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Artificial induction of autogamy in *Paramecium bursaria*

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SUMMARY

Autogamy in *Paramecium* is a sexual process occurring in a single cell. Autogamy brings about complete homozygosity in the progeny and simplifies genetic analysis. As autogamy has not been found to occur naturally in *Paramecium bursaria*, artificial induction of autogamy would be useful for genetic studies. I attempted to induce autogamy in *P. bursaria* by treating the cells with methyl cellulose, because methyl cellulose has been reported to induce not only autogamy in *P. caudatum* but also selfing conjugation in some species of *Paramecium* (*P. bursaria*, *P. caudatum*, *P. tetraurelia* and *P. multimicronuclatum*). When isolated single cells of *P. bursaria* were treated with 1.25% methyl cellulose, most of the single cells underwent micronuclear changes without cell adhesion. This suggests that the cells underwent autogamy. Treatment with methyl cellulose for at least 3 h was necessary for the induction of autogamy. Cytological observations indicated that micronuclear processes in the methyl cellulose-induced autogamy were substantially similar to those in natural conjugation following the mating reaction and that ventral cilia in the autogamous cells degenerated. The observations show that methyl cellulose induces autogamy with normal micronuclear processes in *P. bursaria*. The induction of autogamy will facilitate genetic studies in *P. bursaria*.

Infection process for symbiotic *Chlorella* in *Paramecium bursaria*

Yuuki KODAMA and Masahiro FUJISHIMA (Biol. Inst., Fac. of Sci., Yamaguchi Univ.)

SUMMARY

Cells *of Paramecium bursaria* harbor several hundred symbiotic *Chlorella* in their cytoplasm. *Chlorella*-free aposymbiotic cells can be easily reinfected with *Chlorella* isolated from symbiotic cells. However, the processes of digestive vacuole formation in the host cell and timing of the escape of *Chlorella* from the digestive vacuole have not yet been clarified. By using a pulse label (1.5 min) and chase with bromphenol blue-stained *Saccharomyces cerevisiae* or isolated *Chlorella* at 25ºC, we found that both acidification of the digestive vacuole and digestion of *Chlorella* in the vacuole occurred at an earlier time than in *P. multimicronucleatum* and *P. caudatum*.

Endosymbiotic algae of *Paramecium bursaria* I Isolation and establishment of axenic strains

Shin-ichiro KAMAKO, Ryo HOSHINA and Nobutaka IMAMURA
(Sci. and Eng., Dept. Biosci. and Bioeng., Ritsumeikan Univ.)

SUMMARY

Cells of green paramecium, *Paramecium bursaria*, have several hundred endosymbiotic algae. To investigate the relationship between *P. bursaria* and its symbiotic algae, we attempted to isolate the algae in axenic condition. Algal cells from the paramecia were spread onto C agar medium which contained only nitrate as a nitrogen source. Colonies which seemed microscopically free from bacteria grew poorly on the medium, and colonies growing well were contaminated with bacteria. Colonies that seemed free of bacteria were grown on C + peptone agar medium and the resulting clones were concluded to be axenic from the results of DAPI stain, culture tests for bacterial contamination, and denaturing gradient gel electrophoresis (DGGE) –PCR method. Three axenic strains were established from three *P. bursaria* strains and all strains were unable to grow on C agar medium. Thus, axenic symbiotic algae of *P. bursaria* are not able to use nitrate for growth.

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Endosymbiotic algae of *Paramecium bursaria* II Phylogenetic position inferred from 18S rDNA

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SUMMARY

Small subunit ribosomal RNA gene sequences of endosymbiotic green algae originating from three Japanese *Paramecium bursaria* were analyzed. The DNA contains three group I introns (one IE and two IC1), which results in an unexpected length of 3271bp. There was no substitution in the DNA among the symbionts analyzed here. The phylogenetic tree (exon only) indicates that the symbionts belong to *Chlorella* sensu stricto (Huss, V. A. R., Frank, C., Hartmann, E. C., Hirmer, M., Kloboucek, A., Seidel, B. M., Wenzeler, P., and Kessler, E. (1999) J. Phycol. 35, 587-598), and are genetically closer to the *C. vulgaris* group (including *C. vulgaris*, *C. lobophora* and *C. sorokiniana*) than to *C. kessleri* as previously proposed (Takeda, H. (1995) Phytochemistry 40, 457-459).

Endosymbiotic algae of *Paramecium bursaria* III Utilization of nitrogen compounds

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SUMMARY

Utilization of nitrogen compounds by an axenic endosymbiotic alga isolated from *Paramecium bursaria* was investigated. Since higher plants and algae generally have enzymes for nitrate metabolism, they can use nitrate for their growth. However, the axenic symbiont required peptone and was not able to grow in C medium in which nitrate was the only nitrogen source. Therefore, the symbiont seemed to differ from other plants and *Chlorella* spp. in nitrogen requirement, and utilization of amino acids and ammonium ions was therefore examined. We found that amino acids such as L-Ser, L-Arg and L-Glu, as well as ammonium ions could be used by the symbiont for its growth. Free-living *Chlorella kessleri* does not use such organic nitrogen sources. These results suggest that the symbiont might lack a part of the metabolic pathway from nitrate to ammonium ion. We therefore measured its nitrate reductase activity and no activity was detected.

Changes in nucleoid and cell wall antigen during differentiation to the reproductive form of *Holospora obtusa* of the ciliate *Paramecium caudatum*

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SUMMARY

The macronuclear endosymbiotic bacterium *Holospora obtusa* differentiates from the infectious form to the reproductive form after re-infection to the macronucleus of the host *Paramecium caudatum*. We examind changes in the nucleoid structure and DNA content in the differentiation process to the reproductive form. As a result, it was suggested that the bacterium started DNA synthesis at 12-24 hr after re-infection and that the fluorescence intensity of bacterial DNA increased twice and four times as much as that of the infectious form and the reproductive form, at 30 hr after re-infection, respectively. We also examined changes in localization and amount of the cell wall antigen by immunofluorescence microscopy and immunoblotting with a monoclonal antibody against cell wall antigen. The antigen localized uniformly at the cell wall of the infectious form, but its localization changed in patches at 24 hr after re-infection. Immunoblotting showed that the antigen was not detected in the bacterium at 30-36 hr after re-infection, but it was unclear whether the antigen temporarily disappeared or an epitope of the antigen was not recognized by the antibody.

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Infection of endonuclear symbiotic bacterium *Holospora obtusa* enhances *dad1* gene expression of the host *Paramecium caudatum*

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SUMMARY

The Gram-negative bacterium *Holospora obtusa* is a macronucleus-specific symbiont of the ciliate *Paramecium caudatum.* Differential cDNA display analyses showed that *P. caudatum* changes the gene expressions after infection of H. obtusa. One of the cDNA clones showed 41% identical (63% similar) with *DAD-1* (defender against apoptotic cell death-1). *DAD-1* is one of programmed cell death suppressor gene product (Sugimoto, A., et al., 1995, EMBO J., 14, 4434-4441) and is a subunit of mammalian oligosaccharyltransferase (Kelleher, D. J., et al., 1997, Proc. Natl. Acad. Sci., USA, 94, 4994-4999, Sanjay, A., et al., 1998, J. Biol. Chem., 273, 26094-26099). Northern blot analyses showed that the expression level of the dad1 gene of the *H. obtusa*-bearing cell was the double of that of *H. obtusa*- nonbearing cell. When the infected *H. obtusa* was removed from the *H. obtusa*-bearing cell by treatment with penicillin, resulting cell recovered the expression level of the *dad1* gene in the *H. obtusa*-nonbearing cell. We show here that *H. obtusa* selectively enhances the dad-1 gene expression of the host *P. caudatum*.

