Abstracts of the JSP meeting 2012

Oral presentations

1) Mitochondrial nuclease: a major executor of the programmed nuclear degradation in *Tetrahymena thermophila*
Eriko OSADA

2) Division and death of mitochondria in conjugation of *Tetrahymena thermophila*
Mai NAGASHIMA and Hiroshi ENDOH

3) Studies on the mating-type substances in *Paramecium caudatum*: determination of DNA sequence for Hp-1 which is a ciliary membrane specific protein associated with mating reactivity in *P. caudatum*
Yuta CHIBA and Nobuyuki HAGA

4) Excystment-dependent alteration of protein expression in ciliated protozoan *Colpoda cucullus*
Yoichiro SOGAME, Katsuhiko KOJIMA, Toshikazu TAKESHITA, Eiji KINOSHITA and Tatsuomi MATSUOKA

5) Protein analyses in mucopolysaccharide-containing ciliated protozoan *Colpoda cucullus*: two-dimensional electrophoresis of SDS-containing samples in encystment-induced cells
Ken MOCHIDUKI, Yoichiro SOGAME and Tatsuomi MATSUOKA

6) Analysis of growth controlling activity in the culture medium of green paramecia *Paramecium bursaria*
Yoshihiko YAMASHIN, Eiji HIRAKI, Koyo TETSUKAWA, Yuka ADACHI, Kosuke MATSUBARA, Kozue HAMAO and Hiroshi HOSOYA

7) Electrical responses of *Euglena gracilis* in the electromagnetic field at different electrode distances
Lin CHEN and Toshinobu SUZAKI

8) Feeding habit of *Bodo saltans*
Yurie SHIRAKAWA, Osamu NISHIMURA and Ryuichi SUDO

9) Morphological observation of a novel green *Raphidiophrys* sp.
Masashi M. HAYAKAWA, Rina MATSUMOTO and Toshinobu SUZAKI

10) Relationships between termite reproductives and symbiotic protists: gut flagellates are not harbored in mature queens and kings
Keisuke SHIMADA, Osamu KITADE and Kiyoto MAEKAWA

11) Extension and contraction mechanism of the proboscis in *Lacrymaria* sp.
Ryuji YANASE, Yukinori NISHIGAMI, Teruo SHIMMEN and Seiji SONOBE

12) A representative model of the gliding motion of a diatom, *Bacillaria paradoxa,* predicted from the action of elastic fibrils
Nozomi YAMAOKA, Yasutaka SUETOMO, Teruo SHIMMEN and Seiji SONOBE

13) Three-dimensional analysis of the plasmalemma and cytoplasm dynamics during locomotion of *Amoeba proteus*
Atsushi TANIGUCHI, Yukinori NISHIGAMI, Hiroko KOBAYASHI, Daisuke TAKAO, Teruo SHIMMEN, Shigenori NONAKA and Seiji SONOBE

14) Localization and properties of *Tetrahymena* formin
Fumihide BUNAI and Osamu NUMATA

15) Novel features in the formation and function of the “9 + 2” axoneme revealed by a basal body-deficient mutant of *Chlamydomonas*
Yuki NAKAZAWA, Tetsuro ARIYOSHI, Ritsu KAMIYA and Masafumi HIRONO

16) Phosphoinositide-specific phospholipase C (PI-PLC) of *Amoeba proteus*
Hitoshi YAGISAWA, Ryoko SHINKI-HORIE, Kayoko MAEDA-AKAHANE, Teruo SHIMMEN and Seiji SONOBE

17) Determination of Immaturin gene sequence: an intragenic structure responsible for sexual rejuvenescence in *Paramecium*
Nobuyuki HAGA, Toshinori USUI and Tomoaki ABE

18) Comprehensive search for the nuclear pore complex proteins of ciliate *Tetrahymena thermophila*
Masaaki IWAMOTO, Hiroko OSAKADA, Chie MORI, Koji NAGAO, Chikashi OBUSE, Yasushi HIRAOKA and Tokuko HARAGUCHI

19) Soil testate amoebae fauna in Japan, re-evaluation
Anatoly BOBROV, Satoshi SHIMANO and Yuri MAZEI

20) Analysis of photosynthetic products in green ciliate *Paramecium bursaria*
Hiroshi HOSOYA, Eiji HIRAKI, Koyo TETSUKAWA, Yuka ADACHI, Kosuke MATSUBARA and Kozue HAMAO

21) Specific connections between symchlorosomes and mitochondria in *Paramecium bursaria* as verified by electron tomography and 3D reconstruction from serial sections
Chihong SONG and Toshinobu SUZAKI

22) New method to eliminate germinal chromosome in *Tetrahymena* and observation of the arrangement of meiotic chromosome using nullisomics and unisomics obtained by the method
Toshiro SUGAI and Chie KAJIWARA

23) Prevalence of *Sarcocystis* in slaughtered horses and imported horse meat
Kenji YAGITA

