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Localization of Microtubules during Macronuclear Division in *Tetrahymena* and Possible Involvement of Macronuclear Microtubules in 'Amitotic' Chromatin Distribution

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ABSTRACT. **The ciliated protozoa *Tetrahymena* contains two nuclei, a micronucleus and a macronucleus. In the vegetatively growing cell, the macronucleus divides amitotic while the micronucleus divides by mitosis. It has been indicated that microtubules are involved in macronuclear division and microtubules are observed to exist in the dividing macronucleus. To clarify the localization and the organization of microtubules in the amitotic dividing macronuclei, we used immunofluorescent staining technique. The microtubules were observed in the cytoplasm and macronucleus. The microtubules were organized and dynamically changed their distribution throughout the macronuclear division. We suggest a possibility that these microtubules are involved in 'amitotic' distribution of chromatin throughout the macronuclear division.**

Key words: microtubule/macronucleus/macronuclear division/amitosis/*Tetrahymena*

The ciliated protozoa *Tetrahymena* divides by both amitotic and mitotic process (10). *Tetrahymena* cell contains two types of nuclei, a micronucleus and macronucleus (Fig. 1). The micronucleus serves as a germinal nucleus that is transcriptionally inactive and divides mitotic. The macronucleus serves as a somatic nucleus that is transcriptionally active and divides with amitosis. The evidence for amitosis is that macronuclear division occurs without chromosome segregation, without spindle formation and the genomes are randomly assorted to the daughter nuclei.

Studies on macronuclear division have been started since 1960's. First observation of macronuclear microtubules in *Tetrahymena* was done in 1961 by using electron microscope (6). The macronuclear microtubules were described to localize near the periphery of macronucleus and running in the nucleoplasm (1, 4, 7). Some of the macronuclear microtubules were observed to attach to the macronuclear envelope (9).

Investigation of the function of microtubules in macronuclear division was carried out by disrupting microtubules by colchicine treatment (5, 7, 9) and by a low temperature treatment (8). The inhibition of macronuclear division was induced by a colchicine treatment, suggesting that microtubules are involved in macronuclear division. In spite of these studies, further study on the localization and function of microtubules in *Tetrahymena* macronucleus has remained stagnant. The sequential study of the localization of microtubules through the macronuclear division was never done and previous studies had only shown the partial images of the cell.

The macronuclear division

The macronuclear division in *Tetrahymena* occurs without the nuclear envelope breakdown. Hence, the diverging of macronucleus can be visualized by staining the DNA. To study the macronuclear division in a sequential manner, it is classified into six stages according to the morphology of the macronucleus (Fig. 2). Interphase and stage 1: round macronucleus. Stage 2: oval macronucleus. Stage 3: cylindrical macronucleus. Stage 4: constricted macronucleus. Stage 5: deeply constricted macronucleus, which is right before the constriction. Stage 6: from when the macronucleus divides into two daughter nuclei to the end of cytokinesis. The macronucleus divides with the elongation and the constriction. The mechanism of macronuclear constriction is unknown and no such contractile structures have yet

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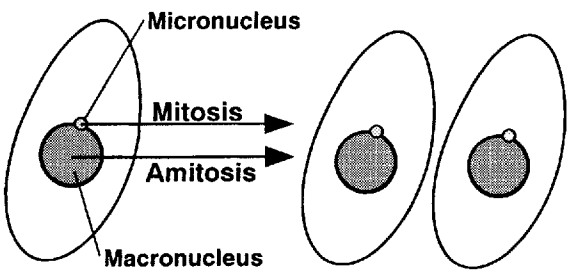

Fig. 1. Nuclear division of *Tetrahymena*. Micronucleus, a germinal nucleus, divides by typical mitosis. Macronucleus, a somatic nucleus, divides by an amitotic process.

