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

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SUMMARY

The Gram-negative bacterium *Holospora obtusa* is an endonuclear symbiont of the ciliate *Paramecium caudatum*. The surface protein with a molecular mass of about 266 kDa was extracted only from the aposymbiotic cells by salt/ethanol treatment, but not from the symbiotic cells. Instead, two surface proteins of 188 kDa and 149 kDa were extracted from the symbiotic cells by the same method. Indirect immunofluorescence microscopy and immunoblot with a monoclonal antibody raised for the 266-kDa protein showed that the antigen was present only on the surface of the aposymbiotic cell but not on the symbiotic cell. When *H. obtusa* were removed from the symbiotic cells by treatment with penicillin-G-potassium, resulting aposymbiotic cells recovered the 266-kDa surface protein but lost both 188- and 149-kDa proteins from the cell surface. These results show that the *P. caudatum* cell changes the surface antigen depending on the presence or the absence of *H. obtusa* in the macronucleus. Although *Paramecium* changes types of the cell surface antigen by various stresses such as temperature-shifts, ionic strength and starvation, the188 kDa and 149 kDa only by the infection of *H. obtusa*.

Stable single nucleotide polymorphism in antigen genes of *Plasmodium falciparum*

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SUMMARY

Since surface antigen genes of the human malaria parasite, *Plasmodium falciparum*, show extensive polymorphism, a rapid evolution of their polymorphism is presumed. However, little is known about frequency of the generation of novel polymorphisms. We are interested in *P. falciparum* populations in Vanuatu, in the southwestern Pacific, where the malaria epidemiological settings are suitable to test whether novel antigen polymorphism evolves rapidly because limited human movement limits the parasite diversity in isolated populations. We analyzed single nucleotide polymorphisms (SNPs) and repeat-length polymorphisms in three major surface antigens, *msp1*, *msp2* and *csp,* in populations from 7 islands of Vanuatu in 1996 to 2002. We also sequenced simple repeat-length polymorphisms at three microsatellite loci, *serca* second intron, TA40 and TA101. Analysis of more than one million bases revealed no de novo SNPs in the three antigen genes in Vanuatu. In contrast, repeat length polymorphism evolved rapidly. Analysis of 'linkage disequilibrium' between pairs of loci revealed a spectrum of population genetic structure, suggesting that some of old antigen alleles have persisted through meiotic recombination events after the appearance of chloroquine resistance in Vanuatu. We argue that SNPs in *P. falciparum* antigen genes are substantially stable in isolated populations.

Gametogenesis in the nonphotosynthetic dinoflagellate *Noctiluca scintillans*

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SUMMARY

Noctiluca scintillans is one of the organisms responsible for harmful red tides and usually reproduces by binary fission. A small fraction of cells sporadically differentiate into a form for sexual reproduction and produce zoospores. Here, I describe the processes in zoospore formation, which is followed by gamete fusion. Initial signs of differentiation were a

change of the cell shape (from eggplant-like to spherical), accompanied by loss of the tentacle, rod organ and cytosome. After this differentiation, the nucleus migrated towards the the cell surface, where it divided twice. Each of the resultant four nuclei, together with some cytoplasm, protruded from the cell surface. Zoospores (gametes) were produced through successive divisions in the protrusions (nucleus–cytoplasm complex). During the divisions of zoospore formation, the nuclei seem to be connected to one another with a thin network of cytoplasm. Mature zoospores were biflagellate and had a semi-spindle shape when they were released from the parental cell ghost. The released biflagellated zoospores fused in pairs, but their subsequent fate is unknown. In the vegetative phase the cells are highly specialized, so they do not have the typical morphology of dinoflagellates, but the zoospores maintain dinoflagellate-like characteristics. Further study of the zoospores will shed new light on the evolution of dinoflagellates.

Effect of Japanese *Paramecium bursaria* on symbiotic algae

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SUMMARY

Cells of green paramecium, *Paramecium bursaria*, have several hundred endosymbiotic algae. To investigate interactions between *Paramecium bursaria* and its symbiont, we attempted to measure the effect of a cell-free extract of Japanese *P. bursaria* on the photosynthetic carbon fixation of the symbiont. A cell-free extract of *P. bursaria* was prepared by filtration of *P. bursaria* cells on a membrane filter. Carbon fixation by the symbiotic algae and by free-living *Chlorella* spp. in solutions of various concentrations of the extract were measured using $NaH[^{14}C]O_3$. The amount of carbon fixed by all algae increased about 3-fold with increasing extract concentration. Since this phenomenon was not affected by elimination of carbon dioxide from the extract, the existence of a host factor that stimulates algal carbon fixation (without species specificity) is suggested. In addition, the host factor would be a heat-stable low molecular weight substance. From a comparison with pH effects on carbon fixation by the symbiotic alga and a free-living *Chlorella* sp., the stimulation of algal carbon fixation seemed not to be caused by a change of intracellular pH. It appears that paramecium regulates photosynthesis of the symbiotic alga via a specific host factor.

Nitrogen utilization by endosymbiotic algae isolated from *Paramecium bursaria*

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SUMMARY

The green ciliate, *Paramecium bursaria,* has several hundred endosymbiotic algae. Symbiont F36-ZK isolated from Japanese *P. bursaria* F36 required some amino acids as a nitrogen source for its remarkable growth, rather than nitrate, which is used by endosymbionts of American and European *P. bursaria* and most of free-living *Chlorella* spp. To elucidate this novel nitrogen utilization by F36-ZK, we studied enzymatic activity related to nitrogen assimilation and amino acid transport. Nitrate reductase (NR) activity was not detectable in F36-ZK, indicating why F36-ZK could not utilize nitrate. Activities of the key enzymes of ammonium assimilation, glutamine synthetase (GS) and glutamate dehydrogenase (GDH), were also assayed. As no GS activity was detectable, it seemed likely that GDH assimilated ammonium to yield Glu and this was utilized for algal growth. However, our previous results showed that Glu could be taken up, but not utilized, by F36-ZK. This suggests the presence of an unknown ammonium assimilative pathway in F36-ZK. Symbiont F36-ZK possesses three active amino acid uptake systems, a basic amino acid transport system (Arg and Lys), a general amino acid transport system (Lys, His, Glu and neutral amino acids) and an alanine transport system (only Ala). The novel character of F36-ZK may have evolved during symbiosis over a long period.

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Effects of host termite colony size on the symbiotic protist fauna.

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SUMMARY

The symbiotic flagellate community of a termite is characterized by host-specific species composition, definite boundary, and clear community structure created by social system of host termite. To investigate the effects of meta-community size on the symbiotic flagellate community, we set up experimental termite colonies of *Reticulitermes speratus* composed of five, 25, 100 and 300 workers with seven replicates. The colonies were kept in an incubator at 22°C, and on the day 60 and 90 we picked up five termite individuals from each experimental colony and investigated the numbers of each flagellate species. We also investigated the flagellate numbers of the original field colony. Fifteen flagellate species were found from the field colony with nearly 100% infection rates. The total numbers of flagellates were significantly smaller in five-termites colonies than in the field colony, but slightly larger in 25- and 100-termites colonies. In the three Trichomonad species with small individual numbers in the community, infection rates and population sizes of the flagellates clearly decreased as the meta-community size decreased. However, some flagellate species of large population sizes did not follow these trends. The diversity of the flagellate community, measured by species number and Shannon-Wiener index, tended to decrease as the size of meta-community decreased.

Phylogenetic analysis, based on the SS rDNA gene, of *Parentodinium* living in the fore-stomach of Hippopotamus

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SUMMARY

It is well known that numbers of ciliate species belonging to the Subclass Trichostomatia live in the digestive tract of large herbivores. Phylogenic relationships of rumen ciliates, including the families Ophryoscoleciidae and Isotrichidae, have been inferred by comparison of DNA sequences of small subunit ribosomal RNA region (SSrDNA). But a comparative study on molecular phylogenetic relationships of the ciliates living in the fore-stomach of hippopotamus, *Hippopotamus amphibius,* has never been done. Furthermore, our previous morphological studies suggested that these *Parentodinium* ciliates do not belong in the family Cycloposthiidae in which the genus has been included. To determine the phylogenetic position of the genus *Parentodinium* more clearly, we examined, for the first time, the SSrDNA of *Parentodinium* and *Cycloposthium* ciliates and compared these sequences with those of the other Trichostomatia ciliates. Using Distance Matrix, Maximum Parsimony and Maximum Likelihood methods, all the results indicated that *Parentodinium* did not belong in any described families of the Subclass Trichostomatia, although the Cycloposthiidae had a close relationship to the Ophryoscoleciidae as a monophyletic clade. This result indicates the need to establish a new family, Parentodiniidae, in the Subclass Trichostomatia.

Effect of aphidicolin on the excystation and metacystic development of *Entamoeba invadens*

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SUMMARY

The effect of aphidicolin, a specific inhibitor of the replicative DNA polymerases, on the excystation and metacystic development of *Entamoeba invadens* was examined. The protein profile of metacystic amoebae and their immunogenicity in the presence and absence of aphidicolin were also examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Excystation, which was assessed by counting the number of metacystic amoebae after the induction of excystation, was inhibited by aphidicolin in a concentration-dependent manner during incubation compared to the controls. Metacystic development, when determined by the number of nuclei in amoeba, was also inhibited by aphidicolin, because the percentage of 4-nucleate amoebae in cultures with aphidicolin during incubation was higher than that in cultures without the drug. The addition of aphidicolin to cultures at day 1 of incubation reduced the number of metacystic amoebae thereafter compared to cultures without the drug. The inhibitory effect of aphidicolin on excystation and metacystic development was reversed by removal of the drug. Cellular proteins of metacystic amoebae with 4 nuclei, which were predominant even at day 3 in the cultures with aphidicolin, reacted strongly with rabbit anticyst serum absorbed with trophozoite proteins. In contrast, those of metacystic amoebae with 1 nucleus, which were predominant at day 3 in cultures without aphidicolin, no longer reacted with the absorbed anticyst serum, suggesting change in the expression of proteins during metacystic development.