Endonuclear symbiotic bacterium *Holospora obtusa* reversibly changes types of surface antigens expressed in the host *Paramecium caudatum*

Yoshimitsu NAKAMURA, Manabu HORI and Masahiro FUJISHIMA
(Biol. Inst., Fac. Sci., Yamaguchi Univ.)

SUMMARY

Holospora obtusa is an endonuclear symbiotic bacterium of the ciliate *Paramecium caudatum*. Infection of *Holospora obtusa* to the host endonucleus alteres gene expression to another serotype of the surface antigen (SAg) G-homolog in the host cell, or no SAgs are expressed after infection of *H. obtusa*. So we attempted to survey expression of SAg in the host cells both aposymbiotic cell (A-cell) and symbiotic cell (S-cell). The problem is whether the SAgG-homolog transforms in A-cell and S-cell using salt/alcohol extraction method. As a result, the SAg-like protein over 250 kDa in molecular mass was extracted from the A-cell. However, expression of SAgG-like protein could not be detected from the S-cell. Instead, S-cell specific SAg was expressed. Indirect immunofluorescence microscopy with a monoclonal antibody specific for the SAgG-like protein showed that the antigen appeared only on the cell surface membranes of the A-cell. Furthermore, S-cell specific SAg reversibly changed to that of A-cell specific SAg after eliminating *H.obtusa* from the host macronucleus.

Genetic analysis of morphological mutant in *Paramecium tetraurelia*

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SUMMARY

We isolated four trichocyst-nondischarge mutants of *Paramecium tetraurelia* by treatment with N-methyl-N-nitorosoguanidine. One clone of them was slow-growing mutant named Bf-4. The cells of Bf-4 showed a normal shape like wild-type cells in mass culture, but they became bigger and more spherical when they were cultured in isolated conditions. In isolation culture, the fission rate of Bf-4 was very low and some cells died. To analyze its genetic characteristic, F1 progeny cells were obtained after cross with the wild type. If conjugation process progressed normally, all F1 progeny ought to express the same phenotypes as wild-type cells. In this case, however, many progeny showed different phenotypes in each ex-conjugant. And most of them showed the parental phenotype. Some progeny showed the medium rate of cell division after conjugation. To analyze the cause of unexpected results of F1 progeny from crossing of Bf-4 and wild type, we induced autogamy in F1 progeny that showed the same phenotype in both ex-conjugants and a different phenotype. Autogamy progeny from F1 of the same phenotype segregated wild type and mutant characters. These

characters contain a trichocyst-nondischarge, a cell division rate and an abnormal shape. On the other hand, autogamy progeny from parental F1 showed the same characters of each F1 progeny. Now we are analyzing the rate of segregated phenotype in autogamy progeny of F1 that showed the medium rate of cell division.

The cloning and molecular analysis of *cnrC*$^+$ controlling behavior of *Paramecium caudatum*

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(Inst. Biol. Sci., Univ. Tsukuba)

SUMMARY

Internal Ca^{2+} concentration regulates the waveform and beat direction of flagella and cilia of various eukaryotes. In Paramecium, Ca^{2+}-dependent ciliary reversal depends on the Ca^{2+} influx through the voltage-gated Ca^{2+} channels on the ciliary membrane. However, little is known about the molecular mechanisms of the Ca^{2+} channels that control ciliary reversal. The *cnrC* mutant, which is one of *P. caudatum* mutants with a defect in the voltage-gated Ca^{2+} channel activity, neither produces Ca^{2+} action potentials nor responds to any depolarizing stimuli. Applying complementation method, the total genomic DNA of the wild type was individually digested with the restriction enzymes and then microinjected into a macronucleus of *cnrC* cells. Of five restriction enzymes, *Bam*HI and *Bgl*II did not cut the *cnrC*$^+$. Since the transformation ability was observed in the 2 to 4-kb size fraction, we made a genomic DNA library using 2 to 4-kb genomic DNA fraction, screened the library for clones which restored Ca^{2+} channel function of *cnrC*, and finally isolated a single clone. The plasmid contained the insert sequence consisting of 2025-bp. Among the 2025-bp DNA, the 1213-bp fragment from wild type possesses an activity to restore Ca^{2+} channel function of *cnrC*. This result suggests the wild type 1213-bp contain c*nrC*$^+$ gene.

Determination of germinal micronucleus division or degeneration in exconjugants of *Paramecium caudatum*

Noriko TAKA, Kazuyuki MIKAMI (EEC, Miyagi University of Education)

SUMMARY

In *Paramecium caudatum* each cell has a germinal micronucleus. There are four presumptive micronuclei in an exconjugant and it is generally believed that only one of these micronuclei remains and the other three degenerate before the first post-conjugational fission. However, we obtained contradictory evidence with immunostaining with α-tubulin antibody and DAPI staining: four micronuclei remained in most dividing cells regardless of nutritional condition, though their frequency of degeneration was higher in cells under continuous well-fed conditions (WF) than in cells that had been in starvation conditions (ST) for 48 hours after conjugation. At the first fission, only one micronucleus divided. This is an exconjugant-specific phenomenon, because two micronuclei can divide during the vegetative phase after nuclear transplantation. The dividing micronucleus was always located along with the central axis of the cell between the oral apparatus at the ventral side and the macronuclear anlagen at the dorsal side. After artificial removal of the dividing micronucleus at the first fission, each cell produced two daughter cells. These daughter cells divided a few times but, with a few exceptions, eventually disappeared. The cells were amicronucleate under WF and micronucleate under ST conditions. In conclusion, the three non-dividing presumptive micronuclei remain morphologically intact but in many cases lose their ability to divide under WF conditions. However, the occurrence of micronuclear division in some cells after micronuclear removal showed that the remaining micronuclei sometimes retain the ability to divide at the first post-conjugational fission.

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Modification pattern of cell size and its relationship with macronuclear DNA content in *Paramecium*

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SUMMARY

Cell size is similar in different animals, but the same body has cells of different size. The size of renal epidermal cells of the salamander depends on the ploidy. In 7 species of ciliate in 5 genera, log cell volume has been reported to be proportional to log macronuclear DNA content (Soldo, A. T., 1981, J. Protozool., 28, 377-383). Our data also show that cell size varies with the macronuclear DNA content in three species of *Paramecium*, *P. tetraurelia* (Pt), *P. caudatum* (Pc) and *P. multimicronucleatum* (Pm). When *Paramecium* cells were grown in a flask culture, it was found that 1) the cell size increased during the early half of the log phase and decreased during the late half, and 2) the cell size halved during the stationary phase. These phenomena were common to Pt, Pc and Pm. The turning point for cell size during the log phase was shown to be related to cell density in the culture. We found that the cell-size increase during a cell cycle occurred in two steps, the first at G_1 stage and the second at S phase. This suggests that the cell size of *Paramecium* is modulated in two ways: DNA-dependent modulation (DDM) and DNA-indepenedent modulation (DIM). We obtained preliminary data suggesting that the cell size halving during the stationary phase was a DDM type, but this needs to be studied further.

