 분열전 및 분열후의 핵과 분열중의 핵을 화학고정방법과 동결교체방법을 이용하여 균사분화과정중의 성상체 미세소관의 미세구조적 동태를 조사하였다. 많은 수의 성상체 미세소관들이 이동중인 분열전의 핵들과 연관되어 있었으며, 분열전의 핵이 이동하는 시기에 SPB의 분리가 진행됨이 발견되었다. 분열전 이동중의 핵과는 대조적으로 SPB의 분리가 더 이상 일어나지 않는 분열중의 이거나 분열이 끝난 핵에서는 성상체미세소관이 덜 발달되어 있었다. 활발히 이동할 것으로 추측되는 분열후의 핵에서 핵과 연관된 성상체미세소관이 잘 발달되어 있지 않음은 특이한 점이다. 분열중의 모든 핵에서 성상체미세소관이 SPB에서 뻗어나오며 인은 핵막에 남아있다. 핵의 분열과 핵의 이동 및 SPB의 분리와 관련된 성상체미세소관의 기능에 대하여 논의하였다.

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