Occurrence of *Cryptosporidium* spp. among reptile hosts

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SUMMARY

Waterborne transmission of *Cryptosporidium* is one of the most prominent public health concerns in drinking-water safety, since the oocysts are highly resistant to chlorine disinfection. In 2001, high levels of cryptosporidial oocysts were detected in a community water treatment plant in western part of Japan, where well water was supplied to the consumers without filtration. Studies by PCR and sequencing revealed that the isolate is identified as the W11 genotype, suggesting the wild reptile(s) to be the source of contamination. This study aimed at obtaining the prevalence of cryptosporidial infection among reptiles in Japan, which might again be microbial contaminants of drinking-water. A total of 598 stool samples from wild reptiles, including snakes, lizards and tortoises, and 524 samples from captive bred reptiles, mostly exotics, were examined occurrence of *Cryptosporidium* by means of sedimentation method followed by fluorescent antibody staining. Only one domestic wild lizard was positive for *Cryptosporidium*, whereas 45 bred reptiles were positive. Until now 14 isolates were genotyped and obtained 4 distinct species/genotypes; *C. serpentis* (1), *C. saurophilum* (9), *C. parvum* bovine genotype (3) and *C. andersoni* (1), though the latter 2 might have originated from the food fed that had transiently been shed into feces.

Molecular cloning and characterization of a protein geranylgeranyltransferase type I from *Entamoeba histolytica*

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SUMMARY

Protein prenylation is essential for the Ras superfamily small G proteins to function. Protein prenylation includes

protein farnesylation and protein geranylgeranylation, which are catalyzed by protein farnesyltransferase (FT), protein geranylgeranyltransferases type I (GGT-I) and type II. FT and GGT-I have attracted attention as an anti-cancer agent, and attempts have been made to use the enzymes as a target of anti-protozoan chemotherapy. Following the study on FT of *Entamoeba histolytica* (*Eh*), we characterized *Eh*GGT-I. The α subunit of *Eh*GGT-I was shared with FT. The β subunit of *Eh*GGT-I had 337 amino acids, and showed 22 to 28% identities to those of other organisms. The recombinant *Eh*GGT-I, expressed by *Escherichia coli*, was a complex of 38 kD and 35 kD proteins. The rabbit anti-*Eh*GGT-I serum did not reacted with rat GGT-I, showing difference in antigenicity. Rat GGT-I geranylgeranylates human mutant H-Ras-CVLL, but does not H-Ras-CVLS. *Eh*GGT-I, however, geranylgeranylated both substrates. In mammals, Ras proteins are farnesylated by FT, and Rac proteins are geranylgeranylated by GGT-I. In contrast, *Eh*GGT-I geranylgeranylated Ras proteins as well as Rac proteins. To inhibitors for mammalian GGT-I, *Eh*GGT-I was much more resistant than rat GGT-I. In conclusion, *Eh*GGT-I was significantly different from mammalian GGT-I in substrate specificity and sensitivity to the inhibitors, and thus *Eh*GGT-I would become a possible chemotherapeutic target of amoebiasis.

Phylogenetic position of Radiolaria based on 18S rDNAs sequences

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SUMMARY

'Radiolaria' (classes Acantharea, Polycystinea, and Phaeodarea) are planktonic protists widely distributed in tropical, subtropical, and even polar marine environments. Some recent studies based on 18S rDNA sequences have reported that the Polycystinea and the Acantharea form a monophyletic group, but the Phaeodarea branch with the phylum Cercozoa, separately from the Polycystinea and the Acantharea (Polet et al., 2004). Here we show the phylogenetic relationships of 'Radiolaria', using the 18S rDNA regions of the solitary shell-bearing polycystines and phaeodarians. Their molecular analyses have never been reported. Phylogenetic analyses of our sequences indicate that the Polycystinea and the Acantharea are monophyletic, and the Phaeodarea diverge with the phylum Cercozoa as already suggested by Polet et al. (2004). Furthermore, the Polycystinea were shown to be a paraphyletic group in the 'Radiolaria'. These conclusions are not consistent with the current taxonomy of 'Radiolaria', and lead us to consider that the collosphaerid, sphaerozoid, and thalassicollid spumellarians and the pterocorycid nassellarians may have evolved from an ancestor with triradiate branched spicules.

Amino acid sequence analysis on *Naegleria fowleri* proteins separated by 2D-PAGE

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SUMMARY

We have established and released partially on a website as database of 2D-PAGE map of *Naegleria fowleri* total proteins obtained by a combination of 2D-PAGE image analysis with direct N-terminal amino acid analysis. Until now, N-terminal sequences of 38 proteins out of 59 major proteins spots examined have been successfully determined. Further, internal sequences of 11 and 24 proteins have, then, been analyzed along either with Cleveland peptide mapping or with EST-IT tandem MS followed by similarity search for known proteins against NCBInr database using Mascot search engine, and 3 and 4 proteins were identified with high confidence as cyclophilin, nucleoside diphosphate kinase and

isocitrate dehydrogenase [NADP], and MP2CL5, *N.fowleri* HSP70, albumin and actin , respectively. In addition, MS/MS spectra have been de novo sequenced by means of PEAKS Studio v2.4 and revealed many peptide candidates from each of 24 spots. The methods reported in the present experiments showed an ultimate advantage for the identification of the proteins from the internal sequences of protein spots especially of which N-terminal are blocked like many of those of *N.fowleri*. The 2D-PAGE database will be updated spot by spot without delay for public access.

Characterization of the 60 kDa microtubule-associated protein from *Tetrahymena pyriformis*

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SUMMARY

In preparing the microtubule-associated proteins (MAPs) of *Tetrahymena pyriformis*, we identified three polypeptides, with apparent molecular masses of 90 kDa, 60 kDa, and 37 kDa, that co-sedimented with taxol-stabilized mammalian microtubules. The 60 kDa MAP was heat-stable. Using this characteristic, the protein was purified to homogeneity, and was further characterized biochemically. Partial amino acid sequences of the 60 kDa MAP were homologous to those of *Xenopus* XMAP215. The 60 kDa MAP enhanced both the initial rate and the plateau level of mammalian tubulin polymerization, in a concentration-dependent manner. Electron microscope observations showed that the assembled structures were normal microtubules. The 60 kDa MAP existed abundantly in the soluble fraction, rather than the membranous one, as revealed by a partial cell fractionation. Immunofluorescence microscope observations using anti-60 kDa MAP antibody also revealed that the 60 kDa MAP existed in the cytoplasm. The 60 kDa MAP appeared as particles, suggesting that the 60 kDa MAP was associated with granules or vesicles. The cellular 60 kDa MAPs were mostly co-localized with cytoplasmic microtubules, while some of the 60 kDa MAPs were situated near microtubules, but without clear associations. Nocodazole treatment resulted in a change in the 60 kDa MAP localization, which probably accompanied the disruption of microtubule networks.

Division inheritance of free cytoplasmic organelles in *Tetrahymena thermophila*

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SUMMARY

Cells of *Tetrahymena thermophila* are full of membrane-bound cytoplasmic organelles such as food vacuoles or lysosomes that are free in the cytoplasm, while all mitochondria are attached to the cell cortex. There is no protoplasmic streaming. When cells were slightly starved, cytoplasmic organelles were found in the posterior half of a cell. When these cells divided, cytoplasmic organelles moved from this posterior position to the surface of the macronucleus (MAC) just after completion of micronuclear division and covered the whole surface of the MAC. During division, cytoplasmic organelles were partitioned almost equally to the daughter cells by hitchhiking on the dividing MAC, and they dispersed into the cytoplasm just after MAC division and before separation of the daughter cells. The MAC is the partition apparatus for cytoplasmic organelles. Similar partition was also observed in *T. pyriformis* W. During the first division of exconjugants after conjugation, there is no MAC division. Organelles located in the posterior part of exconjutants also moved to the central part of the cell and partitioned almost equally to daughter cells. This shows that *Tetrahymena* cells have

precise mechanisms for organelle partitioning.

Determination of the genomic sequence and the upstream sequence of the gamone 1 gene in the ciliate *Blepharisma japonicum*

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SUMMARY

In the conjugation of *Blepharisma japonicum*, cells of complementary mating types I and II participate in the interaction which leads to mating pair formation. Pair formation is induced by conjugation-inducing substances, called gamones, which are secreted by cells of each mating type. Gamone 1 is not constitutively secreted, but is secreted exclusively by moderately starved, sexually mature type I cells. Moreover, synthesis of gamone 1 is further promoted by gamone 2 which is secreted by cells of mating type II. It was clearly shown in our laboratory that the expression of gamone 1 was regulated by its transcription level in various conditions. In this study, we aimed to find the transcription regulatory region of the gamone 1 gene. First, we determined the genomic sequence of the gamone 1 gene, and found that an intron was not included in the gene. Second, we amplified the 5'-flanking region of the gamone 1 gene using inverse PCR, and an upstream sequence of 868 bp was determined. The sequence contained a proximal sequence element (PSE)-like sequence, which is considered to be the transcriptional regulatory sequence for RNA polymerase III, a TATA box sequence, CCAAT box sequence, and several recognition sequences of various transcription factors.

Analysis of glutathione *S*-transferase gene induced by light stimulation in *Blepharisma japonicum*

Yuichi TAKADA and Tatsuomi MATSUOKA (Dept. Biol., Fac. Sci., Kochi Univ.)