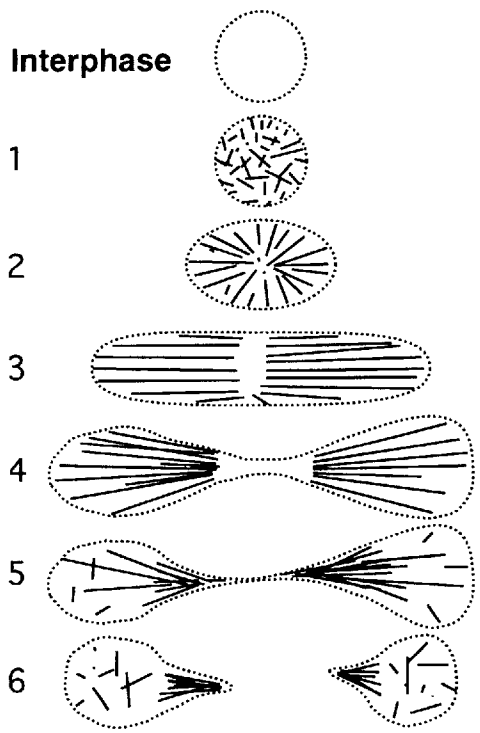

Fig. 2. Changes in macronuclear microtubule distribution during macronuclear division in *Tetrahymena pyriformis*. Numbers refer the stages of macronuclear division. Macronuclear envelope (dotted lines) and macronuclear microtubules (bold lines) are represented. Macronuclear microtubules are not observed in interphase macronucleus. In the beginning of macronuclear division, assembly of microtubules takes place in random orientation, and some near the periphery of macronucleus are tend to localize spiral with one end pointed toward the inner side of macronucleus. With the elongation of macronucleus, microtubules are formed into radial distribution and then parallely distributed with having the middle region of macronucleus sparse in microtubules. With the constriction of macronucleus, distributions of microtubules become radial. At the point where macronucleus separates into two daughter macronuclei, microtubules become dense at the region where the separation of macronuclei occurred.

been observed at the constriction area of macronucleus. With an addition, macronucleus constricts independent to the cell division furrow. The best evidence is the *cdaA1* mutant, a temperature sensitive cytokinesis defect mutant, which does not require the cytokinesis for macronuclear division (2). So far, the microtubule is the only candidate involved in the macronuclear division.

The macronuclear microtubules

In order to study the localization of microtubules, the commonly used typical method of immunofluorescence staining of the alpha-tubulin was largely modified. This enabled the imaging of microtubules in *Tetrahymena* cell and clearly showed the localization and the organization of macronuclear microtubules throughout the macronuclear division (Fig. 2). Immunofluorescence of tubulin inside macronucleus indicated that few microtubules exist in the interphase macronucleus. In the stage 1 macronucleus, newly polymerized short microtubules became randomly localized and microtubules adjacent to the nuclear envelope tended to localize radial. At stage 2, the macronuclear microtubules were radial localized from the center of macronucleus towards the nuclear envelope. In the stage 3 macronucleus, microtubules were parallel localized along the longer axis of the macronucleus. Microtubules were sparse in the middle of macronucleus, while strong fluorescence appeared at the outside of the sparse region. At stage 4, microtubules were running radial toward both ends of macronucleus. In stage 5, the sparse microtubule region disappeared and the radial microtubules were running from the constriction area toward both ends. In the posterior and anterior ends of macronucleus, microtubules showed a random localization. At stage 6, when the macronuclear division was completed, the radial microtubules were extended from the constricted region to the opposite side in the daughter macronucleus. When cytokinesis was completed, macronuclear microtubules were disassembled and microtubules dispersed resembling to the interphase macronucleus. The microtubules are organized throughout the macronuclear division in *Tetrahymena* cell.

The cytoplasmic microtubules

Microtubules are also localized in the cytoplasm, for example, the oral apparatus, the contractile vacuole pore, the cilia, the cell cortical region including the cilia row, and the basal body. In addition to this localization, some microtubules run through the cytoplasm.