Histone modification and developmental regulation of sexual maturity in *Paramecium caudatum*

Naomi KIMURA (Department of Biology, Faculty of Science, Kanazawa University)

SUMMARY

Coordinated gene expression during mammalian development is thought to be regulated by histone modifications such as acetylation-methylation balance. In *Paramecium caudatum*, a maternal macronucleus is fragmented into some 40 pieces during conjugation. Microsurgical removal of the new macronucleus during the stages later than the 5th cell cycle produced many clones which regenerated the maternal macronucleus. These clones unexpectedly showed no mating reactivity for about 50 fissions, as if they became sexually immature, implying that 're-programming' of the maternal macronucleus did occur. This observation leads us to the assumption that normal 'programming' of the new macronucleus or a reset of aging occurs at this critical period. Here, we demonstrate that the initiation of the maternal macronuclear degeneration occurs at about the same fifth cell cycle using acridine orange staining. In addition, to investigate whether histone modification plays a crucial role in the expression of mating reactivity, we treated immature Paramecium cells with Trichostatin A (TSA) that is a histone deacetylase inhibitor. Resultant histone hyperacetylation by TSA-treatment leads to a partial recovery of mating reactivity, i.e. the appearance of sexually mature cells, although they were essentially still in immaturity period. This suggests that histone acetylation-methylation is involved in the

The changes in the ability of *Paramecium* cells to regenerate cilia according to clonal aging

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SUMMARY

The ability to regenerate cilia called ciliary regeneration is an important character in *Paramecium*. Ciliary regeneration is brought about in exconjugant cells to repair the deciliated area in the ventral surface of the cell. Ciliary regeneration is also observed in the cells that are artificially deciliated. However, it is unknown whether the ability to regenerate cilia is a deteriorative character in clonal aging or not. In this report we examined the ability to regenerate cilia in the artificially deciliated cells and compared the time course of regeneration and swimming velocity of cells after regeneration

of cilia between young and old Paramecium. First, to measure the time course of ciliary regeneration, we examined the correlation between swimming velocity and the length of cilia and found that it was proportional. Then, by using swimming velocity of cells after the treatment of deciliation, we made the profile of the time course of ciliary regeneration. The comparison of the correlation coefficients between young and old cells indicated that there were statistically significant differences in both KNZ and SOS strains. In both cases the ability of ciliary regeneration was reduced in old cells. On the other hand, the swimming velocity of cells after completion of ciliary regeneration that was measured at 24 hours after deciliation indicated that there were no statistical differences between young and old cells in both strains. Our results suggest that reconstructive activity of cilia is a deteriorative character but the locomotive activity of reconstructed cilia is not. Our findings would give a new point of view in the consideration of clonal aging and would provide a new experimental approach to understand the molecular mechanisms underlying clonal aging of the cell.

High speed video recording of ciliary reversal and cell body contraction in *Paramecium* induced by slow photolysis of caged calcium

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SUMMARY

Cell body contraction and ciliary reversal in *Paramecium caudatum* are controlled by intracellular calcium concentration. We injected caged calcium into *P. caudatum* cells and applied ultraviolet (UV) light to the cell continuously. The UV was filtered through neutral density (ND) filters to raise the intracellular calcium concentration slowly. When UV was applied through a 1/64 ND filter, the Paramecium cell began to contract just after the start of UV application and the contraction continued for about 4 s. Whereas, ciliary reorientation began 1.5 s after the start of UV application. The period required for ciliary reorientation was about 100 ms. In the case when UV light was applied through no ND filter, the Paramecium cell began to contract just after the start of UV application and finished the contraction after 0.3 s. Whereas, ciliary reorientation began 8 ms after the start of UV application. The period required for ciliary reorientation was about 100 ms as is the case of 1/64 ND filter. These results strongly suggest that (1) cell body contraction takes place in an intracellular calcium concentration dependent manner and (2) ciliary reversal takes place in an all-or-nothing manner in living *P. caudatum*.

Molecular cloning of the integral membrane protein IP39 of *Euglena gracilis*

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SUMMARY

Flagellates of the genus *Euglena perform* a characteristic movement called “euglenoid movement”. Intramembrane proteins called IP39, which are regularly and densely arranged in the plasma membrane of the pellicular strip have been implicated in this movement (Suzaki and Murata, 1997). We carried out PCR-based cDNA cloning of IP39 from *Euglena gracilis*. As a result, we found two types of cDNA (α- and β-types). The α- and β-type cDNAs consisted of 792- and 795-base pairs, respectively. Molecular weights of both types of IP39 protein were predicted to be 29 kDa, which is much smaller than the 39 kDa estimated by SDS-PAGE. Between α- and β-type cDNAs, differences were found over the whole sequence. However, between the deduced amino acid sequences, differences were restricted to the C-terminal region, except for two residues in the middle part. Predicted secondary structures of both types of IP39 suggest that they are multipass transmembrane proteins with two or four transmembrane regions.

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Analysis of proteins involved in the process of feeding in the heliozoon *Actinophrys sol.*

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SUMMARY

Actinophrys sol captures food organisms using axopodia that radiate from the spherical cell body. Beneath the cell surface of axopodia and the cell body, there are many extrusomes, which discharge their contents when prey make contact with the axopodial surface. A 40-kDa glycoprotein (gp40) is stored in the extrusome and is considered to play an important role in prey recognition and ingestion. Using Con A-affinity chromatography, gp40 was purified and its N-terminal amino acid sequence was determined. We cloned and sequenced a PCR product that was amplified using degenerate primers designed from the N-terminal amino acid sequence of gp40. The sequence of the PCR product was not identical to the amino acid sequence of gp40, but showed high identity with the sequence of TPPI (tripeptidylpeptidase I), one of the lysosomal enzymes. Antibodies were raised against the N-terminal amino acid sequences of gp40 and TPPI of *A. sol* Immunofluorescence microscopy and immunoelectron microscopy with these antibodies showed localization of the proteins. Gp40 was localized around the cell body and on the axopodia, especially in the extrusomes, and anti-TPPI antibodies recognized small vesicles and food vacuoles.

Phototaxis rhythms in *Volvox carteri*

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SUMMARY

It is well known that the alga *Volvox* shows positive phototaxis and swims in the direction of light. This movement, however, has not been analyzed quantitatively so far. First, the intensity of phototaxis was quantitatively determined by counting how many *Volvox carteri*, in a rectangular pool divided into light and dark regions, accumulated in the light area. Second, we examined the intensity of phototaxis shown by *V. carteri* maintained on a 12-hr light / 12-hr dark cycle. It was found that *V. carteri* showed positive phototaxis during the light period, but not in the dark period. The peaks of positive phototaxis and non-phototaxis were respectively around 8 hours after the light was turned on or off. The intensity data fitted a sine curve. Third, this entrained daily rhythm of phototaxis was tested to see if it persisted under constant light conditions. It was found that the rhythm persisted for at least 1.5 days under constant light. Now we have tested if it persists on a 1-hr light / 1-hr dark cycle. In this test we found that *V. carter*i on a LD=1/1 cycle behaved as if under constant light after a 12-hr light period, but as if under constant dark after a 12-hr dark period. This suggests the existence of a switching or hysteretic mechanism of phototaxis. These results strongly indicate that *V. carteri* has a circadian clock.

A stable and long term culture method for the branched Vorticellidae in the presence of green algae

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SUMMARY

The peritrich ciliate, *Vorticellida* sp., is usually cultivated with a nutrient extract like lettuce juice and Vita-shrimp infusion in the presence of a bacterium such as *Aerobacter*. However, when, *Carchesium* sp., one of the branched peritrich ciliates, is cultured, it sometimes proliferates as solitary organisms, not as branched ones. Furthermore, there is no report of laboratory culture of *Zoothamnium* sp., another species of branched vorticellidae. We have recently developed a method to cultivate *Carchesium polypinum* and *Zoothamnium procerius* in the presence of the green alga, *Chlamydomonas reinhardtii*, along with bacteria as food. These peritrich ciliates grew and proliferated stably and successfully for a long

period when *Chlamydomonas reinhardtii* was employed as a second nonaxenic food. A symbiotic green vorticellidae (*Vorticella chlorellate*) can be stably cultivated in the presence of small amounts of nutrient with a bacterium. This may be possible because of the intracellular presence of Zoochlorella .