SUMMARY

The ciliated protozoan *Blepharisma japonicum* possesses a photosensitive pigment called blepharismin that causes the generation of reactive oxygen species (ROS) such as hydroxyl radicals and singlet oxygen. In order to elucidate the mechanism of resistance to photooxidative stress caused by ROS, cDNA clones induced by light stimulation were isolated from a cDNA library of *B. japonicum* by a differential screening method, and their light-dependent expression was checked by semi-quantitative RT-PCR analysis. Sequence analysis showed that one of these clones encodes glutathione *S*-transferase (GST), which is known as an antioxidative enzyme. Further cDNA library screening with a *B. japonicum* GST (BjGST1) cDNA probe revealed the presence of another GST cDNA (BjGST2), and the two BjGST (BjGST1 and BjGST2) clones showed 86% sequence identity at the amino acid level. Both BjGST sequences were overexpressed in *Eschericia coli*, and the quaternary structure was investigated using gel filtration chromatography and Native-PAGE. It was found that BjGST could form homodimers and heterodimers. Furthermore, Southern blot analysis showed that the two *BjGST* genes were tandemly arranged in the *Blepharisma* genome. These results indicate that *Blepharisma* GSTs could act cooperatively in response to photooxidative stress.

Molecular biological analyses of IP39, the intramembrane protein of *Euglena gracilis*

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SUMMARY

The euglenoid flagellates perform a characteristic movement called euglenoid movement. Intramembrane proteins called IP39, which are regularly and densely arranged in the plasma membrane, have been implicated in this movement. We carried out PCR-based cDNA cloning of IP39 from *Euglena gracilis* in our previous studies, and found two types of IP39-encoding cDNAs (α- and β-types). The α- and β-type cDNAs consisted of 792 and 795 base pairs, respectively. 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. To examine the expression of these two types of IP39 mRNA, we carried out northern blot analyses of mRNA extracted from cells in the logarithmic growth phase. The specific DNA probes were designed from the 3' untranslated region of the IP39 mRNA. Results of northern blotting showed that both mRNAs are expressed in the cell and suggested that expression levels of the two types of IP39 mRNA were similar.

Appearance and disappearance of the 140 kD protein of the ciliate, *Sterkiella cavicola*, and the ultrastructural changes of micronuclear chromatins during encystment and excystment

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SUMMARY

Hongo (1984) has discovered a cyst specific 140 kD protein in the ciliate, *Sterkiella cavicola*, assuming its localization in the outermost layer of the cyst wall. Himura (1993) has made clear that this protein localizes in the cystic micronuclear chromatins by immunoelectron microscopy. Kikukawa (1996) has shown that the cysts contain 2 types of micronuclei, with thin compact chromatins and with thick rough chromatins, and that the 140 kD protein is recognized only in the micronuclei with thin compact chromatins. In order to elucidate the function of the 140 kD protein, we compared the appearance and disappearance of the protein by Western blotting technique and the ultrastructural changes of micronuclear chromatins during encystment and excystment. The results of the Western blots indicated that the 140 kD protein appeared at the stage 4-5 of the encystment, and remained in the ciliates immediately after excystment but disappeared during a few hours after excystment. Ultrastructurally the thin compact chromatins were first found at the stage 5 of the encystment, and remained immediately after excystment but was not found in the cells a few hours after excystment. We supposed that the 140 kD protein condensed micronuclear chromatins and the condensation of chromatins might protect genetic information of the cyst against adverse environmental conditions.

Suppressing factors for encystment in *Colpoda* sp.

Akemi KIDA and Tatsuomi MATSUOKA (Inst. Biol, Fac. Sci., Kochi Univ.)

SUMMARY

Ca^{2+}-induced encystment of *Colpoda* sp. is cancelled by bacterial products such as proteins and peptides in the surrounding medium. In the presence of bovine serum albumin (BSA), suppression of Ca^{2+}-induced encystment occurred concomitantly with uptake of BSA into endocytic vesicles. Such suppression of encystment was not affected when endocytosis was inhibited by chlorpromazine. The result suggests that the suppression of encystment is not correlated with endocytosis and/or nutrient supply via endocytosis. In order to elucidate whether the *Colpoda* cells detect the specific

conformation of the proteins or peptides, we examined the suppression effects of BSA, peptides obtained by digestion of BSA, or a mixture of the total amino acids constituting BSA, on Ca^{2+}-induced encystment. All of them had a marked encystment-suppression effect, implying that the receptors involved in suppression of encystment might recognize side chains of amino acids or certain structures common between amino acid residues and free amino acids.

Evidences for the increase of telomere length with clonal aging in *Paramecium caudatum*

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SUMMARY

Telomere shortening is associated with proliferative senescence in cultured human cells. However, stable maintenance of telomere length is found in proliferative senescence in *Paramecium tetraurelia.* In previous studies of *P. caudatum*, we found that the length of telomeres increased for about 700 fissions after conjugation. In this study we altered the conditions of Southern hybridization by changing both the non-RI detection system and the length of telomere probes. The biotin-labeled telomere probes (TTGGGG: 4 repeats) enabled us to increase the hybridization temperature from 42.5°C to 68°C. Under these conditions, we have reconfirmed that the signals obtained in the previous experiments were from the terminal restriction fragments. Furthermore, under the new experimental conditions, we could estimate the telomere elongation ratio as about 2.5 bp per cell division in KNZ52 strain and about 1.0 bp per division in SOS2. However, there was no statistically significant difference between the two strains. Paramecia have detectable telomerase reverse transcriptase (TERT) activity during sexual maturation and until about 170 fissions after conjugation. Although there is no information about TERT activity in senescent cells, this enzyme is one of the candidates for telomere elongation. In some cell lines, homologous recombination is thought to be an alternative mechanism. Our results suggest that telomere length may be useful as a molecular indicator of the clonal age in *P. caudatum*. For an understanding of the biological meaning of telomere elongation, it would be necessary to establish methods of artificial manipulation of telomere length in future.

The effects of parental age on the aging of offspring in *Paramecium tetraurelia*

Tomoyoshi OGINO and Isoji MIWA (Dept. Biol., Fac. Sci., Ibaraki Univ.)

SUMMARY

It is known that the germinal age of the parent affects the initial mortality of offspring after fertilization in *Paramecium tetraurelia*. In this experiment, we examined the length of autogamy immaturity, life span and the fission rate, of offspring induced by autogamy for various parental ages and generations. When we examined the initial mortality of offspring induced at each parental age, we found, as did the previous study, that the initial mortality of offspring induced from aged parents increased rapidly. Furthermore, the offspring induced from cells after 30 fissions in the second generation showed a lower initial mortality than that of the parent. The length of autogamy immaturity, life span and aging speed of offspring, however, were not affected by parental age, and they maintained a constant value in all offspring induced from every generation. These results indicate that parental age may be reset in a viable offspring induced by autogamy.

Ca^{2+}-sensitivity of centrin is important for the activity of the voltage-gated Ca^{2+} channels in *Paramecium caudatum.*

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

SUMMARY

Internal Ca^{2+} concentration regulates the waveform or beat direction of flagella/cilia of various eukaryotes. Ciliary reversal in *Paramecium* depends on a Ca^{2+} influx through 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. One of the voltage-gated Ca^{2+} channel mutants in *P. caudatum*, *cnrC*, neither produces Ca^{2+} action potentials nor responds to any depolarizing stimuli. Previously, we reported that the *cnrC*$^{+}$ gene product is *P. caudatum* centrin (Pccentrin1p), a member of the calmodulin superfamily. Like calmodulin, centrin possesses four Ca^{2+}-binding sites called EF-hand. To examine whether Pccentrin1p controls the voltage-gated Ca^{2+} channels in a Ca^{2+}-dependent manner *in vivo*, the curing effect of Pccentrin1p genomic DNA that had mutations in the EF-hand was examined. The DNA was microinjected into *cnrC* cells, and then the responses of the cells to depolarizing current were investigated. The results showed that the Ca^{2+} sensitivity of Pccentrin1p is important to the regulatory mechanisms of the voltage-gated Ca^{2+} channels. In particular, it was suggested that the cooperative work of EF-hand 3 and 4 in the presence of Ca^{2+} is indispensable to the Ca^{2+} channel activity.

Expression of gamone 1 gene during sexual maturation in the progeny clones of the ciliate *Blepharisma japonicum*

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SUMMARY

The ciliate *Blepharisma japonicum* switches the reproductive process from asexual (binary fission) to sexual (conjugation) when cells are deprived of food . This switching mechanism has not been elucidated yet. Conjugation in *B. japonicum* is induced by interaction between cells of complementary mating types I and II. Substances that act as signaling molecules for conjugation are called gamones. The glycoprotein gamone 1, produced by mating type I cells, is a key factor that triggers interaction. In this study, we examined the influence of nutritional conditions on the expression of gamone 1. We found that mature mating type I cells do not transcribe gamone 1 in the logarithmic phase, but start transcribing it when they are starved, and that the level of transcription increases as starvation progresses. We isolated progeny clones and examined their level of maturation and the expression of gamone 1 at different clonal ages. We showed that most progeny clones differentiated into mating type I, becoming mature at about age 25 and continuing maturation gradually. The expression of gamone 1 was examined at the clonal ages of 10, 18, 25 and 39 by northern blot analysis. Gamone 1 mRNA was not detected in immature cells (clonal age of 10 and 18), first faintly detected at age 25, and became distinct at age 39. Gamone 1 activity in cell-free fluid prepared from each sample corresponded to the level of gamone 1 mRNA. These results suggest that the transcription of gamone 1 is strictly linked to the process of sexual maturation and clonal age.

Analysis of the feeding system in the heliozoon *Actinophrys sol*

Soichiro KAKUTA and Toshinobu SUZAKI (Dept. Biol., Fac. Sci., Kobe Univ.)

SUMMARY

Actinophrys sol is a predatory protozoan which captures prey organisms with its axopodia. When capturing prey organisms, *A. sol* discharges the contents of extrusomes, which are secretory granules located beneath the cell membrane. It has been reported that extrusomes contain concanavalin A (Con A)-binding materials. At least two proteins are known to be involved in feeding of *A. sol*. The first is a 40-kDa glycoprotein (gp40) that binds Con A, and gp40-adsorbed agarose beads induce phagocytosis by *A. sol*. This suggests that gp40 functions in prey recognition. Another protein involved in feeding is TPPI , which is deduced to be a peptidase involved in lysosomal digestion. By immuno-microscopic observation, it was found that TPPI was localized in lysosomes and discharged into late phagosomes. From the results of a homology search, it is conceivable that TPPI is a peptidase belonging to the sedolisin family. Immuno-electron microscopy confirmed that gp40 was localized in extrusomes. After gp40 was purified with Con A-agarose, and then digested with N-glycosidase, SDS-PAGE and western blotting showed that its molecular mass, without the Con A- binding sugar chain, was 37.5 kDa.

