

Abstract

In most organisms, microtubules are involved in the migration of the pronuclei during fertilization. Fertilization process of *Paramecium caudatum* includes two stages of nuclear migration. One is a selection of meiotic products. The other is a nuclear exchange. Microtubules may be involved in these nuclear behaviors but the roles of microtubules on these events of *Paramecium* were not yet fully elucidated.

In the part 1, to ascertain the dynamics of microtubules at the stage of the selection of meiotic products in *P. caudatum*, the localization of microtubules was analyzed by indirect immunofluorescence using a monoclonal anti- α -tubulin antibody. After meiosis, four haploid meiotic products are formed in a conjugating cell. The immunofluorescence showed that the cytoplasmic microtubules appeared between some meiotic products and the paroral region, and then surrounded a meiotic product survived in the paroral region. Furthermore, the nuclear movement to the paroral region was inhibited by injection of the anti- α -tubulin antibody and all the meiotic products out of the paroral region were degenerated. These results suggest that the cytoplasmic microtubules are essential for the migration of meiotic products to the paroral region.

In the part 2, to investigate the possible roles of microtubules on the behavior of pronuclei during the nuclear exchange in *P. caudatum*, I observed the localization of microtubules in relation to the pronuclear behavior by laser scanning confocal microscopy and the detail of the organization of microtubules by transmission electron microscopy. The survived meiotic product divides once and produces the migratory and the stationary pronuclei. The migratory pronucleus was surrounded by the cytoplasmic microtubules, and seemed to be pushed to the cell junction by the cytoplasmic microtubules. Then the migratory pronucleus extended

through the cell junction into the partner cell. An electron-microscopic observation showed that the cytoplasmic microtubules assembled at the back of the migratory pronucleus and the intranuclear microtubules of the pronucleus aligned along the direction of the extension at exact moment when the migratory pronucleus was crossing the cell junction. Moreover, the transfers of migratory pronucleus was inhibited by injection of the anti- α -tubulin antibody. These results suggest that both cytoplasmic and intranuclear microtubules are necessary for the dynamic behavior of gametic pronucleus across the cell junction of conjugating pair.

MTOCs are involved in the assembly of microtubules. γ -tubulin specifically localizes at MTOCs and is a highly homologous protein in a wide variety of eukaryotic cells. In ciliates, the localization or the role of γ -tubulin related to nuclear behavior has not been studied.

In the part 3, in order to analyze the role of γ -tubulin on the nuclear behavior of conjugants in *P. caudatum*, I produced a polyclonal antibody raised against *P. caudatum* C-terminal- γ -tubulin (Pcg 3) and investigated the localization of γ -tubulin during conjugation, especially at the selection of meiotic products and the nuclear exchange. Fluorescence of γ -tubulin dots was able to be detected in the micronucleus at all stages of the cell cycle, while the macronucleus was only labeled with Pcg 3 during cell division. After meiosis, the dots stained by Pcg 3 were accumulated only around a survived meiotic product. During the nuclear exchange, the dots stained by the antibody were accumulated around the migratory pronucleus. The fluorescence of γ -tubulin was not observed around the stationary pronucleus. In the amiconucleate cell of conjugant, however, these dots were not accumulated, and the cytoplasmic microtubules were not assembled around the paroral region. The movement of the meiotic products and the nuclear exchange were inhibited by injection of Pcg 3. These results suggest that γ -tubulin localized in cytoplasm is essential for the behavior

of germinal nucleus at the stages of the selection of meiotic products and the nuclear exchange. I also suggest that the appearance of these cytoplasmic microtubule assemblies and accumulations of γ -tubulin around the paroral region are dependent on the existence of the germinal nuclei by using conjugating pairs between amiconucleate and micronucleate cells.