

General Introduction

Microtubules are hollow tubes that are assembled by polymerization of α - / β - tubulin hetero-dimers, and are found in an array of morphologically distinct structures in eukaryotes. Since α - and β - tubulins are highly homologous in a variety of eukaryotes. Microtubules play key roles in cell division, intracellular transport of organelles, morphogenesis, and cilia and flagellar motions (McIntosh, 1983). Fertilization is well known genetic processes and includes many events such as maturation of egg and sperm, acromosomal reaction, cortical reaction, nuclear fusion of both gametes and so on. Migration of the male and the female pronuclei is one of these very important events. The sperm centrosome nucleates a microtubule structure (sperm aster) and the microtubules of the aster elongate to the two pronuclei in most organisms (Hamaguchi and Hiramoto, 1980; Navara *et al.*, 1995). Then the male and female pronuclei migrate to the center of the zygote.

P. caudatum is a unicellular organism, and has two types of nuclei, a germinal micronucleus and a somatic macronucleus. The process of fertilization in unicellular organisms is called conjugation. Conjugation of *Paramecium* has two stages of nuclear movement. One is a selection of meiotic products (Wichterman, 1946; Sonneborn, 1954; Skoblo and Ossipov, 1968), and the other is a nuclear exchange before the formation of fertilized nuclei (André and Vivier, 1962; Inaba *et al.*, 1966; Jurand, 1976). These processes can be easily analyzed in *P. caudatum*. Because its conjugation can be induced highly synchronously, the pronucleus of living cell can be observed under the phase-contrast microscopy, and living-conjugating cells can be micromanipulated without any difficulties. As the same as fertilization of eggs with sperm in higher organisms, conjugation begins with specific cell adhesion. During conjugation in *P. caudatum*, the micronucleus undergoes meiosis to produce four haploid

nuclei (Fig. 1). One of the four nuclei lies in the paroral region around the degenerated oral apparatus and survives, while the other three nuclei degenerate (Wichterman, 1946; Sonneborn, 1954; Skoblo and Ossipov, 1968).

In an abnormal strain of *P. tetraurelia* in which all of the meiotic products degenerate, none of the four haploid nuclei get into the paroral region (Sonneborn, 1954). This suggests that the paroral region plays a crucial role for the survival of the one nucleus. Yanagi (1987) reported that when the prospective surviving nucleus located in the paroral region of *P. caudatum* was removed by micromanipulation, one of the prospective degenerating nuclei reached the paroral region and survived. This result suggests that there are no differences among the four post-meiotic products in the ability of moving to the paroral region and surviving. What mechanism is involved in movement of a prospective surviving nucleus to the paroral region? By using immunofluorescence, Numata *et al.* (1985) suggested that *Tetrahymena* filamentous protein (49-kDa protein) was involved in the selection of one meiotic product among four nuclei after meiosis in *T. thermophila*. Yanagi and Hiwatashi (1985) proposed the possibility that the microtubules play a role at the selection of the meiotic products. Their proposal was based on the inhibition of the nuclear movement by vinblastine, microtubule-disrupting drug, on *P. caudatum*. When cells in the second meiotic anaphase were treated with vinblastine, no meiotic products entered the paroral region and all the four nuclei became pycnotic and degenerated. However, morphological observation to support this idea was not provided in this report. In *T. thermophila*, microtubules were observed around the meiotic products during its movement to the junctional area after meiosis using a fluorescent microscopy (Gaertig and Fleury, 1992). The junctional area of *Tetrahymena* corresponds to the paroral region of *Paramecium*. However, the mechanism of the selection of meiotic products has not been

analyzed.

After the selection of meiotic products, the micronucleus that survives in the paroral region divides to form two pronuclei in *P. caudatum* (Fig. 1). One is a migratory pronucleus and the other is a stationary pronucleus, corresponding to male and female pronuclei in higher organisms. A reciprocal exchange of migratory pronuclei occurs through a small opening on the membranes of the junction between the two conjugating cells in the paroral region (Inaba *et al.*, 1966). Then, the migratory pronucleus fuses with the stationary pronucleus of the conjugating partner to form a fertilized nucleus. André and Vivier (1962) reported about the nuclear exchange of *Paramecium*. They observed that the migratory pronucleus of *P. caudatum* formed a pseudopodia-like figure as amoeba using electron microscopy, though the exact moment of crossing the cell junction was not revealed. In *P. multimicronucleatum*, Inaba *et al.* (1966) succeeded in observation of the migratory pronucleus at the exact moment of migration by electron microscopy. The pronucleus migrated through the small opening of the cell junction as a pseudopodial extension, and a bundle of fine fibrils were stretched through the construction from the main body of the pronucleus to the pseudopodial extension. This is the reason why they concluded that the migratory pronucleus seemed to make migration by amoeboid movement in *P. multimicronucleatum*. Moreover, Jurand (1976) reported using electron microscopy in *P. aurelia* that both stationary and migratory pronuclei contained numerous microtubules scattered, and short microtubules elements existed outside the migratory pronucleus. When the migratory pronucleus passes through the cell junction, the nuclear envelope of the migratory pronucleus forms discrete pseudopodia at the cell junction side of the pronucleus. Although microtubules were observed in or out the migratory pronucleus during the nuclear exchange, previous studies have agreed that the nuclear exchange occurs through an opening on the

membrane of the cell junction by amoeboid movement in *Paramecium* (Inaba *et al.*, 1966; Jurand, 1976).