Toxic effect of heavy metal ions on the axopodia of heliozoon *Raphidiophrys contractilis*

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SUMMARY

In this study we have observed the effect of zinc, lead, copper, mercury and cadmium ions on the heliozoon *Raphidiophrys contractilis*. The effects of heavy metal ions on the axopodial length, food uptake mechanism and axopodial contraction were examined, and we found that the axopodial length decreased significantly, the food uptake mechanism and axopodial contraction prolonged. The half strength effects of these heavy metals are around 1 μM, i.e., in 1 μM heavy metal ions axopodial length became almost half. In the same concentration of these heavy metal ions, the affect of mercury is higher than those of zinc, lead, copper and cadmium. In high concentration of these heavy metals, it was observed that axopodia were disappeared and cells were disrupted very quickly.

Isolation of a gene encoding ADF/cofilin in *Tetrahymena thermophila*

Nanami SHIOZAKI, Kentaro NAKANO and Osamu NUMATA (Inst. of Biol. Sci., Univ. of Tsukuba)

SUMMARY

Cytokinesis is one of the most important events for cell growth and is accomplished by contraction of the contractile ring. Although the contractile ring is mainly composed of actin filaments and myosin, it is not well understood how these proteins are assembled in the ring. Therefore, we decided to study the function of actin-regulating proteins in *Tetrahymena thermophila*, and identified an ADF/cofilin-homologous gene, *ADF73*, from its genome project. ADF/cofilin is a protein that severs and depolymerizes actin filaments and plays an important role in establishing cytokinesis in many eukaryotic cells. First, we cloned *ADF73* and deduced its encoded amino acid sequence. It was revealed that Adf73p is a 136-amino acid protein and is most similar to starfish and *Schistosoma* ADF/cofilin. Next, we determined that the molecular weight of Adf73p is about 14.5 kDa, by subjecting its recombinant protein, expressed in *E. coli*, to SDS-PAGE. In addition, we confirmed the intercellular interaction of Adf73p with actin from the fission yeast, *Schizosaccharomyces pombe*. In future, we will study the biochemical reactivity of Adf73p to actin in vitro, and the function of Adf73p in cytokinesis in *Tetrahymena*, by knocking out its gene and by immunofluorescence microscopy.

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Role of phospholipase C in the motility of *Amoeba proteus*

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SUMMARY

We have examined the role of phospholipase C (PLC) in the cell movement of *Amoeba proteus*. Although mechanisms of regulation of actin-based cell motility by phosphoinositide in mammalian cells have been well explored, those of *Amoeba proteus* still remain poorly understood. The *Amoeba* movement was examined as a model system to dissect locomotion of mammalian cells, such as macrophages, neutrophils as well as some cancer cells, exhibiting a rapid amoeboid movement. *Amoeba* cell lysates showed the PtdIns(4,5)P_2 hydrolyzing activity. Microinjection of Ins(1,4,5)P_3, one of the products of PtdIns(4,5)P_2 hydrolysis, into the cytoplasm of resting *Amoeba* caused pseudopod extension and cell movement. We have isolated cDNA clone encoding a functional PLC from *Amoeba* by RT-PCR cloning method. The deduced amino acid sequence and the domain structure show a marked similarity to the eukaryotic PLCs, especially those of mammalian delta type isoforms except that the structure includes an additional C2 domain at the N-terminus instead of the pleckstrin homology (PH) domain. We designate the gene as *Applc* (*Amoeba proteus phospholipase C*).

Genetical research on cell proliferation at low temperature in *Paramecium caudatum*

Hajime SASAKI and Nobuyuki HAGA (Dept. of Biotech., Ishinomaki Senshu Univ.)

SUMMARY

The question of how paramecia survive the winter season involves many interesting problems in the fields of ecology, physiology and genetics. Using growth experiments under low temperature, we have examined the importance of heritable characters for both proliferation and survival. To elucidate genetic effects on cell proliferation and survival in low temperature cultivation, we have examined the acclimation effect, a short-term physiological adaptation, by changing the cooling rate leading to low temperature cultivation. Two proliferative lines (KNZ2S1 #24, #26) showed growth curves after acclimation treatment (20 days incubation with a cooling rate of 1°C per day from 25°C) similar to the growth curves obtained in rapid-cooling conditions; and two non-proliferative lines (#11, #22) still showed no proliferation after acclimation treatment. These results indicate that neither proliferation nor survival under low temperature depend on the cooling rate. Average survival ratio and average proliferative activity in the progeny of a selfing conjugation of KNZ2, the original low-temperature proliferative stock, showed positive correlation. However, we could not obtain clear segregation ratios in either proliferative ability or survival in these experiments. We must therefore consider the possibility of genetic complexity underlying the mechanisms of low-temperature acclimation, and hence the wintering mechanisms.

A new type of temperature sensitive mutant of *Paramecium tetraurelia* that has long autogamy immaturity and short clonal life span

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SUMMARY

We have isolated a new type of *Paramecium tetraurelia* mutant, named d4-312, that has a long immaturity period until autogamy. As such a mutant, we previously isolated the stock d4-RK (Komori, R., et al., 2004). These two mutants

had some additional common features such as dependence of the occurrence of autogamy on the temperature, involvement of a single recessive gene, lower fission rate and shorter clonal life span. However, d4-312 was considered a new type mutant distinguishable from d4-RK because of their different natures of temperature sensitivity. First, the temperature at which they resembled the wild-type phenotype was low (19°C) in d4-312, although it was high (32°C) in d4-RK. Second, the clonal life span of d4-312 at 25°C was similar to that of the wild-type, but it was extremely shorter at 32°C than at 25°C, although it was similarly shorter at both temperatures in d4-RK. Third, the difference of the fission rate between the mutant and wild-type was greater at 32°C than at 25°C in d4-312, although it was similar at both temperatures in d4-RK. The age at sexual maturation did not correlate, within species, to the maximum clonal life span as proposed among species.

On the axenic culture of the ciliate *Tetrahymena* sp. found in a dead mosquito larva

Yuko HISATOMI, Ryoko IWAMI, Ryoko MATSUDA, Yumi AKAGAWA, Masako UCHIDA and Tadao TAKAHASHI (Biol. Lab., Nishikyushu Univ.)

SUMMARY

We succeeded in axenically culturing the ciliate *Tetrahymena* sp., which was found in a dead mosquito larva, using PY medium (1% proteose peptone, 0.5% yeast extract containing antibiotics) at 23°C (Hisatomi et al., 2003). Under the culture conditions, the cells grew logarithmically and reached the stationary phase at day 9. At that time, the cell densities reached 174,000 cells/ml. However, from day 11, some of the cells gradually assumed a round shape and subsequently underwent cytolysis. Therefore, the purpose of this work was to modify the culture method to prevent cytolysis. As a first step, the population growth was examined in the following four culture media: 1% proteose peptone (1%P), 0.5% yeast extract (0.5%Y), 1% proteose peptone with 0.1% yeast extract (P with 0.1%Y), and 1% proteose peptone with 0.01% yeast extract (P with 0.01%Y). In 1%P, P with 0.1%Y, and P with 0.01%Y, the cells grew logarithmically until day 7, and their cell densities reached 8,900 cells/ml, 28,000 cells/ml, and 18,000 cells/ml, respectively. In 0.5%Y the cells grew continuously until day 21, but the cell density reached only 7,000 cells/ml. It is clear that further modification of the culture method is required, because cytolysis occurred gradually from day 22 to day 24 in the first three media.

Encystment-inducing factors in *Colpoda* sp.: Cell-to-cell interaction and effect of components contained in exhausted medium

Takahiko AKEMATSU and Tatsuomi MATSUOKA (Inst. Biol, Fac. Sci., Kochi Univ.)

SUMMARY

Cells of the ciliated protozoan *Colpoda* sp. gradually encyst in stationary phase culture. Candidates for encystment-inducing factors could be as follows: (1) an increase in cell density; (2) molecules accumulated in exhausted medium; or (3) a decrease in encystment-suppressing elements contained in fresh culture medium (cereal infusion). We found that an increase in cell density up to 3,000 cells/ml strongly induced encystment, and replacement of the living cells with polystyrene latex particles (PLP) or glass particles also induced encystment. Cell-free exhausted medium also showed strong encystment-inducing activity, and boiling destroyed this activity. These results indicate that factors inducing encystment of the cells during the stationary phase include cell-to-cell contact due to an increase in cell density, and molecules that are unstable to heat which accumulate in exhausted medium.

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Screening of the gene controlling mating type in *Paramecium caudatum*

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SUMMARY

In *Paramecium caudatum*, when the Odd (O) mating-type and Even (E) mating-type cells are mixed together under appropriate condition, they make mating clumps that called mating reaction leading to conjugation. The genes controlling mating types were investigated by cross breeding analyses and proposed three gene hypotheses (Tsukii & Hiwatashi, 1983). According to this hypothesis, co-dominant allele *Mt* at the *Mt* locus determines E mating type, and *MA* and *MB* at the *MA* and *MB* loci determine O type. However, little is known about the substances controlled by three genes. Here, we report two attempts for molecular identification of genes involved in mating types. One attempt is the cloning by complementation cloning method. First, we tried to find the proper restriction enzyme for preparation of sub-genomic library. When E^1 DNA digested with *Dra*I was microinjected to E^3 macronucleus, only a few recipient cell lines expressed very weak E^1 type. We are searching the better restriction enzyme to prepare the library. The other attempt is differential display method for screening the genes involved in controlling mating types. Comparing the band patterns between mating reactive and non-reactive cells, several bands that expressed only in mating reactive cells were detected.