24) Transfer mechanism of spasmin–Ca^{2+} binding energy to novel elastic protein spaconnectin
Hiroshi ASAI and Yasushige MORIYAMA

Poster presentations

P1) Function of glycocalyx in cell adhesion and cell locomotion on *Amoeba proteus*
Yukinori NISHIGAMI, Hisato IKEGAYA, Rika SHIBATA, Atsushi TANIGUCHI, Teruo SHIMMEN and Seiji SONOBE

P2) Isolation of actin filaments from a diatom, *Craticura* sp.
Yuuka KISHIMOTO, Nozomi YAMAOKA, Teruo SHIMMEN and Seiji SONOBE

P3) Ultrastructural changes during rapid axopodial contraction in heliozoon *Raphidiophrys contractilis*
Risa INOUE, Midori EDAMATSU and Motonori ANDO

P4) Screening of microbial anti-trypanosomal compounds using *Bodo saltans*
Koichiro SUMITANI and Nobutaka IMAMURA

P5) Gametogenesis in *Noctiluca scintillans* under light-dark cycle
Takumi KITAMURA and Hiroshi ENDOH

P6) Construction of foreign protein secretion system in *Tetrahymena thermophila*
Kohei MASUDA and Hiroshi ENDOH

P7) Measurements of phagocytic activities of *Paramecium bursaria* using GFP-labeled yeast
Takashi MIURA and Sosuke IWAI

P8) Role of the two-headed inner dynein arm in the ciliary movement in *Paramecium tetraurelia*
Takuya KATOH, Manabu HORI, Kanako INOUE, Osamu KUTOMI and Munenori NOGUCHI

P9) Maltose release mechanism of symbiotic *Chlorella* from Japanese *Paramecium bursaria*
Aika SHIBATA and Nobutaka IMAMURA

P10) Culturing condition and isolation of micronuclei in *Blepharisma japonicum*
Ryota KOKUTANI and Hiroshi ENDOH

P11) Do the mating pheromones of genus *Blepharisma* play an important role in speciation?
Mayumi KOBAYASHI, Kiyono SHINOHARA, Mari TAKUSAGAWA and Terue HARUMOTO

P12) Study on the amino acid residues which are involved in the stop codon recognition in ciliate eRF1s
Ying LI, Oanh KIM T.P., Do Thi HONG, Adriana VALLESI and Terue HARUMOTO

P13) Species identification of wild strains and molecular phylogenetic analysis of *COI* gene in the genus *Blepharisma*
Kiyono SHINOHARA, Mayumi KOBAYASHI and Terue HARUMOTO

P14) Nickel-induced genes in *Paramecium caudatum*
Yasuhiro TAKENAKA, Nobuyuki HAGA and Ikuo INOUE

P15) Properties of *Paramecium bursaria* cells in the stationary phase
Sosuke IWAI and Takashi MIURA

P16) Species composition and structure of symbiotic protist community in the termite *Coptotermes formosanus*
Osamu KITADE

P17) New bio-remediation technique for radioactive cesium in contaminated soil using *Paramecium bursaria*
Chisato YOSHIMURA and Toshinobu SUZAKI

#1

Mitochondrial nuclease: a major executor of the programmed nuclear degradation in *Tetrahymena thermophila*

Eriko OSADA

(Div. Life Sci., Grad. Sch. Nat. Sci. Technol., Kanazawa Univ.)

SUMMARY

During conjugation of *Tetrahymena thermophila*, three meiotic products out of four and an old macronucleus are programmed to degrade in different stages, following to differentiation of new macronuclei. Mitochondria are involved in this programmed nuclear death (PND) as a main player. Prior to the nuclear degradation, a lot of mitochondria lose their membrane potential and are incorporated into autophagosome. The sequestered mitochondria move to the old macronucleus, where they release many apoptotic molecules, such as apoptosis-inducing factor (AIF) and yet-unidentified nucleases. To identify these nucleases, I previously searched multiple mitochondrial proteins from *Tetrahymena* that have nuclease activity using SDS-DNA-PAGE. Especially, a protein of approximately 15 kDa showed the highest and predominant activity. Mass spectrometry analysis revealed three candidate genes of the nuclease (TTHERM_00426240: called Tn1 provisionally here, TTHERM_00666370: Tn2 and TTHERM_00825290: Tn3). All of these proteins were confirmed to localize in mitochondria. Gene disruption of Tn1 resulted in 1) inhibition of degradation of the meiotic products and 2) delay of degradation and resorption of the old macronucleus. Actually, Tn1-deficient mitochondria showed a drastically decreased nuclease activity, when compared with wild type mitochondria. These observations strongly suggest that Tn1 is one of the major executors of PND.

#2

Division and death of mitochondria in conjugation of *Tetrahymena thermophila*

Mai NAGASHIMA[1] and Hiroshi ENDOH[2]

([1]Dept. Sci. Engn., [2]Grad. Sch. Nat. Sci. Technol., Kanazawa Univ.)