In *T. thermophira*, the exchange of gametic pronuclei was blocked by inhibitor of microtubule assembly (Hamilton and Suhr-Jessen, 1980). In *Heliophyra eahadi*, numerous microtubules were observed around the prospective migratory pronucleus, ran in different directions and were associated with fuzzy coating materials (Lanners, 1980). The stationary pronucleus was also surrounded by the microtubules, but the amount of the microtubules was less than these around the migratory pronucleus. In *T. termophila*, Orias *et al.* (1983) showed that the migratory pronucleus is surrounded by microtubules. Then the microtubules become more densely packed, and the migratory pronucleus acquires the biconvex lens shape. Finally, the junction is locally disrupted, and the pronucleus is pushed by the cytoplasmic microtubules through the junction into the conjugating partner. Furthermore in *T. termophila*, the immunological observation revealed that the prospective stationary pronucleus was surrounded by microtubules, while the prospective migratory pronucleus anchored to the cell junction and was surrounded by microtubules (Gaertig and Fleury, 1992).

In *T. termophila*, the 49-kDa protein was associated with the pronuclei prior to the third prezygotic division and persists until the fertilization (Numata *et al.*, 1985), and colocalized with microtubules (Takagi *et al.*, 1991). A fenestrin and a *Tetrahymena* Ca²⁺-binding protein of 25 kDa (TCBP-25) localized around the stationary and the migratory pronuclei during the nuclear exchange (Nelsen *et al.*, 1994; Hanyu *et al.*, 1995). Thus these proteins are thought to be involved in the pronuclear behavior. However, no immunological study about the nuclear exchange during conjugation of *Paramecium* has been carried out, and little is known about what kinds of proteins work on the nuclear behavior. What are the roles of microtubules involved in the behavior of

the pronucleus during conjugation of *P. caudatum*?

Ciliates do not have centriole and special organization such as centrosome and spindle pole body, except for basal bodies required for duplication of cilia. In eukaryotic cells, the number, polarity and organization of cellular microtubules are controlled by the MTOCs. γ -tubulin is a highly conserved member of the tubulin family, and involved in microtubule nucleation. γ -tubulin localizes to the MTOCs such as centrosomes in animal cell (Stearns *et al.*, 1991; Zheng *et al.*, 1991), spindle pole bodies of fungi (Oakley *et al.*, 1990; Horio *et al.*, 1991), and acentriolar spindle poles of land plants and mouse oocytes (Liu *et al.*, 1993; Gueth-Hallonet *et al.*, 1993; Palacios *et al.*, 1993). Microinjection studies on mammalian cells with an antibody raised against γ -tubulin have provided *in vivo* evidences for a role of γ -tubulin in microtubule nucleation (Joshi *et al.*, 1992). A primary location of γ -tubulin is toward the minus end of microtubules. Immunogold-labelling, however, indicates that some γ -tubulin localizes along the microtubules in both plant and animal cell (Liu *et al.*, 1993; Lajoie-Mazenc *et al.*, 1994; Hoffman *et al.*, 1994). For example, these γ -tubulins are involved in the assembly of the microtubules forming the midbodies of animal cell (Julian *et al.*, 1993), and distribution of cortical γ -tubulin has also been noted in mouse and *Xenopus* oocytes (Palacios *et al.*, 1993; Gard, 1994). Recent immunological studies reveal that γ -tubulin localizes in micronucleus, the basal bodies of cirri, cilia and oral apparatus in interphase cell of *Euplotes octocarinatus*, *T. thermophila* and *P. tetraurelia* (Liang *et al.*, 1996; Ruiz *et al.*, 1998). In mitotic and meiotic micronuclei of *E. octocarinatus*, γ -tubulin localizes to both extremities of the spindle during anaphase, and then the separation spindles (Curtenaz *et al.*, 1997). Furthermore, in *P. tetraurelia*, inactivation of γ -tubulin genes led to inhibition of basalbody duplication and cell growth (Ruiz *et al.*, 1998).

Thus, microtubules are expected to play important roles in the migration of nuclei during conjugation in *Paramecium*. However, detail of the localization and the role of microtubules have not yet been elucidated.

In this thesis, I showed that the cytoplasmic microtubules are essential for the migration of meiotic products during conjugation, and both the cytoplasmic and the intranuclear microtubules are necessary for the nuclear exchange during conjugation. Moreover, I reported that the γ -tubulin located in cytoplasm is involved in the nuclear behavior during conjugation.

This study provides the basis for the mechanism of the conjugation of *P. caudatum*. I developed a tool that allows us to study the behavior of nucleus, the postconjugational differentiation, cell division, reproduction of oral apparatus and so on.