Our observations have shown that there are numbers of microtubules running in the cytoplasm. These cytoplasmic microtubules were mainly seen during the cell

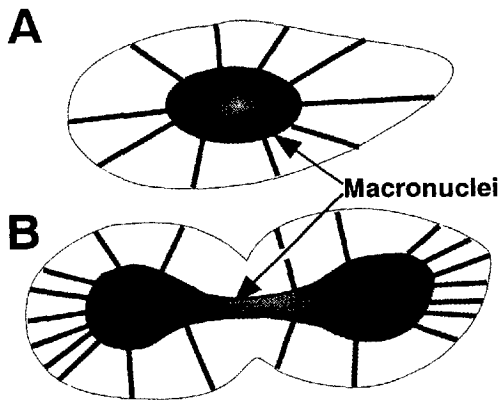

Fig. 3. Changes in cytoplasmic microtubules during macronuclear division. The cytoplasmic microtubules are localized almost equally through out the cytoplasm at the beginning of macronuclear division (A). When the macronucleus elongates, the cytoplasmic microtubules tend to localize frequently at posterior and anterior sides of elongated macronucleus (B).

division (Fig. 3). From stage 2, when the macronucleus starts to elongate, the cytoplasmic microtubules were localized linking between the macronucleus and some area near the cell cortex. These cytoplasmic microtubules were especially localized at the elongating ends of macronucleus linking to the cortical region of the cell (Fig. 3B). The macronuclear division seemed to be cooperatively carried out by these macronuclear microtubules and cytoplasmic microtubules.

Distribution of DNA and macronuclear microtubules

Throughout the macronuclear division, distribution of chromatin or the DNA in macronucleus changed its distribution pattern. DNA dense region appeared in the middle of macronucleus from stage 2 to 4. In the stage 4 macronucleus, the sparse region of DNA appeared at both posterior and anterior side of DNA dense region. These DNA distribution patterns were disrupted and diffused through macronucleus by treatments of microtubule disrupting drugs. The relation between macronuclear microtubules and DNA was focused. The macronuclear microtubules were localized relatively few at the DNA dense region through stage 2 to 4. In stage 4 macronucleus, the macronuclear microtubules were radial localized from the DNA sparse region to the elongated side of macronucleus. These observations suggest relationship between distributions of macronuclear microtubules and DNA in amitotic dividing macronucleus.

For the explanation of this correlation between dense microtubules and sparse DNA, two models have arisen. The first model, the microtubules have over taken the region in macronucleus and interfered DNA to distribute in this region. The second model, DNA was moved out from this region indirectly by the microtubules. For the second model, the chromatin body or the granular structure of chromatin has been observed to localize on macronuclear microtubules (4), suggesting the interaction between microtubules and DNA.

Anti-microtubule drug treatment

To investigate the characters of microtubules in macronuclear division, dividing cells were treated with anti-microtubule drugs, colchicine, benomyl, nocodazole, and taxol. Colchicine treatment disrupted both the macronuclear microtubules (1) and cytoplasmic microtubules. Nocodazole treatment and benomyl treatment both disrupted the macronuclear microtubules and cytoplasmic microtubules. However, in the microtubule disrupting process, the benomyl treatment showed a difference in its effect. The benomyl treatment disrupted the macronuclear microtubules before the cytoplasmic microtubules while the nocodazole and colchicine treatment disrupted both the macronuclear and cytoplasmic microtubules almost simultaneously. This result suggests that the macronuclear microtubules and cytoplasmic microtubules are different in their biochemical properties. The taxol treatment seemed to promote the localization of microtubules adjacent to the macronucleus. This could be observed through the macronuclear division. The unique localization of microtubules was observed in the macronucleus before the elongation. In stage 1, the microtubules localize spiral swirling around the macronucleus. It would be very interesting to study how these microtubules are organized spiral and their biological properties in the macronuclear division.

The localization of microtubules in *Tetrahymena* is very unlike to the known microtubules involved in nuclear division in other species. The morphology of macronuclear division looks similar to the nuclear division of the fission yeast, though the localization of microtubules is far different (3). The revealing of the localization of microtubules in macronuclear division may provide a novel model of nuclear division or the division of the intracellular compartment. There is still much mystery that how these microtubules function in macronuclear division, how they are organized, and how they are regulated through the macronuclear division. It is interesting that no other structures, for example actin or the contractile structure, has not yet been observed in the macronuclear division. The microtubule is the sole structure for macronuclear division. Further studies on the macronuclear microtubules and microtubule binding proteins of *Tetrahymena* would make clear the machinery of macronuclear division.

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