A role of mitochondria in programmed nuclear death during conjugation of *Tetrahymena thermophila*

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SUMMARY

Protozoan ciliates, represented by *Tetrahymena thermophila*, have two morphologically and functionally different nuclei in a single cytoplasm. One is a germinal micronucleus and the other is a somatic macronucleus. During conjugation, new macro- and micronuclei for the next generation are differentiated from a synkaryon (fertilized nucleus). Once the new macronucleus is differentiated, old parental macronucleus begins to degenerate. The nuclear degradation is so similar to that of the nucleus in apoptosis or programmed cell death (PCD) that this is called “programmed nuclear death (PND)”. The death process is divided into three stages, depending upon the degraded DNA sizes: 1) Initial generation of high-molecular-weight DNA fragments (>30 kb), 2) oligonucleosome-sized ladder formation, 3) Eventual complete degradation of the DNA. Previously we identified caspase-like activities in PND. Here, we show association of mitochondria with PND using two mitochondria specific dyes, DePsipher and MitoTracker. Mitochondria are incorporated into autophagosome together with the parental macronucleus prior to an entire resorption. In addition, we demonstrate that mitochondria retain a DNase activity similar to mammalian endonuclease G. Taking the mitochondrial DNase activity and the timing of the autophagosome formation into consideration, we presently conclude that the DNase activity might play a role for the oligonucleosomal fragmentation during PND.

Analysis of non-coding regions in the micronuclear DNA of *Paramecium caudatum*

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SUMMARY

Paramecium caudatum exhibits nuclear dimorphism. Each cell contains a germ micronucleus and a somatic macronucleus. During the conjugation, micronuclei underwent meiosis, followed by reciprocal exchange of gametic nuclei and fertilization. The zygotic nucleus divided three times. Four of the division products differentiated into macronuclei. It is known that chromosomal rearrangement occurs during the development by the caryonides, that is, the third cell cycle after conjugation. We analyzed the upstream region of the *pap* gene to elucidate the structure of micronuclear chromosome. When the micronuclear DNA amplified by inverse-PCR with two primers, which was designed according to the macronuclear sequence of the *pap* gene, a 6.5-kbp fragment was obtained. This fragment contains two ORFs (0.7-kbp and 0.6-kbp), and non-coding regions (6.5-kbp in total). Consequently ORFs were not clustered close in the 6.5-kbp fragment, through six ORFs were concentrated in the 6.5-kbp downstream of the pap gene. In the non-coding regions other than ORF like region CAAT boxes, GC boxes and TATA-like sequences were found.

Studies on water accumulation by contractile vacuole in *Amoeba proteus*

Eri NISHIHARA, Teruo SHIMMEN, and Seiji SONOBE (Grad. Sch. Life Sci., Univ. Hyogo)

SUMMARY

In fresh water, the osmolality of the cytoplasm is higher than that of the extracellular medium, and water always enters the cell along the osmotic gradient across the plasma membrane. The cell volume of protozoa is controlled by contractile vacuoles (CV). However, the mechanism of collecting water into the CV is poorly understood. The present study is aimed at elucidation of the mechanism of water accumulation by the CV in *Amoeba proteus*. CVs released from cells were analyzed *in vitro*. When CVs were treated with a hypertonic medium, their volume quickly decreased. This result suggests that the CV membrane is semi-permeable and that fluid is collected along the osmotic gradient *in vivo*. The water permeability of the CV membrane was calculated from the rate of osmotic volume change. The value was high, suggesting that the CV membrane is equipped with water channels. To observe CV dynamics *in vivo*, living cells were vitally stained with FM4-64. It strongly stained the plasma membrane and CV. Just after systole, the membrane of the CV was flattened. During diastole, a part of the flattened membrane expanded to a few vesicles and they fused with each other before reformation of CV.

Generation of extra contractile vacuole complex in *Paramecium*

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SUMMARY

Paramecium species normally have two contractile vacuole complexes (CVC) in a single cell. However, the number of CVC per cell (N_{CVC}) increased when a hypoosmotic and/or a Ca^{2+}-rich external condition. The CVCs of third and after were generated more remarkably in starved cells than in growing cells. The generation of extra CVCs was independent of a cell cycle progression, while the replication of CVCs occurs only when just before cell division. In the cytoplasm of growing cells, there are two clear regions that are prohibited to generate extra CVC, and the replications occur in these two regions at the same time prior to cell division. In the cytoplasm of starved cells, on the other hand, these regions might be canceled, and then extra CVCs could spread along the dorsal axis. The N_{CVC} is larger in the bigger individuals and in the bigger species.

Immunocytochemical analysis of the mastigonemal protein in zoosporic

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plant pathogen *Phytophthora*

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SUMMARY

The stramenopiles are one of the main groups of eukaryotes and characterized as having hair-like appendages called mastigonemes on the surface of their flagella. It has long been thought that the mastigonemes play an important role in cell motility, especially in flagellar function. There is, however, currently almost no information on the proteins that form the mastigonemes. *Phytophthora* and other Oomycetes which belong to the stramenopiles are known as notorious plant pathogens which cause the worldwide devastation of plant diseases. For most species of *Phytophthora*, the motile biflagellate zoospore is the main infective agent, which is responsible for rapid dissemination and initiation of infection of potential host plants. In this study, as a first attempt to elucidate the function of mastigonemes in zoospore motility at the molecular level, we have tried to produce monoclonal antibodies by immunization of isolated and purified flagella of *Phytophthora*. Consequently, we successfully obtained antibodies against various kinds of zoosporic surface components. Immunofluorescent and immunoelectron microscopy using the mastigoneme-specific antibody showed that the antigenic protein scattered throughout the mastigonemes, not on the plasma membrane or inside the flagellum. Furthermore, the antigen was also found to be localized in a certain granular organelle in the cytoplasm.

Elucidation of the energy-supplying mechanism for ciliary motility in *Tetrahymena* I. Localization of axonemal adenylate kinase in the ATP-regenerating system

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SUMMARY

The function of adenylate kinase, which has been detected in *Tetrahymena pyriformis* cilia, is to potentiate the utilization of ATP for motility. This study has shown, by low ionic strength dialysis (1 mM Tris and 0.1 mM EDTA buffer, pH 8.0), that adenylate kinases associated with ciliary axonemes differ in solubility. Adenylate kinases were solubilized together with other axonemal components such as outer- and inner-arm dyneins, radial spokes and the two central-pair microtubules, and were further separated into 16 S and 4 S fractions by sucrose density gradient centrifugation. By native-gel electrophoresis, followed by SDS-PAGE analysis, the active band of the 16 S fraction was found to consist of six polypeptides and that of the 4 S fraction of a few bands above 21 kDa. Insoluble axoneme components also possessed adenylate kinase activity. Furthermore, affinity-purified polyclonal antibody against adenylate kinase from chicken muscle (MW 21) was cross-reacted with the 21-kDa band from the insoluble axonemal proteins on immunoblots. Immunoelectron microscopy of the insoluble axoneme component suggested that immunogold-labelled adenylate kinase is located at the inner side of the outer doublet microtubules. These data suggest that the three types of adenylate kinases are located beside the axonemal proteins that require ATP.

A macronuclear division specific globular chromatin in *Tetrahymena*

Masaki ENDO and Toshiro SUGAI (Dept. Mate. Biol. Sci., Fac. Sci., Ibaraki Univ.)

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SUMMARY

In *Tetrahymena* cell, there are two kinds of nuclei, the macronucleus (MAC) and the micronucleus (MIC). MAC divides amitotically and MIC divides mitotically. In the interphase MAC, there are diffused chromatin and numerous condensed heterochromatin called chromatin body while loosely condensed chromatin aggregate of various size appears in the dividing MAC. We observed large globular chromatin of similar size in the dividing MAC in living cell and studied conditions for inducing clear globular chromatin; culturing cells in a medium containing EDTA and inducing cell division in Tris buffer containing benomyl, a microtubular drug, were effective. The globular chromatin appeared at MIC division and disappeared just after moving of the MIC from cell surface to the MAC in separated daughter cell. We compared *T. thermophila* and *T. pyriformis* and found the MAC of the former had clear globular chromatin. At first cell division of exconjugant when no MAC division occur, globular chromatin appeared in the MACs, indicating appearance of the globular chromatin is related to cell division, not to MAC division itself. It seemed that the globular chromatin is the unit of DNA distribution during division as it's numer seemed to be halved before and after division.

Determination and degeneration of a germinal micronucleus in exconjugants of *Paramecium caudatum*

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

SUMMARY

In *Paramecium caudatum* each cell has a germinal micronucleus. Artificially produced bi-micronucleate cells did not reduce to a uni-micronucleate state, at least for some fissions. However, there are four presumptive micronuclei in an exconjugant and only one of them divides at the first post-conjugational fission. There must be an exconjugant-specific reduction mechanism for the nuclear division. It is generally accepted that four of the eight postzygotic micronuclei derived from a synkaryon appear as micronuclei, and then three of them degenerate by the first post-conjugational fission. However, we obtained contradictory evidence by immunofluorescence using an anti-α-tubulin antibody and DAPI staining. Most of the four micronuclei remained even after the first post-conjugational fission, regardless of nutritional condition or stock difference. Artificial removal of the dividing micronucleus at anaphase of the first post-conjugational fission showed that the remaining micronuclei had the ability to divide at the following fissions, because each cell of the resulting clones had a micronucleus. In some clones, however, the micronuclei sometimes seemed to be degenerative because the clones produced some amicronucleate cells and were composed of both micronucleate and amicronucleate cells. To determine at what time the micronucleus is committed to divide, a micronucleus was transplanted from a vegetative G1 cell into an exconjugant. It was shown that the selective decision for nuclear division occurs between about 10 h and 24 h after the critical stage of macro- and micronuclear differentiation.