Axenic cultivation and encystment induction of the ciliate *Tetrahymena* in a dead mosquito larva

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SUMMARY

The ciliate, *Tetrahymena* sp., which was found in a dead mosquito larva, grew well in lettuce infusion (0.5 g/1,000 ml) with a piece of poultry-meat (2 g/1,000 ml), and then some transformed to cysts at the stationary phase of growth. When the logarithmically growing cells were transferred to CFF (cell-free fluid) which was prepared by centrifugation (1,500 rpm, 5 min), pre-filtration (DP70), and Millipore-filtration (0.45 μm in diameter) of the cell suspension from a stationary growth phase, more than 40% of the cells transformed to the cysts. This suggests that a certain factor which is responsible for the encystment of the ciliate is included in the CFF. In order to purify such an encystment-inducing factor, it is important that the ciliate is axenically cultured with any medium. Thus, the ciliate which had been cultured by lettuce infusion with a piece of poultry-meat, was washed 8 times with an inorganic salt solution SMB-II, and then transferred to PY medium (1% proteose peptone, 0.5% yeast extract) containing antibiotics (4,000 U/ml penicillin G, 200 μg/ml streptomycin sulfate and 5μg/ml vancomycin), and was maintained at 23±1°C. We, however, could not prevent the growth of contaminating bacteria by such a culture method. Thus, we washed the ciliate with SMB-II, and then applied 100 μl cell suspension on a 0.9% PY agar plate containing antibiotics, and then the PY agar plate was maintained at 23±1°C. Two days later, a pure colony of the ciliate on the agar plate was transferred to a 10 ml Erlenmeyer's flask containing 2 ml PY medium with antibiotics. As a result, we succeeded in axenically culturing the ciliate. We will try to purify the encystment-inducing factor from the axenic culture in the near future.

Encystment-suppressing factors in *Colpoda* sp

Chiharu YAMASAKI and Tatsuomi MATSUOKA
(Inst. Biol, Fac. Sci., Kochi Univ.)

SUMMARY

In *Colpoda* sp. isolated from fallen leaves, encystment induced by suspending the cells in a saline solution (1 mM $CaCl_2$, 1 mM KCl, 5 mM Tris-HCl, pH 7.2) was cancelled by bacteria suspended in the surrounding medium. Continuation of uptake of polystyrene latex particles into the food vacuoles caused a slight suppression of encystment. When the cells of *Colpoda* containing a large number of food vacuoles filled with bacteria were transferred into a fresh saline solution, the contents of food vacuoles were promptly expelled prior to encystment. Supernatant obtained from bacterial suspension completely suppressed encystment, and dialysis (MWCO: 10,000) of the supernatant reduced encystment-suppressing effect. The present results indicate that the essential factors for suppression of encystment were certain components derived from bacteria and continuation in simple formation of food vacuoles.

Pre-treatment conditions of the soil samples to the estimation of biomass of soil ciliates using the MPN method

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SUMMARY

The purpose of the work is to clarify the role of soil ciliates in the soil ecosystem, and to establish a method for the evaluation of soil environment by using ciliates' fauna and biomass. Takahashi et al. (2002) suggested that the soil ciliates' biomass could be estimated by using the modified MPN method. They did not, however, examine the most efficient method for separating the soil ciliates' cysts and active forms from the soil particles. Either a vibratory mixer or an ultrasonic cleaner was used to disperse the soil particles. The survival ratio of the free-swimming ciliate, *Paramecium caudatum*, was more than 90% by any of these treatments. In contrast, when the soil samples were treated by using the ultrasonic cleaner, the number of the detected ciliates was less than half that detected using the vibratory mixer. Furthermore, it was suggested that 3-5 minutes of treatment using the vibratory mixer is more efficient in separating the soil ciliates from the soil particles than are the other treatments.

Dynamics of soil protozoa and bacterial population in upland soil with slurry application

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SUMMARY

Effect of manure application on soil protozoan fauna and bacterial populations was examined in the upland soil with 0, 60, 150 and 300t/ha of slurry application levels, which field was located in Natl. Agr. Res. Cent. Kyushu Okinawa Reg., Miyakonjo-city, Miyazaki prefecture. Soil samples were collected from the surface Kuroboku soil (0-15 cm) on June 5th 2003. Bacterial population was enumerated with the plate dilution method. Protozoan fauna was examined with a modified MPN (most probable number) method by Takahashi et al. (2003) to enumerate viable number of each identified taxonomical groups. In this study, the amoebae were categorized to 4 morphological groups. The ciliates were as identified species levels. The total count was examined in the flagellate. As increasing the slurry application levels, bacterial and fungi populations were significantly increased in 60, 150 t/ha plots. Flagellate, amoeba and ciliate number were also increased. The identified group number of amoeba was also increased in the slurry applied plots. The biodiversity of ciliate increased with slurry application levels, 3, 10, 11 and 14 species for 0, 60, 150 and 300 t/ha plots, respectively. The modified MPN method could be one of valuable tools to analyze the protozoan community structure in soil ecosystems to investigate effect of manure application or other agricultural activities.

Comparison of soil testatate communities under the various environments (I)

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SUMMARY

We conducted a preliminary study of enumerating soil testate amoebae by culture method (easy-to-use method) and direct counting method, and of species inventory by SEM observation. Soil samples were taken from the several environments around this conference space (Nippon Veterinary and Animal Science University) before four months ago. The soil sample were taken from 5 materials: 1) the dry gravel of bicycle-parking space of the university , 2) the moss on the *Castanopsis* tree trunk near bicycle-parking, 3) fresh fallen leaves of the *Castanopsis* tree, 4) the soil (A_1 layer) of the

plantation of the entrance turn in the front of the train station, 5) fresh fallen leaves (A_0 layer) of the *Zelkova* tree mainly in the plantation. The population of the tastacea included plasma body g^{-1} by culture method were 20, 667, 713, 806 and 1520 for the *Castanopsis* litter, the moss of the *Castanopsis* tree trunk, the *Zelkova* litter, the soil of the plantation and the dry gravel of bicycle-parking, respectively. The population of the tastacea included plasmabody g^{-1} by direct counting method were 36, 152 and 316 for the *Zelkova* litter, the soil of the plantation and the moss on the *Castanopsis* tree trunk, respectively. The other samples included only empty test. The following species were identified from the soil of the plantation with SEM observation: *Assulina* sp.; *Centropyxis* af. *minuta* Deflandre; *Centropyxis* sp.; *Cyclopyxis* sp. 1; *Cyclopyxis* sp. 2; *Plagiopyxis declivis* Thomas; *Trinema encherys* Dujardin. Also, the following species were identified from the moss on the *Castanopsis* tree trunk with SEM : *Centropyxis elongata* Penard; *Centropyxis minuta* Deflandre; *Centropyxis* sp.; *Cyclopyxis* cf. *eurystoma* Deflandre; *Euglypha compressa* Carter; *Euglypha cristata* Leidy; Euglypha *tuberculata* Dejardin; *Trinema encherys* Dujardin.

