MICRONUCLEAR FUNCTION DURING VEGETATIVE GROWTH IN *TETRAHYMENA THERMOPHILA*. I. INDUCTION AND OBSERVATION OF AMICRONUCLEATE.

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A ciliate has two kinds of nuclei, macronucleus and micronucleus(MIC) in a cell. MIC, once believed to be inactive, plays some role during vegetative growth. In *Tetrahymena thermophila*, amicronucleate cells never give rise clones, suggesting critical role of MIC.

It was reported that cell population of very aged strain called star strain(*), C* in this case, contains irregular shaped cells which were amicronucleate and lack oral apparatus(OA). We confirmed those results on C* and, moreover, found that even ciliary rows disappeared. The same results obtained from other aged strains A* and B*, suggesting this is general character of star strains. To know whether this phenomena depend on the absence of MIC irrespective of age of cells, we induced amicronucleate cells in young clone by treatment with an antitubulin drug nocodazole. It was easier to determine the presence or absence of the MIC in young cells compared to star strains which have very small and variable size of MIC. We also found loss of OA, then loss of ciliary rows in young amicronucleate cells. Amicronucleate lost its OA within about 8 hours. Destruction of OA began first in undulating membrane. Long term observation of singel cell showed OA disappeared even without division.

Our results clearly show the critical role of the MIC during vegetative growth; maintenance of cortical structure, especially of OA.

EFFECTS OF HEAT-SHOCK ON REGENERATION OF AMICRO-NUCLEATE FRAGMENTS IN THE CILIATE PSEUDOUROSTYLA LEVIS.

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It has been already reported that in multinuclear ciliate Pseudourostyla levis micronuclei may share some important role for maintenance of normal oral structure (Takahashi, 1988; Takahashi & Suhama, 1991). Nevertheless, the essential somatic function of micronuclei has hardly been clarified yet. To solve this problem, effects of heat-shock treatment on regeneration of amicronucleate (ami) fragments were examined in the present work. In the experiments, many dividing cells were selected from exponentially growing culture at 23 °C, and the cells were transected into two fragments at posterior to the mouth at 2h after the completion of cell division. These fragments were exposed to the heat-shock treatment at 38.5 °C for 20min in the water bath. Then, they were fixed at intervals of 30 min and impregnated with protargol technique. When the fragments were not exposed to heat-shock treatment, regeneration of micronucleate (mi) fragments completed for about 5h and that of ami ones also finished within 6h after the transection. On the contrary, completion of regeneration delayed for about 4-5h in heat-shocked mi fragments and ami opimers. Almost all of the heat-shocked ami promers did not undergo regeneration until 10h after the transection. The problem as to why only heat-shocked ami promers could not respond to the injury is remained to solve in the future.

THE STURUCTURE OF ORAL APPARATUS IN LH AND RH CELLS OF GLAUCOMA (CILIOPHORA)

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Living left handed (LH) and right handed (RH) cells of Glaucoma scintillans were extracted with Triton X-100 by adding glutaraldehyde simultaneously. Their oral apparatuses (OAs) and oral primordia (OPs) were taken out from cell surface lamina and observed with scanning electron microscope. The mature OA was usually isolated as a bowl-like mass in which three membranelles (M1-3) and undulating membrane (UM) were included, at 10.0 % Triton X-100. The structural pattern of cytoskeleton connecting among M1-3, the arrangement of basal bodies of M1-3 and UM, and the deep fiber bundle tapering from the base of OA were visible from the outside of the bowl. The sculpture was observed at the anterior tip of each membranelle. Six basal bodies separated from the anterior end of M2 on the way of development and made a sculpture corresponding to X-body. The pattern of cytoskeletal frame work in inverted OAs of LH cells was basically almost the same as that of RH cells except for the fact that the former rotated about 180°.

IMMUNOELECTRON MICROSCOPE DETECTION OF THREE POLYPEPTIDES IN THE CYST WALL OF THE CILIATE, *HISTRICULUS CAVICOLA*.

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Ultrastructural studies of the ciliate, *Histriculus cavicola* have clarified that the cyst wall of the ciliate are made up of three layers, ecto-, meso- and endocyst, from the outer surface. Analysis using SDS-PAGE have suggested the presence of at least 8 specific polypeptides in the cyst wall. Immunoelectron microscopically, ectocyst was recognized by an antiserum against 70KD polypeptide, one of the 8 polypeptides. The antiserum was proved to be mono-specific for the polypeptide by Western-blot. By an antiserum against 52KD polypeptide, which was mono-specific for the polypeptide, the inner area of mesocyst was decorated. Endocyst was recognized specifically by an antiserum against 190KD polypeptide, which was mono-specific for the polypeptide. Structures of about 1000 nm long and about 100 nm wide, containing electron dense thin disks, were formed in the cells of early stage of encystment. The structures have been considered as ectocyst precursors. The disks in the structure were decorated by anti-70KD antiserum, indicating that the bodies are indeed precursors of ectocyst. Tubular bodies of about 100 nm in diameter with varying length, containing thin filamentous materials, were found in the cells of mid to late stages of encystment. They have been presumed to be the mesocyst precursors. Because the anti-52KD antiserum decorated the tubular bodies, they may be the mesocyst precursors. Small vesicles of about 300 nm in diameter, containing amorphous materials, were found in the cortical cytoplasm of the cells in late stage of encystment. These vesicles have been supposed to be precursors of either endocyst or granular layer. Immunoelectron microscopy using anti-190KD antiserum demonstrated heavy deposits of gold particles on the vesicles, indicating them as endocyst precursors.