Function of the micronucleus at vegetative phase of *Paramecium caudatum*

Satoko TAKEDA and Kazuyuki MIKAMI (EEC, Miyagi Univ. of Edu.)

SUMMARY

In *Paramecium caudatum*, removal of the micronucleus causes some abnormal symptoms: structural changes of oral apparatus, decline in food vacuole formation and increase of inter-fission time, so it appears likely that the micronucleus has a specific function in stomatogenesis. To investigate whether the micronucleus is involved in forming the new oral apparatus at cell division, the length of cell cycle was compared between “proter” and “opisthe” after elimination of the micronucleus. At cell division, the new oral apparatus is formed at the opisthe and the existent one stays with the proter. Delay in the occurrence of fission appeared about three cell cycles after micronucleus removal. But the length of the two to three cell cycles after micronucleus removal was no different between proters and opisthes. This means that the mi-

cronucleus was not directly involved in stomatogenesis. In the next experiment, the cells were starved after micronucleus removal so that the time of fission was postponed. The starvation treatment delayed the fissions of both micronucleate and amicronucleate cells, but the delay was more conspicuous in amicronucleates and appeared one to two cell cycles after micronucleus removal. Appearance of the amicronucleate-specific abnormality depends not on the times of cell division but on the elapsed time after micronucleus removal.

Classification systems of phylum Ciliophora – Review of changing systems in recent years –

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SUMMARY

The ciliated protozoa are widely distributed in nature. To classify these ciliates, various classification systems have been proposed so far. Of these, three main systems were compared between each other in this work. 1) A classification system based on the oral morphological criteria was systematically constructed by Corliss (1979). In present time, this system is often used for identification of ciliates. However, this concept becomes to be hardly adopted in classification. 2) The system based on the ultrastructural morphology on the somatic kinetids was established by Small and Lynn (1981, 1985). This system, however, contained some questionable taxa, because the classification criteria more biased toward the characters of somatic kinetids. The system, thereafter, has been modified by some additional ultrastructural and molecular information. As a result, in this system the phylum Ciliophora consisted of two subphyla; Postciliodesmatophora and Intramacronucleata. 3) Lynn (2003) established a new classification system based on the molecular information, especially SSrRNA gene sequences. This system includes two “riboclasses”, which is a new concept taxon based on the molecular information. However, this system includes severe problems, that is, it is a chimeric system without reasonable explanations. From these analyses, we concluded that Lynn and Small’s system (2000), which seems to be more accomplished system than others, should be adopted for a practical use at the present time.

Opalinids as an ancestral chromalveolate inferred from alpha-, beta-tubulin genes and 18S rDNA sequence

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SUMMARY

Opalinids are enigmatic endosymbionts principally found in the large intestine of anuran amphibians. They are multinucleate and uniformly covered with numerous flagella (or cilia). Their appearance is somewhat similar to that of ciliates, so that opalinids were initially classified in ciliates. However, based on their monomorphic nuclei, absence of a ciliate-like life cycle characterized by conjugation, and an interkinetal fission mode, opalinids were subsequently transferred into the zooflagellates. Currently, opalinids are favored to be placed in the Class Opalinea, within the heterokont of the Kingdom Chromista, based on the ultrastructural characteristics of the flagellar base called transitional helix shared with the proteromonads. However, the question of their classification has not been fully resolved, because of the possession of a double-stranded ciliary necklace shared with ciliates, and lack of molecular information. Here, we show their phylogenetic position inferred from 18S rDNA and alpha-, beta-tubulin gene sequences. The 18S rDNA analysis gave the opalinids an ancestral position in heterokonts, together with the proteromonad flagellates, as suggested by the previous morphological studies. In contrast, alpha-, beta-tubulin gene analyses suggest an affiliation of opalinids to alveolates, not to heterokonts. Taking account of a few characters dispersed in the large taxon “Chromalveolata”, the present results may reflect an early branching of opalinids from ancestral chromalveolates.

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Comparison of soil testate amoeba communities under the various environments (II). – Improvement of treatment condition to estimate soil testate amoeba fauna –

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SUMMARY

This study was aimed to improve direct or viable counting methods to evaluate testate amoebae communities in upland soils with slurry application (the 0, 60, 150 or 300 t/ha). The direct counting method: Soil samples were dispersed by stirring to isolate testate amoebae. Then samples on membrane filters were stained by the EQPS method and examined with a microscope or a confocal laser scanning microscope. The viable counting method: Three gram of soil samples were inoculated on 1.5% agar plates poured by water, MNAS (Modified Neff's Amoeba Saline) [6] or KCMt [5] with or without *Enterobacter aerogenes*. Viable counts of testate amoebae were determined after 50 days incubation at 20°C. Although the active forms of testate amoebae were not detected in the 0 and 60 t/ha plots by the direct counting method, they existed 43 and 69 active cells/g wet soil in the 150 and 300 t/ha plots, respectively. The shapes of stained cells were enough clear to identify testate amoebae communities. Viable counts increased with slurry application levels. The identified species number and viable counts with KCMt media were significantly higher than those with other media. These results suggest that these counting methods would be valuable tools to examine dynamics of testate amoebae in soil.

Species Composition of Soil Ciliates and their Population Size in the Upland Soils with Slurry Application. II. Seasonal and Yearly Change.

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SUMMARY

We have already reported that the biomass of soil ciliates can be estimated by using the modified MPN method (MPN-SIPSE: Most Probable Number with Species Identification and their Population Size Estimation) (Takahashi et al., 2003). In this work, we attempted to examine the ciliate fauna and their biomass relative to seasonal and yearly changes by using the MPN-SIPSE method. From analyzing the soil samples, which were collected from the upland field with slurry application of NARC Kyushu-Okinawa Reg. Miyakonojo Branch in August 2003 and 2004, it was found that the population size of soil ciliates significantly increased with increase of the applied slurry volume in both soil samples. Further, the water content of soil samples also indicated significant positive correlation with the applied slurry volume. In May and August in 2004, the population size of soil ciliates and their biomass in soil obtained from a 60t/ha slurry-applied field were greater than those of other soils collected from 0, 150, and 300t/ha slurry-applied fields. These results indicate that the MPN-SIPSE method might be more suitable than the MPN method to estimate the population size of soil ciliates and their biomass.

Investigation of the protozoan distribution in the Nakaumi Lake

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SUMMARY

Protozoa were observed from 2002 to 2003 in relation to salinity, water temperature, pH, COD etc. in Nakaumi Lake. This lake, a typical brackish water lake, is known to have a large change in salt concentration through the year. It has become clear from our research in recent years that various protozoans exist in Nakaumi Lake. However, hardly anything is known about composition or density of species in this habitat. Therefore, 12 stations were set up to check factors which influence protozoan species composition. Inflow of seawater from the Sea of Japan into Nakaumi Lake changes sharply according to season. It is also known that the amount of inflow from the upstream Shinji Lake depends on rainfall etc. It was found that distributions of *Cinetochilum* sp., *Helicostoma* sp., and *Mesodinium* sp. positively correlated to salt concentration, and those of *Euplotes eurystomus* and *Halteria grandinella* negatively correlated to salt concentration. Moreover, *Chlamydomonas reinhardtii* and *Cinetochilum* sp. negatively correlated with water temperature, while *Gymnodinium paradoxum*, *Mastigina* sp., and *Ochromonas* sp. showed positive correlation to water temperature.

Morphological variation of the symbiotic algae in *Paramecium bursaria*

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SUMMARY

Paramecium bursaria has hundreds of symbiotic algae in its cytoplasm, and is often used for the study of symbiosis. In the light, *P. bursaria* exchanges metabolic products with its symbiotic algae (mutualism). However, in the dark, the symbiotic algae come to depend completely on the paramecium for their nutrition. To see how the morphology of the symbiotic algae is affected by light, *P. bursaria* was cultivated in continuous darkness (DD), continuous light (LL) or alternating light/dark (LD)conditions. In this study, four stocks of P. bursaria syngen 1, KN-1(mating type I), BWK-16 (II), KN-21 (III) and BWK-4 (IV) were used. After cultivating the four stocks for one week under DD conditions, the stocks were transfered to LL conditions. Morphological variations of symbiotic algae were observed using a microscope and compared in LD, DD and LL conditions over time. The size and fluorescence intensity of the symbiotic algae of stock BWK-4 were measured using a flow cytometer. In DD conditions, the size and fluorescence intensity of the symbiotic algae decreased markedly when compared with those of the symbiotic algae in LD conditions. When the paramecia cultured in DD conditions were transferred to LL conditions, the symbiotic algae recovered their fluorescence intensity and size again within one week.

Biotoxicity of acrylamide on the ciliate *Paramecium bursaria*

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SUMMARY

Monomeric but not polymeric form of acrylamide induces toxicity like carcinogenic or neurotoxic effects. Recent studies revealed that high acrylamide concentrations were found in starch-containing foods cooked at high temperatures (Press Release, WHO, 2002). However, the mechanisms of acrylamide toxicity to living organisms including human have not been well elucidated. We have introduced a convenient bioassay system using a green paramecium, *Paramecium bursaria*, possessing several hundreds of endosymbiotic algae to evaluate acrylamide toxicity of various kind of

environmental chemicals. Using this system, acrylamide toxicity has been evaluated on the *P. bursaria* (Takahashi *et al.*, 2004 (Toxicol. in Vitro 19, 99-105)). It has been also reported that a herbicide paraquat, which is known as a generator of reactive oxygen species (ROS), can produce alga-free paramecia. In this study, a production of ROS was investigated to elucidate mechanisms of the toxicity of acrylamide on the ciliate *P. bursaria*.

