

General discussion

Excreted gamones and cell-bound mating-type substances in ciliates may have different characteristics. In order to attract complementary mating-type cells on the appropriate condition, gamone excretors have to excrete continuously a large amount of gamones which are always diluted in the environment. From the point of economy, relatively stable and small molecules like amino acid derivatives appear suited to be excreted gamones. Most of so far isolated gamones in contemporary ciliates, such as blepharhormone 1 in *Blepharisma japonicum*, gamones 1, 2, 3, and 4 in *Euplotes octocarinatus*, and Er-1, Er-2 and Er-10 in *E. raikovi* are small proteins. Cell-bound mating-type substances may be less constrained. Since they are carried on the cell, smaller amounts of them may suffice for interaction with the complementary cells. In addition, they are better protected and easier to form stable conformation in the cellular environment. Thus cell-bound mating-type substances are freer in increasing their size as well as their complexity to exploit larger variability and specificity. In this study, I directly proved that mating-reactive O³ mating-type substances exist in E³ cells expressing E³ mating-type substances on their cilia and supported the hypothesis that O mating-type substances are precursor

molecules of E mating-type substances. The results obtained with monoclonal antibody XomO also suggest that there are common domains between O and E mating-type substances. It may be the case in *P. caudatum* that O and E cells may use the same molecule to synthesize O and E mating-type substances with different conformations. To gain mating reactivity of O and E mating-type substances, they may expose different mating-reactive domains of O and E mating-type substances.

Immunological approach is the best way to identify and monitor the biosynthesis pathway of a molecule in a cell. Relying on monoclonal antibodies whose antigens are suggested to be O mating-type substance of *P. caudatum*, cellular localization of mating-type substance was visualized for the first time. Moreover, the detection of antigens in the E cells triggered the study on the relationship between O and E mating-type substances. The fact that mating-reactive O mating-type substance exists in E cytoplasm expressing E mating-type substance was proved directly and gave us a more clear understanding about O and E mating-type substances. Using monoclonal antibody XomO, no matter what the antigen molecules may be, the special localization of antigen on a cilium provides us valuable information on transporting pathway on a cilium. The event of transporting cargoes along a cilium is a

dynamic changing process but not a simple loading-unloading operation. Although we know that the cells become mating reactive under a slightly starved condition, but the exactly cues to inducing mating reactivity are not known. Using these monoclonal antibodies, the answers of it and what are effectors involving this event may be monitored under varied conditions.