Symbiotic flagellate faunae of two termopsid termites from Laos

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SUMMARY

The termite family Termiosidae, consists of five genera, is known to have diverse symbiotic flagellate faunae. In this study, we investigated symbiotic flagellates from two termopsid species, *Archotermopsis* sp. and *Hodotermopsis sjoestedti* from northern Laos. Stained specimens of intestinal contents were observed on three colonies of the former and on four colonies from the latter. The materials were fixed with 1% OsO_4 and Schaudinn's fluid and subjected to protalgol staining. *A.* sp. possessed five flagellate species that belong to five genera, sharing *Trichomitopsis* and *Protrichonympha* with *A. wroughtoni* in northern India. It also shared four flagellate genera with *Zootermopsis* spp. in North America, suggesting these two host genera are closely related taxa. *Hodotermopsis sjoestedi* from Laos retained 20 species that belong to nine genera. The fauna was identical to that of populations from Japan, China, and Taiwan and was greatly different from that of *Archotermopsis* spp. and *Zootermopsis* spp. A preliminary analysis of host phylogeny based on COII gene showed good correspondence to the similarity patterns of symbiotic flagellate faunae.

Expression system of the conjugation-inducing substance (gamone 1) in the ciliate *Blepharisma japonicum*

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SUMMARY

Conjugation in the ciliate *Blepharisma japonicum* is induced by interaction between complementary mating-type cells, types I and II. When mature cells are moderately starved, they start expressing the conjugation-inducing substances, gamones. Gamone 1 produced by type I cells is a glycoprotein consisting of 272 amino acids and 7 sugars. Gamone 2 produced by type II cells is a small molecule that was identified as 3-(2'-formylamino-5'-hydroxybenzoyl) lactate. In this study, we precisely analyzed the structure of gamone 1 to speculate on the functions of gamone 1 in the conjugation-inducing process. The results showed that gamone 1 has several interesting motifs including four possible attachment sites of N-linked oligosaccharide and five phosphorylation sites. The prediction of secondary structure indicated that gamone 1 is composed of two domains, one of which consists of alpha-helices and beta-sheets, the other of only beta-sheets. We also estimated the tertiary structure of gamone 1 by the 3D-1D method, which showed that the tertiary structure of gamone 1 has significant homology to that of cathepsin B-like cysteine protease. We constructed an expression system of His-tagged gamone 1 by integrating mature gamone 1 cDNA with pQE60 vector (QIAGEN).

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The influence of wheat germ agglutinin on mating pair formation in *Paramecium caudatum*

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SUMMARY

Conjugation is a sexual process in ciliates induced by mixing complementary mating type cells. Previous studies indicated that concanavalin A (ConA) inhibits mating pair formation in *Paramecium caudatum*, and wheat germ agglutinin (WGA) inhibits mating pair formation in both *P. tetraurelia* and *P. primaurelia*. In the mating reaction, cells adhere at the anterioventral surface (holdfast union) and later the adhesion spreads over the whole ventral site (paroral union). It has been also shown that labeling of the cell by FITC-WGA starts at the anterioventral adhesion sites during the holdfast union stage and later spreads over the whole ventral site. The aim of this study was to investigate the mechanism of mating pair formation by using WGA and FITC-WGA in *P. caudatum* mating cells. A low concentration of WGA (above 1.5 μg/ml) inhibited mating pair formation. This indicates that the WGA binding sites are involved in intercellular adhesion and cell fusion at conjugation in *P. caudatum*. In conjugating cells at early stages (0-2h after mixing cells), appreciable staining was not detectable. But conjugating cells at the holdfast union stage began to be stained at the anterioventral adhesion site and later FITC-WGA staining spread over the whole adhesion site. After the mating pair separated, cells were stained only at the oral regions. At more advanced stages, appreciable staining was not detectable. It seems that WGA binding sites may be transported from the intracellular region to the cell membrane.

Comparison of the evolutionary distance among the syngens and the genetic species of the genus *Paramecium* using hsp70 sequences

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SUMMARY

The morphological species *Paramecium aurelia* includes fourteen biological (=genetic) species that are sexually isolated from each other. These biological species were initially defined as syngens, because they are sibling species which could be categorized previously only by mating tests. The mating-type groups of the other *Paramecium* species, such as *P. caudatum*, are also called syngens for the same reasons. Following Sonneborn's establishment of classification criteria based on isozyme analysis, the syngens of *P. aurelia* became recognized as genetical species. However, molecular markers for the classification of the syngens of the other *Paramecium* species is not yet established. Previously, we demonstrated that the relationships among the syngens of *P. caudatum* were not clear based on analysis of hsp70 sequences. In the present study, to investigate the evolutional relationships between the syngens and the biological species, we analyzed the cytosolic hsp70 genes from 44 strains of *P. aurelia* complex, *P. caudatum* and P. *multimicronucleatum*. We constructed phylogenetic trees for each set of sequences using ME methods. The resulting tree had shown that the divergent time among the biological species of *P. aurelia* complex is evidently earlier than that of among the syngens of *P. caudatum*.

A chimera theory on the origin of dicyemid mesozoans: Evolution driven by frequent lateral gene transfer (LGT) from the host to parasites

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SUMMARY

The phylogenetic status of the enigmatic dicyemid mesozoans is still uncertain. Are they multicellular protozoans or degenerate triploblastic animals? Presently, the latter view is favored. A phylogenetic analysis of 18S rDNA sequences placed dicyemids within the animal clade, and this was supported by the discovery of a Hox-type gene with a lophotrochozoan signature sequence. This molecular information suggests that dicyemid mesozoans evolved from an ancestral animal degenerately. Considering their extreme simplicity, they might have come from an early embryo via

neoteny. In spite of this molecular information, dicyemid mesozoans retain many protistan-like features, such as double-stranded ciliary necklace, tubular mitochondrial cristae, endocytic ability from the outer surface, and the absence of collagenous tissue, while they do not share noticeable synapomorphy with animals. In addition, the 5S rRNA phylogeny suggests a closer kinship with protozoan ciliates than with animals. If we accept this clear contradiction, dicyemids should be regarded as a chimera of animals and protistans. Here, we propose a new “chimera” theory: Dicyemid mesozoans are exposed to a continual flow of genetic information via eating host tissues, because of their absolute dependency on the host for nutrients. Consequently, many of their intrinsic protozoan genes have been replaced by host-derived genes through lateral gene transfer (LGT), implying that LGT is a key driving force in the evolution of dicyemid mesozoans.

Differentiation of mitochondria and structural change of mtDNA during early development in the mesozoan dicyemids

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SUMMARY

Mitochondria of mesozoan dicyemid carry minicircle DNAs, on each of which a single gene is encoded. Unfortunately, whether the minicircles reflect on a unique form of mitochondrial genome or a form of differentiated mtDNA is still an open question. In this study, a search for the original mtDNA different from the minicircles was carried out. I tried identification of such a mtDNA by two different methods: 1) Separation of the mtDNA through CsCl-Hoechst density gradient and 2) Southern blot analysis. The results obtained from these experiments showed that a very poor amount of high-molecular-weight (HMW) mtDNA exists in mitochondria of dicyemids as well as abundant minicircles. These two forms might correspond to differentiated mtDNA (minicircle) and undifferentiated original mitochondrial genome (HMW), respectively. Furthermore, in situ hybridization revealed that amplification and the subsequent dilution of the minicircle mtDNAs during early development do occur. These observations lead me to the following "amplification-dilution" model: only mitochondria in stem cells, such as axoblasts or germinal cells, preserve the undifferentiated mtDNA in a large size. In the early embryogenesis, amplification and rearrangement of the mtDNA would occur concomitantly with germline and soma differentiation, resulting in the formation of the numerous minicircles. This model inevitably predicts the lack of replication of the minicircles. As mitochondria increase their number by divisions, the amplified minicircles in somatic cells would be diluted, reflecting on the lack or faintness of in situ hybridization signal in somatic mitochondria such as those in the jacket cells.