Search for symbiosis-related genes of symbiotic algae from *Paramecium bursaria*

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(Department of Biological Science, Faculty of Science, Hiroshima University)

SUMMARY

Paramecium bursaria harbors several hundreds endosymbiotic algae in its cytoplasm. To investigate the symbiotic association of *P.bursaria* with its symbiotic algae, we made both aposymbiotic cell strains of *P.bursaria* and the cloned strains of symbiotic algae isolated from *P.bursaria*. We have revealed that some of the cloned strains of symbiotic algae have infective activities into aposymbiotic *P.bursaria*, but the others are non-infective for aposymbiotic *P.bursaria*. In this study, we tried to search for possible symbiosis-related genes of symbiotic algae from *P.bursaria* by comparing the genetic differences between the infective and the non-infective algal strains. We have obtained three genes expressing more highly in an infective algal strain (SA-4b) and one gene expressing more highly in a non-infective algal strain (SA-4a).

Symbiosis-related genes of endosymbiotic algae of *Paramecium bursaria* syngen 1

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(Biol. Inst., Grad. Sch. of Sci., Hiroshima Univ.)

SUMMARY

The green paramecium *Paramecium bursaria* has several hundreds of endosymbiotic algae in its cytoplasm. But it is not clarified how this symbiotic association can be established and maintained. To elucidate the mechanism of symbiosis, we had removed these endosymbiotic algae from paramecium using the herbicide paraquat and symbiotic algae-free strains were prepared (Hosoya et al. 1995, Tanaka et al. 2002). Using several clones of symbiotic algae isolated from green paramecia, we have already established the conditions to let them re-infect into symbiotic algae-free paramecia (Nishihara et al. 1998). Interestingly, these cloned symbiotic algae contained two kinds of strains, infective or non-infective for algae-free paramecia, respectively. Here we compared DNA patterns developed in the infective and non-infective strains of cloned algae using a differential display and northern blot method. As a result, four cDNA fragments, which show different patterns of expression in the infective and the non-infective clones, were obtained.

Phylogeny of symbiotic algae of *Paramecium bursaria*

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SUMMARY

We have made progress on the study of phylogeny of paramecian symbionts based on the 5' half ribosomal DNA operon. All symbiotic algae we used (6 Japanese, 1 Chinese, 2 American and 1 German samples) belong to *Chlorella*

sensu stricto (Huss et al. 1999 [3]) based on the 18S rDNA phylogeny. An analysis based on internal transcribed spacer 2 unveiled two phylogenetic groups J-C-A (Japanese, Chinese and American) and German, which suggests the event of algal symbiosis have occurred more than one time.

Influence of cultivation period of symbiotic *Chlorella* on reinfection in *Paramecium bursaria*

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SUMMARY

Symbiotic algae-free *Paramecium bursaria* can be re-infected with isolated algae through ingestion via cytopharynx. It is believed that cell cycle of algae play some roles in the host-algae recognition. In the present work, we re-examined whether cell cycle and culture age of algae affect on the re-infection phenomena of *P. bursaria*. We cultivated algae for 2 weeks under 16h light (L)-8h dark (D) condition and obtained semi-synchronized algae (CA1). In 6 weeks culture under the same condition, however, growth of algae was asynchronous (CA2). When CA1 and CA2 algae were ingested by aposymbiotic paramecia, rate of algae-retaining paramecia and number of retaining algae per paramecia was significantly much when determined at 12 and 24 hours after infection. This suggests that the culture age influence on the re-establishment of paramecia-algae relationship. When algae of CA1 and CA2 were prepared at the start and the end of L-period and ingested in paramecia, infection rate was not significantly different, indicating that reassociation of algae to paramecia was not influenced by cell cycle of algae.

The mechanism of escape of symbiotic *Chlorella* sp. from the host *Paramecium* digestive vacuoles

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SUMMARY

In *Paramecium bursaria*, each symbiotic alga is enclosed in a perialgal vacuole derived from the host digestive vacuole (DV), which protects the alga from lysosomal fusion. Algae-free cells can be easily reinfected with algae isolated from algae-bearing cells by ingestion into DVs. By pulse label for 1.5 min and chase, we showed that an alga can successfully escape from the host's DV after acidosomal and lysosomal fusion with the vacuole has occurred, in order to produce endosymbiosis. When symbiotic *Chlorella* sp. isolated from *P. bursaria* were ingested into the DVs, each algal cell was pinched off into the cytoplasm and enclosed within the DV membrane, irrespective of whether the ingested cells were living or had been killed by boiling. However, this phenomenon was not observed when labeled yeast *S. cerevisiae*, India ink or latex spheres were ingested into the DVs. This suggests that a contact between the DV membrane of *P. bursaria* and the cell walls of the algae may participate in this phenomenon. Effects of various temperatures, pHs, and enzymes on abilities for escaping from the DV and for maintenance in the alga-free cell was examined.

Ecological process of producing endosymbiotic relation between *Chlorella vulgaris* and *Tetrahymena thermophila* in microcosm composed of *C. vulgaris*, *E. coli*, and *T. thermophila*

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SUMMARY

A microcosm composed of three species, including algae (*Chlorella vulgaris*), bacteria (*Escherichia coli*), and protozoa (*Tetrahymena thermophila*, containing two different mating type), was fabricated to investigate an ecological process producing the evolution of endosymbiotic relation between algal and protozoan cells. The microcosm, containing 250 ml culture medium in a glass-bottle, was cultured over 1,200 days under 12h-light/12h-dark condition at 30°C. *T. thermophila* cells containing algal cells within the vacuoles (TC cells, hereafter) were observed from the beginning of microcosm culture, whose frequency was maintained around 50% in the *T. thermophila* population until about 100th day. However, the TC cells increased in the frequency in the population, which reached about 90% until about 260th day, and maintained the frequency during the experimental period. Measurements of dynamical changes in other components of the microcosm revealed that photosynthetic activity of the algal population began to decline after 60th day, and bacterial activity of decomposing the detritus of dead cells began to increase after that. These indicate that the concentration of DO (dissolved oxygen) in the microcosm decreased at the late phase of the microcosm culture. These results and other data suggest the possibility that TC cells increased in frequency due to an adaptive advantage of obtaining oxygen from intracellular algae in a low DO environment.

Investigation of Infection Process of *Holospora obtusa* with Monoclonal Antibodies Specific for Special Tip of the Bacterium and for Actin of the Host *Paramecium caudatum*

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SUMMARY

The endosymbiotic bacterium *Holospora obtusa* infects the macronucleus of the ciliate *Paramacium caudatum*. This bacterium enters into the target nucleus with a tip of the periplasmic region first, but never with the other tip, suggesting that this special tip may have an important function for infection. In order to clarify the substances in the special tip, a monoclonal antibody against the tip was developed, and the antigen of 89k was purified from 2D-SDS-PAGE gel of the bacteria. After determination of the partial amino acid sequence of the 89k protein, a gene coding the protein was cloned from the bacterial genome using oligonucleotides against the amino acid sequence of 89k protein as proves. The ORF of the protein encoded 750 amino acids and possessed two actin-binding motifs at the N-terminal. Indirect immunofluorescence microscopy with an anti-*P. caudatum* actin antibody and anti-89 k antibody labeled the bacteria escaped from the host digestive vacuoles into the cytoplasm. The anti-89k antibody also labeled several dots on the macronuclear envelope if the nuclei were infected with the bacteria. Transmission electron microscopy showed fibrous materials around the tip in infection. Correlation between the 89k protein and the host actin was discussed.

Micronucleus-specific bacterium *Holospora elegans* enhances stress gene expressions of the host *Paramecium caudatum* ireversively

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Jpn. J. Protozool. Vol. 38, No. 1. (2005)

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SUMMARY

The bacterium *Holospora elegans* is a micronuclear-specific symbiont of the ciliate *Paramecium caudatum*. *H. elegans*-bearing paramecia could survive well compared with *Holospora*-free paramecia under the heat shock condition. And symbiont-bearing paramecia expressed high levels of hsp60 and hsp70 mRNA even at 25°C. To determine whether infection with *H. elegans* confers heat shock resistance on its host, we had prepared aposymbiotic paramecia that removed the symbiont with penicillin. However, aposymbiotic paramecia could survive at 37°C and also express high levels of hsp60 and hsp70 mRNA at 25°C. Furthermore, in order to determine whether unknown substance originated from *H. elegans* is in a micronucleus, we had established amicronucleate cell that removed a micronucleus from aposymbiotic cell by microsurgery. Surprisingly, amicronucleate cells still expressed high levels of hsp60 and hsp70 mRNA as same as aposymbiotic cells. These results suggest that the host Paramecium was made to transform the heat-tolerance ireversivly by *H. elegans*.

Cloning of the genes specifically expressed during the induction of conjugation in mating type II cells of *Blepharisma japonicum*

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SUMMARY

Conjugation of *Blepharisma japonicum* is induced by interaction between complementary mating-type cells I and II. Both mating-type cells which received complementary gamones undergo morphological changes and start to unite. It was reported that protein synthesis drastically increased during this time, and this protein synthesis was indispensable for the formation of conjugating pairs. However, it is still unknown the genes expressed specifically in the induction of conjugation. To identify genes involved in these processes, we isolated genes expressed specifically in conjugation-induced type II cells by a cDNA subtraction method. To induce mating-pairs, we treated type II cells for 4 hours with cell-free fluid of type I cells containing gamone 1, then purified polyA$^+$RNA. PolyA$^+$RNA was subjected to cDNA synthesis, and the cDNA was subtracted between such treated cells and untreated cells.We have done subtraction twice and obtained 8 products. We sequenced some of them. Homology search revealed that the two of these fragments showed significant homology to CDK family (Cdc2 and Cdk2). Northern hybridization demonstrated that the transcription occurred specifically during the induction of conjugation. We also found that the transcript already appeared 2 hours after gamone 1 treatment.

Development of a transformation system with mitochondrial plasmids for *Paramecium caudatum*

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SUMMARY

Mitochondrial plasmids found in *Paramecium caudatum* in 1994 have been so far studied for their structure, but their physiological functions are still unknown. To clarify this, we attempted to introduce isolated plasmid DNA into plasmid-free recipient cells. Cells were treated with ice-cold calcium chloride and the plasmid DNA. After the treatment, cells

were cultured for about three weeks, and then DNA was isolated from the cells to conduct PCR using primers constructed from known sequences of the plasmid (type Ia). As a result, plasmid-specific sequences were amplified from the isolated DNAs in 7/61 samples, indicating that plasmid DNA was introduced into the recipients. Furthermore, plasmid-specific sequences were detected in cells which were cultured for three months after the treatment, suggesting that imported plasmids can multiply within the recipient cells. Fractionation of the cells suggests that the imported plasmids are present in the cytoplasm (outside of mitochondria).

