Abstract

In *Paramecium*, when cells of the odd (O) and even (E) complementary mating types are mixed together under appropriate conditions, they undergo massive agglutination, which eventually leads to conjugation. This agglutination, which results from syngen-specific cell-to-cell sexual recognition, refers to the mating reaction (Sonneborn 1937). Gilman (1954, 1962) reported 16 syngens (i.e. sibling species, groups of complementary mating types) in *P. caudatum*. The substances involved in the mating reaction are called mating-type substances. They are thought to exist on the ciliary membranes of the ventral surface of highly mating-reactive cells (Cohen and Siegel 1963; Hiwatashi 1961), and assumed to be proteins (Kitamura 1988). No one has so far succeeded in identifying them from the cilia, while the biosynthetic processes of both mating-type substances are not known. The research on mating types and mating-type substances provides beneficial investigations on the foundation and evolution of the species, one of the most important and general biological problems in *Paramecium*. Moreover, mating reaction between complementary mating-type cells in *Paramecium* is one of the best models in studying inter-cellular interaction and sexual recognition of unicellular organism.

Determination and inheritance of mating types in *P. caudatum* have been extensively analyzed by inter-syngenic

crosses, revealing that three genes (i.e. *Mt, MA,* and *MB*) are involved in these events (Tsukii and Hiwatashi 1983). *Mt* is epistatic to *MA* and *MB* and determines the syngen-specific E mating type. *MA* and *MB* are expressed only in the recessive homozygote (*mt/mt*) at the *mt* locus and determine the O mating-type specificity of each syngen. However, the relationship between these three genes, and O and E mating-type substances is not known.

To identify the mating-type substances, five monoclonal antibodies (i.e. OmA, OmB, OmC, OmD and OmE) had been isolated and they specifically inhibited the mating reactivity of all strains belonging to O mating type of syngen 3 (O^3) without ciliary immobilization (Azuma et al. 1996). The localization of the antigens on the ventral surface cilia of the O^3 cells was completely consistent with the localization of the mating-type substance so far postulated (Hiwatashi 1961). Furthermore by indirect immunostaining, the antigens were detected only in the cilia of the mating-reactive cells. These results strongly suggested that these monoclonal antibodies were raised against the O mating-type substances, although direct identification of the antigen molecules using these monoclonal antibodies has not yet been achieved (Azuma 1999). Using these antibodies, Kumakura (1997) examined antigens in the different culture and clonal age, and found that the antigens were highly associated with the mating reactivity of the cells used. Unexpectedly, she

found that there were substances recognized by these monoclonal antibodies in E^3 cells, but she did not determine the cellular localization of them. This result was inconsistent with the O mating-type specific inhibiting effects on the mating reactivity of these monoclonal antibodies, and raised two questions. One is whether these antibodies were really raised against the O mating-type substances. The other one is what is the molecular nature of the antigens detected in E^3 cells.

In the part I, I directly proved that the O^3 mating-type substances exist in the mating-reactive E^3 cytoplasm. At first, I determined that the detection of the antigen as dot-blot signals was associated with the mating reactivity of O^3 cells. This result confirmed the previous studies by Kumakura (1997). Then, the cellular localization of the substance detected by these antibodies was clarified by both dot-blot analysis and indirect immunostaining. The antigen was detected in E^3 cytoplasm but not on its cilia. Comparable results were obtained among other 3 syngens. Furthermore, I discovered that O^3 mating-type substances were contained in E^3 cytoplasm. These results strongly support the precursor hypothesis that O mating-type substance is a precursor molecule of E mating-type substance (Butzel 1955).

In the part II, in order to identify mating-type substances, a new monoclonal antibody, XomO, was isolated. In the isolation

of this antibody, mating-reactive membrane vesicles reconstituted from O^3 cilia were used as antigen to obtain more varied epitopes. The monoclonal antibody XomO inhibited the mating reactivity of both O^3 and E^3 mating types. The unusual characteristic is the localization of the antigen, which particularly exists only on the root portion, but not on the whole length of the ventral surface cilia. Moreover, the antigen was also correlated with the mating reactivity of both mating-type cells. These results suggest that the antigens of antibody XomO are substances involved in mating reaction. Furthermore, XomO had syngen specificity and did not inhibit the mating reactivity of both mating types of syngen 1, syngen 5, syngen 6, and syngen 12. This result suggests that the antigens are the mating-type substances, which undergoes conformational change on the cilia.