Observation of fertilization and synkaryon in living *Tetrahymena* cells

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SUMMARY

During conjugation in *Tetrahymena thermophila*, many important nuclear events occur within a rather short time. These nuclear events have been defined by staining of chromatin only. In the present study, fertilization and synkaryon were observed in living cells. After nuclear exchange, both the migratory nucleus and stationary nucleus changed to spindle shapes and attached to the conjugation junction by one end of each nucleus. The nuclei contacted each other at regions near the junction and fused within 20-30 seconds to give rise to a synkaryon. The synkaryon detached from the junction and was positioned just anterior to the macronucleus. In the spindle-shaped synkaryon at interphase, two chromatin threads, derived from the gametic nucleus, lay parallel along the long axis of the nucleus. This figure looks like a dividing nucleus and is thought to be the nucleus undergoing fertilization. The synkaryon rounded and chromatin dispersed homogeneously just before the first postzygotic division. When the conjugation junction was partially destroyed under Ca-poor conditions, two synkarya sometimes contacted and fused to give a 4n nucleus. Fertilization involving three gametic nuclei has been reported. It seems that, in this species, many nuclei in the common cytoplasm could fuse at this stage. *Tetrahymena* is a good subject for the study of fertilization.

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Screening of novel myosins from *Tetrahymena*

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SUMMARY

The superfamily of myosins found in eukaryotic cells were known to contain at least 18 different classes. Members were classified based on the phylogenetic analysis of the head domains located at the amino terminus of the polypeptide. Using degenerated primers that had been designed from bases of class II myosin motor domain, 7 myosin cDNAs (MYO1, myo2-myo7) were cloned from *Tetrahymena* by RT-PCR. We extended 6 myosin nucleotide sequences (myo2-myo7) by 3'-, 5'RACE and inverse PCR. Moreover, we searched TIGR (The Institute for genomic research) *Tetrahymena thermophila* sequence BLAST Search (http://tigrblast.tigr.org/er-blast/index.cgi?project=ttg), using myo2-myo7 sequences. We found 12 myosin genomic DNAs in the *Tetrahymena* genome database. Five myosin cDNAs were identical with MYO1, myo3, myo4, myo5 and myo7, respectively. Seven myosin genomic DNAs were novel Tetrahymena myosins. myo2 and myo6 did not exist in this *Tetrahymena* genome database, because the database did not cover all *Tetrahymena* genome. Thus, we found that *Tetrahymena* has at least 14 myosin genomic DNAs. Moreover, 5 myosin sequences (MYO1, myo3, myo4, myo5, myo7) were aligned with those of other organism myosins, and produced a phylogenetic tree. In this tree, MYO1, myo3, myo4, myo5 and myo7 were not contained to 18 classes, although these 5 myosins were clustering a novel class. Therefore, we propose that the novel class containing MYO1, myo3, myo4, myo5 and myo7 refer to as class XIX myosin.

Localization of tetrin and tetrin-associated protein during cell division in *Tetrahymena*

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SUMMARY

To investigate the molecular mechanism of oral morphogenesis in ***Tetrahymena***, we prepared five monoclonal antibodies, HM12, HM04, HM03, HM54, and HM44, using isolated oral apparatus. A 12 kDa protein recognized with HM12 was localized in basal bodies in the oral apparatus and the cell surface. An antigen recognized with HM04 was localized in doughnut-like structures surrounding basal bodies. These localizations did not change during cell division. Three monoclonal antibodies, HM03, HM54, and HM44, reacted to 72 kDa, 78 kDa and 140 kDa proteins, respectively. In interphase cells, these three proteins were localized in the undulating membrane and three membranelles in the oral apparatus. During cell division, these proteins dispersed in the cytoplasm and were localized in old and new oral apparatuses. Interphase localizations of those proteins were similar to those of tetrin 1-4 which are components of the fibrillar network of the oral apparatus. To examine cross-reactivity among HM03, HM54, HM44 and tetrins, we purified tetrins from *Tetrahymena*. HM03 and HM54 reacted to tetrins, while HM44 reacted to 140 kDa protein in tetrin fractions. These results suggest that 72 kDa and 78 kDa proteins are tetrins and that 140 kDa protein is a tetrin-associated protein.

Homopolar doublets in *Tetrahymena thermophila*: induction of homopolar doublets and their conjugation

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SUMMARY

A cell of *Tetrahymena thermophila* has one oral apparatus. Sometimes a cell called a homopolar doublet occurs which has two sets of cell cortex with the same polarity. Conjugation of homopolar doublets has not been reported. As it has two oral apparatuses, a doublet could possibly conjugate with two other cells. If so, two conjugation junctions would influence the behavior of the nuclei, as the junction is important in normal conjugation. In this study, we devel-

oped an easy and quick method to transform normal cells to homopolar doublets. Conjugation of doublets was induced and we found that the doublet could conjugate with two other cells forming a triplet. Nuclear behavior was observed using Giemsa staining which reveals cell cortex structure and nuclei simultaneously. At the macronuclear anlagen stage, n, 2n and 3n micronuclei were found in one triplet but in other triplets all cells had 2n micronuclei. At the third prezygotic division, an anaphase micronucleus was observed attached at both ends to the two junctions. This suggests that the two products of the third division became migratory nuclei and the doublet cell contained no stationary nucleus. If normal fertilization in the two normal cells and fertilization between two migratory nuclei in the doublet cell occurred, then all cells would have 2n micronuclei.

Tetrahymena multifunctional proteins III: localization of hsp70 during conjugation

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SUMMARY

In *Tetrahymena*, a series of dynamic nuclear events occurs during conjugation, for example, elongation of micronuclei (crescent formations), meiosis, exchange configuration of micronucleus, fertilization, differentiation of macronucleus, and disappearance of the old macronucleus. These nuclear events are correlated to dynamic changes of microtubules and cytoskeletal structures. The 70-kDa heat shock protein (hsp70) is a highly conserved protein from bacteria to mammals, and is known to assist folding of immature proteins in normal condition and to repair abnormal proteins under stressed conditions, such as heat shock or chemical shock. To analyze functions of hsp70 during conjugation, we examined localization of hsp70 in Nonidet P40 (NP-40) extracted Tetrahymena cell model during conjugation using immnofluorescent staining with anti-human Hsp70 monoclonal antibody (3a3). Immunoblot analysis with 3a3 showed that the amount of hsp70 was more than that of the 70-kDa heat shock cognate protein (hsc70) in NP-40 extracted cell models prepared from cells during starvation, costimulation and conjugation. Immunofluorescent microscopy showed interesting localizations of hsp70 in (i) macronuclei and micronuclei during costimulation, (ii) elongated micronuclei during the crescent stage, (iii) macronuclei and micronuclei during meiosis, gametogenesis, and fertilization. Furthermore, hsp70 disappeared from the old macronucleus and appeared in the new micronuclei and macronuclei during macronuclear differentiation. These results strongly suggest that hsp70 plays crucial roles in nuclear events during conjugation.