A reverse flow of genetic information from the somatic Mac to germinal mic suggested by the presence of a processed pseudogene of a glyoxysomal citrate synthase gene in *Tetrahymena thermophila*

Atsushi MUKAI (Div. Life Sci., Grad. Sch. Natural Sci. and Technol., Kanazawa Univ.)

SUMMARY

Two glyoxysomal citrate synthase genes, a functional gene and a pseudogene, were identified in *Tetrahymena thermophila*. Phylogenetic analysis revealed that this gene was directly derived from bacteria such as green sulfur bacteria or proteobacteria via lateral gene transfer (LGT). On the other hand, the pseudogene was characterized by the loss of introns. The absence of the introns indicates a duplication from the functional counterpart via mRNA intermediate. The discovery of a processed pseudogene suggests that genetic information reversely moves from the macronucleus to micronucleus mediated by reverse transcription. Because of the low fidelity of reverse transcriptase, this direction of gene flow may facilitate acceleration of the evolutionary rate of protein-coding genes in ciliates by accumulating mutations into the micronuclear gene via homologous recombination.

Highly clustered gene organization in the *Paramecium caudatum* somatic macronuclear genome

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SUMMARY

Each cell of *Paramecium caudatum* has a somatic macronucleus and a germinal micronucleus. Ploidy of the macronucleus is approximately 3,400n (Soldo *et al*., 1981) and assure all gene expression. So far, only 25 genes in *P. caudatum* have been registered in database (NCBI). To expect the overall structure of the *P. caudatum* somatic genome, I randomly cloned 6 fragments of the macronuclear DNA ranging from 6 to 10 kb, and sequenced totally ~50 kb. Sequence analysis revealed at least 24 known and potential protein-coding genes, suggesting highly cluster gene organization. Most of the the genes were arranged in the same direction beside one gene, and intergenic regions are short (42-1212 bp). All of introns identified in this study are also short, as expected previously. This pilot genome analysis suggests that *P. caudatum* retains the highest coding density (78%) on chromosomes among eukaryotes including *P. tetraurelia* (74%) and budding yeast (70%). This clustered organization must be the result of adaptation for achieving an extremely high copy number (3,400 copies) in the macronucleus.

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Transposition of artificial Tc1 transposon into macronuclear genome in *Paramecium*

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SUMMARY

In the ciliate, *Paramecium tetraurelia*, thousands of internal eliminated sequences (TA-IESs) are excised from the germ-line micronuclear DNA during macronuclear differentiation. Based on the resemblance of TA-IES end sequences to Tc1 transposon termini, it has been proposed that TA-IESs might have degenerately evolved from Tc1 family transposons. To know the mechanism of the transposon invasion into macronuclear genome and evolutionally into the micronuclear genome, using a *Tetrahymena* metallothionein gene (*MTT1*) UTR as a promoter, I constructed an artificial Tc1 transposon which can move within the genome of *Paramecium*. After plasmids carrying the artificial Tc1 transposon was microinjected into the macronucleus of *P. caudatum*, the injected cell were treated with $CdCl_2$, expression of the Tc1A transposase was confirmed by RT-PCR, indicating that the *MTT1* promoter is functional in *P. caudatum*. Although not so efficient, successful amplification of the introduced transposon suggests that transposition into the macronucler genome did occur. The ultimate aim of this study is to make a reproduction of an evolutionary process in laboratory from invasion and fixation of transposons. The first step of this study has been cleared by the success of the artificial transposon.

Contraction deficiency of *Carchesium polypinum* by SH-modifying reagent and its 200 kDa protein

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SUMMARY

It has been thought for a long time that the chemical modification of cysteine amino acid residues in *Vorticellidae* spasmonemes does not cause any loss in Ca^{2+}-driven contractility. One of the reasons is that spasmin, which belongs to one of the calmodulin superfamilies, and is the most important protein in contractile spasmonemes, does not contain any cysteine residues. Another reason is that nobody, including us, has used glycerinated *Carchesium* sp. for contractility experiments. It is well known that an old stalk of *Carchesium* sp. is easily and naturally deteriorated and loses its contractility. We have recently succeeded in collecting many young colonies of *C. polypinum* and cultivating them in the presence of *Chlamydomonas reinhardtii* and bacteria as food. We have tried to modify the young stalks of glycerinated *C. polypinum* using DAM (N-7-dimethyl-amino-4-methyl-coumarinyl-maleimide) as a fluorescent cysteine-group modifying reagent. We discovered that the contractility was significantly reduced even in young stalks of *C. polypinum*. The spasmoneme in a stalk of *C. polypinum* was fluorescently labeled. On the other hand, the spasmoneme in a stalk of *Vorticella* sp. was not fluorescently labeled. A 200 kDa protein extracted from *Carchesium* spasmonemes was also fluorescently labeled. These results mean that both the novel 200 kDa protein and spasmin, as a Ca^{2+}-binding protein, play important roles for spasmoneme contraction. In another experiment, a novel 50 kDa protein in the spasmoneme of *Vorticella* sp. was found to be important for its contraction. It is thus suggested that the 200 kDa protein in *Carchesium* spasmonemes is evolutionarily a tetrameric form of the 50 kDa protein in *Vorticella* spasmonemes.

Distribution of centrin-like proteins in the ciliate *Spirostomum ambiguum*

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SUMMARY

The large heterotrichous ciliate *Spirostomum* is known for its remarkable rapid cell contraction that is triggered by external stimuli, and the cytoskeleton-based motile systems of contraction and subsequent re-elongation have been studied in order to understand its underlying molecular machineries. The myoneme in the cortex is thought to generate the force for the Ca^{2+}-dependent contraction, and may be similar to the spasmoneme in the stalk of *Vorticella*. In *Vorticella*, an antibody to centrin 1 has been reported to recognize the spasmoneme. In view of these facts, we have examined the possible existence of centrin in *Spirostomum* using immunohistological and immunobiochemical techniques. The specimens of *Spirostomum* were electrophoresed and blotted, and the antibody to centrin 1 specifically stained a single band of 18 kDa in an extract of *Spirostomum*. When frozen thin sections of *Spirostomum* were stained with anti-centrin 1, fluorescent signal or deposits of tetramethylbenzidine (TMB) substrate were detected at the cell cortex. These results suggest that a centrin 1-like protein may exist in the cortex of *Spirostomum*. Further studies with electron microscopy are needed to understand the precise localization of the centrin 1-like protein in *Spirostomum*.

The occurrence of pathogenic *Naegleria* amoebae in thermal waters in Japan

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A survey to document the presence of pathogenic *Naegleria* species was conducted for the thermal waters of 25 prefectural areas in Japan. A tota1 of 1,173 samples were collected from hot springs, public spas and thermal discharge from those sources or industry. Test plates were incubated at 42°C for up to 3 days to isolate thermophilic *Naegleria* species. Amoeba isolates were morphologically identified to genus level. *Naegleria* isolates were further characterized genetically by means of PCR/RFLP and DNA sequence analysis of Internal Transcribed Spacer (ITS) regions of ribosomal DNA. Amoebae were isolated from 357 (30.4%) of the total samples. *Naegleria* positive samples represented 14.7% of the total samples. *N. lovaniensis*, a non-pathogenic species, was recovered from 20 out of 25 areas. Although *N. fowleri*, a human pathogen, was not isolated from any samples tested, *N. australiensis*, experimentally fatal pathogen to mice, was recovered from 10 areas, mostly isolated from thermal discharge samples with high level of bacteria. The prevalence of these amoeba was correlated with a concentration of residual chlorine of the samples. As the results of pathogenicity test on *N. australiensis* strains, six of 16 strains killed the intracerebrally infected mice. Other 3 strains limited to cause some types of abnormal behavior of the intracerebrally infected mice.

Early evolution of extrusomes in ciliates

Akio MIYAKE

SUMMARY

Pigment granules (pigmentocysts) are spherical, morphologically-simple extrusomes. They are widely distributed among the two groups of primitive ciliates, heterotrichs and karyorelictids and carry out chemical defense against predators by means of the toxic pigments which they contain. Cortical granules are morphologically similar to pigment granules and are widely present in heterotrichs. Some of them were found to carry out the chemical defense by means of

colorless toxins. Based on these recent findings (Miyake, A., Jpn. J. Protozool., 35: 97-11, 2002) and previously known studies (Rosati, G. & Modeo, L., J. Eukaryot. Microbiol., 50: 383-402, 2003) on extrusomes, it was assumed that extrusomes in ciliates started as toxin-containing cortical granules and evolved as organelles of predator-prey interaction.

Comparison of phylogenies between symbiotic flagellate genus *Teranympha* and host termites

Takeshi KONDO and Osamu KITADE (Faculty of Science, Ibaraki University)

We examined SSUrRNA gene sequences of symbiotic flagellates of the genus *Teranympha* derived from four *Reticulitermes* termite species distributed in and around the Japan Archipelago. The inferred phylogenetic tree using neighbor-joining method was comprised of three monophyletic groups supported with more than 80% bootstrap probabilities. For the most parts, the tree topology was consistent with both the phylogeny of host termites inferred from mitochondrial genes and a paleogeological hypothesis of the formation process of the Japan Archipelago, suggesting the co-speciation between host termites and *Teranympha* symbionts. However, clones of *Teranympha* obtained from *R. speratus* of the Aichi population and those from *R. kanmonensis* were separately included in different clusters. If such clones were not artifacts, possible causes of the results might be lateral transfers of flagellates or lineage sorting among alleles of the SSUrRNA gene.