Comparison of the nuclear protein composition between vegetative and cystic cells of the stichotrich ciliate, *Sterkiella cavicola*

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SUMMARY

In the stichotrich ciliate, *Sterkiella cavicola*, Himura (1993) has demonstrated a cyst specific 140 kD micronuclear polypeptide, supposing it as a protective peptide for micronuclear chromatin against environmental stresses. Gutierrez (1985) has reported in *Gastrostyla steinii* that cystic nuclear proteins are arginine-rich and has suggested that these arginine-rich proteins may induce cystic macronuclear chromatin condensation. Since then, however, only few biochemical data on the cystic nuclear proteins have been presented. In the present study, we tried to identify cyst specific nuclear proteins other than 140 kD polypeptide using S. cavicola. Cystic nuclei were separated from the nylon mesh filtrate of the cystic homogenate by Percoll gradient centrifugation. Vegetative cell nuclei were obtained by similar manner from Triton X-100 cell lysate. Nuclear proteins were separated by SDS-PAGE or 4M urea SDS-PAGE. Basic proteins were extracted and separated by acetic acid urea PAGE. The purity of the nuclear fraction was confirmed after staining with aceto-orcein or DAPI. All electrophoretograms demonstrated the existence of polypeptides specific to or rich in vegetative cell nuclear fraction but not in cystic nuclear fraction. Disappearance or decrease of these polypeptides during encystment might have functions as suggested by Himura (1993) or Gutierrez (1985), but the exact function of these peptides remains to be clarified.

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Sorocarp developmental stages of the aggregative ciliate *Sorogena stoianovitchae*

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SUMMARY

The sorocarp-forming ciliate *Sorogena stoianovitchae* is a rare instance of showing mullticellularity in ciliates and its life cycle is similar to that of the cellular slime molds, although both organisms have evolved independently. In fact, multicellularity has evolved many times even in the kingdoms, Monera and Protista. Recent discovery of the adherens junction and a β-catenin homologue in the cellular slime mold *Dictyostelium discoideum* has facilitated a special interest how *Sorogena* has developed its multicellularity. In this regard the sorocarp development is an interesting phenomenon for studying the strategy. Based on the morphological changes, stages of the sorocarp development are divided in the following distinct five stages; aggregation stage 1, aggregation stage 2, secretion stage, rising stage, completion stage. This division of these stages was consistent with that obtained by CHX treatment. To examine an influence of light induction, the sorocarp formation was analysed by changing the length of dark periods from 10 to 23 h. The tendency was observed that the longer the dark periods become, the larger the aggregates get in the beginning of light period, but 23 h dark period completely inhibited the progress from the aggregation stage 2 to the subsequent stages. These results suggest that a material responsible for aggregation is synthesized during the dark period, and that the aggregate in the stage 1 or 2 requires light stimulation for the transition from the stage 2 to the secretion stage.

Isolation of contractile vacuoles from *Amoeba proteus*

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SUMMARY

Contractile vacuole (CV) of *Amoeba proteus* is an osmoregulatory organelle. Electron microscopy revealed that a contractile vacuole of *Amoeba proteus* is surrounded by many small vesicles (about 0.1 μm), suggesting that these vesicles first collect cytoplasmic water and then fuse with each other to form a contractile vacuole. In immunoblot analysis, an antibody against a subunit of V_1 sector of V-ATPase recognized a 70 kDa polypeptide in crude extract of amoebae. The molecular mass was consistent with that reported for V-ATPase in other materials. Immunofluorescence microscopy and immunoelectron microscopy suggested the presence of V-ATPase in the membrane of the CV and that of small vesicles surrounding CV. We tried to isolate CV. When amoebae were centrifuged at 10,000×g for 10 min in 35% Percoll, cell fragments containing CV were obtained. CVs could be released from the cell fragments by homogenization. They were stained with anti-V-ATPase antibody, confirming that CVs could be successfully collected. This success opened a way to the biochemical analysis of CV functions.

Genotyping of *Naegleria* isolates from thermal waters in Japan

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SUMMARY

The free-living amoebae, possible aetiological agents of amoebic meningoencephalitis, appear to be ubiquitous organisms in a variety of thermal waters. The distribution of the pathogenic species is not well documented in relation to those of non-pathogenic. A total of 713 thermal waters, mainly from whirlpool baths and hot spring spas from 16 districts in Japan were examined the occurrence of the genus *Naegleria* that were tolerate ≥42°C. Approximately 38% of the water samples examined were positive for variety of amoebae species. By the use of PCR-RFLP and/or DNA sequencing of the internal transcribed spacer (ITS) regions of the rRNA gene, *Naegleria* isolates were clustered into several cladistic groups, including *N. lovaniensis*, *N. australiensis*, *N. philippinensis*, *N. italica*, *N. andersoni*, *N. clarki N. jamiesoni* and others. The 5.8S rDNA with flanking ITS sequences of the isolates gave high similarities with the pub-

lished sequences: *N. lovaniensis* (100%), *N. australiensis* (100%), *N. philippinensis* (98.1%), *N. italica* (97.9%), *N. andersoni* (99.7%), *N. clarki* (100%), *N. jamiesoni* (99-100%) and *Naegleria* sp. PNMA-1 (98-99%) of which scientific names have not yet been given. Those isolates closely related to known pathogenic species such as *N. philippinensis* and *N. italica* were tested for their pathogenicity to mice. They were injected intracerebrally into mice, but did not kill the

2D-PAGE and western blotting analysis on *Naegleria fowleri* total protein

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SUMMARY

Naegleria fowleri, a causative agent of PAM is morphologically indistinguishable from non-pathogenic *N. lovaniensis*. We compared proteins of closely related these species by analyzing the protein spots into amino acid level after separation by 2D-PAGE (ImageMaster). The protein spots that were common between *N. fowleri* and *N. lovaniensis* were 88 and the similarity between two was 38.6%. A total of 102 proteins were detected that were specific to *N. fowleri*. Among the spots common in 5 strains of *N. fowleri*, 53 spots were analyzed N-terminal amino acid sequences. According to their sequences of about 20 residues, 10 spots were those already known, including MP2CL5 and others. For further characterization of *N. fowleri* specific proteins, the protein spots were blotted on PVDF membrane after being separated by 2D-PAGE and reacted with either a rabbit anti-actin antibody or a *N. fowleri* specific monoclonal antibody according to the method using DyeChrome Western blot stain kit. Of 5 spots labeled with the rabbit antibody, four were identical to those detected on the CBB stained 2D-PAGE. Similarly, 5 spots were labeled with Nf-5D12u, and 2 of them were identified to those detected on the CBB stained gel from the localization analysis of the spots.

Molecular characterization of farnesyltransferase of *Entamoeba histolytica*

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SUMMARY

Farnesylransferase (FT) catalyzes farnesylation, which is one of the post-translational lipid modifications of proteins. Since farnesylation is necessary for Ras to function, FT has attracted attention as a target for cancer chemotherapy. We have already cloned the gene of FT of *Entamoeba histolytica* and expressed the protein in *Escherichia coli*, showed its enzyme activity, and identified one of its intrinsic substrates (*Eh*Ras-CVVA) belonging to Ras superfamily proteins. Phylogenetical analysis revealed that *Eh*FT was independent of FTs from other species. Among the Ras superfamily proteins of *E. histolytica*, *Eh*Ras-CVVA located philogenetically between Ras/Rap and Rho/Rac families. *Eh*FT showed highly resistant to the inhibitors for human FT. These results suggest that *Eh*FT is remarkably different from human FT in binding to substrates and inhibitors, which highlight this enzyme as a novel target for the development of chemotherapeutics against amebiasis.

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Effect of calcium antagonists and calmodulin inhibitors on *Entamoeba* excystation and metacystic development

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SUMMARY

The effect of calcium ions (Ca^{2+}) and calmodulin (CaM) on the excystation and metacystic development of *Entamoeba invadens* was examined by transfer of cysts to a growth medium containing calcium antagonists and CaM inhibitor. Excystation, which was assessed by counting the number of metacystic amoebae after induction of excystation, was inhibited by the calcium chelators ethyleneglycol bis (β-aminoethyl ether)-N,N'-tetraacetate (EGTA) and ethylenediaminetetraacetate (EDTA), with EDTA being more potent than EGTA. The inhibitory effect of higher concentrations of these chelators on excystation was associated with reduced viability of cysts. Metacystic development, when determined by the number of nuclei in an amoeba, was delayed by EGTA, because the percentage of four-nucleate amoebae was higher than in controls at day 3 of incubation. EDTA made metacystic development unusual by producing a large number of metacystic amoebae with more than ten nuclei. The inhibition of excystation by these chelators was partially abrogated by their removal. A putative antagonist of intracellular calcium flux, 8-(N,N-diethylamino) octyl-3,4,5-trimethoxybenzoate (TMB-8) also inhibited the excystation and metacystic development, but had little effect on cyst viability. The slow Na^{+}-Ca^{2+} channel blocker bepridil but not verapamil inhibited the excystation and metacystic development, associating with reduced cyst viability at higher concentrations. The inhibitory effect of bepridil on excystation was abrogated by removal of the drug. The CaM inhibitor trifluoperazine (TFP) inhibited the excystation and metacystic development. The inhibitory effect of TFP on excystation was also abrogated by removal of the drug. These results indicate that extracellular calcium ions, amoebic intracellular calcium flux, calcium channels, and a CaM-dependent process contribute to the excystation and metacystic development of *E. invadens*.

Filamentous Bridging Structure; A Role Playing in the Morphogenesis of *Trypanosoma evansi*

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SUMMARY

Even after 16 minutes of pronase incubation of 500 micrograms at final concentration per 1 ml of 0.15M Na-K phosphate buffer (pH 7.2), the rivet structures (maculae adherence : m.a.) of *Trypanosoma evansi* were confirmed to remain in situ with a transmission electron microscope, although Frevert, U. et al. (1986) described in *T. congolense* that m.a. were completely removed after 2 minutes incubation with pronase, at the same final concentration as this work. The flagellum became detached from the parasite body of *T. evansi* in this work, to my surprise, was proved to be accompanied by the architecture of m.a. Hemphill et al. (1991) demonstrated clearly in *T. brucei* that there was a filamentous bridging structure (FBS) joining the paraxial rods of the flagellum to the subpellicular microtubules of the parasite body. FBS appears to be an adhesive structure appendant to the m.a. as seen in the transmembrane linker of mammalian desmosomes. Therefore, the flagellar detachment from the parasite body in *T. evansi* would be responsible for not the digestion of m.a but that of the FBS. Moreover, the cause of morphological alteration, from the slender form to the stumpy form, would be also sought to not the flagellar internalization and the accumulation of cell organelles at the former posterior end of the parasite body but the digestion of the FBS, because the intermediate filaments and the FBS (or transmembrane linkers) together with the m.a. (or cytoplasmic plaques) are considered to relate to the cell shape and the localization of cell organelles including a kinetoplast as well as the flagellum-to-cell joining, by the analogy with mammalian desmosomes.

Seroepidemiological survey of Encephalitozoon infection in dog in the Kanto district

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SUMMARY

Encephaltitozoon cuniculi is the obligate intracellular protozoan parasite belonging to the phylum Microsporidia that infect a wide range of invertebrate and vertebrate hosts including humans. After AIDS virus emerged in1983, many cases of human encephalitozoonosis caused by *E. cuniculi* infection have also been reported from various developed

countries for the last 10 years, except for Japan. More recently, it was revealed that two genotypes exist in human *E. cuniculi* isolates, i.e. genotype I and genotype III. These two types have host specificity respectively. Genotype I was isolated from rabbits and genotype III from dogs. Whereas rabbit *E. cuniculi* infection commonly is seen throughout the world, the prevalence state of dog *E. cuniculi* infection is obscure to us, in Japan in particular, since probably that has not been surveyed up to now. Therefore, we started to survey seroepidemiologically the current conditions of *E. cuniculi* infection in dogs. This report shows the results of serosurvey in the Kanto area. We tested 159 dogs' sera by ELISA, and obtained only one positive serum. The results indicated that *E. cuniculi* infection in dogs might be poorly prevalent as far as we surveyed by serological means.

Amoebae isolated from some ornamental fish in Thailand

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SUMMARY

Recently, oscar fish faced with disease, which is difficult to control. It caused productive and economic losses. A small pieces of gills and kidney of oscar fish were taken from the fish and inoculated on a NNE-plate. *Amoebae* isolated were classified into three different groups. The first group isolated from the kidney had two stages, trophozoites and cysts, and was classified in the genus *Acanthamoeba*. The second group was isolated from the gills. Trophozoite showed typical fan-like shape with about 20 μm in size. This amoeba was classified in the genus *Vannella*. The third group was isolated from the gills and water. This group formed three stages as trophozoite, flagellum and cyst. This study is a first report on isolation and identification of various group of amoeba from oscar fish.

Isolation of new *Chlamydomonas* mutants deficient in basal body formation

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SUMMARY

We have isolated a *Chlamydomonas* mutant, bld10, which totally lacks basal bodies. The fact that the mutant is viable without basal bodies demonstrates the usefulness of *Chlamydomonas* as a tool for studying the mechanism of basal body/centriole assembly by using a variety of mutants. To isolate new basal-body mutants, we produced mutants by insertional mutagenesis and screened for strains showing similar phenotypes to those of bld10, such as the defects in flagellar formation and mitosis. Of about 25,000 transformants, eight strains that fulfill the criteria were isolated. Immunofluoresence microscopy using anti-centrin, anti-tubulin, anti-Bld10p, and anti-tektin antibodies revealed that four strains (No. 135, 214, 2E7, 1B9) show abnormal staining patterns of the basal bodies and its related cytoskeletons. Of these four strains, strain 135 and 214 were found to be new alleles of bld10 by crossing with several known basal body mutants. Electron microscopic observations of the rest two strains revealed that strain 2E7 has truncated basal bodies and strain 1B9 has multiple basal bodies in the apical region of the cell. These observations suggest that strain 2E7 has a defect at a step in the elongation of the basal body during the assembly process and strain 1B9 has in the regulation of duplicating the basal body.

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Rumen ciliate protozoal fauna of lesser mouse deer (*Tragulus javanicus*) in Malaysia

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SUMMARY

The rumen ciliate fauna of the lesser mouse deer in Malaysia was surveyed. The ciliate composition was simple; only four species belonging to the genus *Entodinium*, *E. simplex*, *E. parvum*, *E. dibardi* and *Entodinium* sp., were found. *Entodinium* sp. had a slender body, length 50.3 μm and width 25.1 μm on average. Its macronucleus was rod-shaped and 26.9 μm in length on average, and a micronucleus was situated at the antero-ventral side of the macronucleus. A distinct groove runs from the anterior end to the posterior end of the body at the ventral-left side of the macronucleus. A contractile vacuole was situated at the ventral side of the groove. In the SEM, the groove was observed as well as many striations on the body surface running obliquely to the body axis. Comparison of this species with morphologically related species suggests that it should be described as a new species. Since the composition rate of this species was high, 30-80% of the total ciliates, this species might have evolved in the environment of the mouse deer.