SUMMARY

Tetrahymena thermophila have a unique apoptotic event in conjugation, in which only parental macronucleus is selectively degraded, but newly differentiated micro- and macronuclei are unaffected. Therefore, this is called programmed nuclear degradation (PND). Mitochondria are involved in this process as a major death executor which carries various death effectors in its inside. During PND, many mitochondria lose membrane potential, and then migrate to the parental macronucleus destined to die, after incorporated into autophagosomes. It is unknown that mitochondrial death is accompanied with their division or not. To ask if mitochondria are programmed to divide prior to death, we treated conjugating cells with ethidium bromide (EtBr), which is known an inhibitor of mitochondrial DNA polymerase "gamma", at certain time points. Addition of EtBr before meiosis resulted in inhibition of meiosis I, but the macronuclear condensation occurred, irrespective of no new macronuclear differentiation. When EtBr was added at fertilization stage, postzygotic nuclear divisions (PZD) were disturbed. Addition after PZD, EtBr inhibited the degradation, where the parental macronucleus suspended before the stage of degradation. The former 2 inhibitions might be due to those of nuclear divisions directly, whereas the latter is likely to have resulted from inhibition of mitochondrial divisions and death.

#3

Studies on the mating-type substances in *Paramecium caudatum*: determination of DNA sequence for Hp-1 which is a ciliary membrane specific protein associated with mating reactivity in *P. caudatum*

Yuta CHIBA and Nobuyuki HAGA

(Dept. Biol. Engn., Fac. Sci. Engn., Ishinomaki Senshu Univ.)

SUMMARY

In *Paramecium*, mating type substances play a role to recognize mating types in mating reaction. Previous studies suggested that mating type substances are the integral ciliary membrane proteins (Kitamura et al. 1988). However, no one has so far identified these substances. In this study, we prepared ciliary membrane fractions (CMFs) with treatment of Triton X-114. SDS-PAGE analysis indicated that a polypeptide with molecular weight of about 52 kDa was specifically detected in CMFs prepared from mating reactive cilia, but not in ones from mating non-reactive cilia. We named this polypeptide as Hp-1 (hypothetical protein #1), and have attempted to analyze its function. Based on three partial amino acid sequences from mass spectrometric data, *Hp-1* DNA sequence was determined. The DNA sequence of Hp-1 was found to be a novel gene, which contains one serine/threonine kinase c domain and four EF hand motifs. Semi-quantitative RT-PCR showed that Hp-1 mRNA was detected in mating reactive cells under the conditions which cannot detect mRNA in non-reactive cells. Western blotting analysis using anti-Hp-1 polyclonal antibody showed that Hp-1 was strongly detected in mating reactive CMFs, but was not so strong in non-reactive ones. These results suggest that the PKC domain with four EF hands in Hp-1 may has an essential role in the expression of mating reactivity.

#4

Excystment-dependent alteration of protein expression in ciliated protozoan *Colpoda cucullus*

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SUMMARY

SDS-PAGE of the total proteins contained in excystment-induced *Colpoda cucullus* showed enhancement of the expression levels of 65-kDa, 60-kDa and 44-kDa proteins at 10 min after the onset of excystment induction. Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis showed that the p60 was DEAD-box RNA helicase-like protein. In the later stage (30–60 min after the onset of excystment induction), alterations in the expressions of 50-kDa and 49-kDa proteins (mitochondrial ATP synthase β chain and elongation factor 1α, respectively) were observed; p50, which had disappeared during encystment, was expressed again, while p49, which is expressed during encystment, disappeared.

#5

Protein analyses in mucopolysaccharide-containing ciliated protozoan *Colpoda cucullus*: two-dimensional electrophoresis of SDS-containing samples in encystment-induced cells

Ken MOCHIDUKI, Yoichiro SOGAME and Tatsuomi MATSUOKA

(Dept. Biol. Sci., Fac. Sci., Kochi Univ.)

SUMMARY

When the samples obtained by a direct solubilization of *Colpoda cucullus* cells with an IEF sample buffer without SDS were analyzed on 2-D PAGE, any clear protein spots were not obtained. The 2-D PAGE of a bacterial (*Klebsiella pneumoniae*) protein sample mixed with *Colpoda* cell components also failed to obtain the clear spots. On the other hand, 2-D PAGE of bacterial proteins mixed with lysozyme-treated *C. cucullus* components was successful at protein focusing. These results suggest that protein aggregation through mucopolysaccharides may prevent proteins from isoelectric focusing. When *C. cucullus* samples were pre-treated with a SDS sample buffer for SDS-PAGE and mixed with an IEF sample buffer, the spot resolution improved in 2-D PAGE. We showed, using the above-mentioned method, alteration of the expression level of the several proteins during encystment of *C. cucullus*.

#6

Analysis of growth controlling activity in the culture medium of green paramecia *Paramecium bursaria*

Yoshihiko YAMASHIN, Eiji HIRAKI, Koyo TETSUKAWA, Yuka ADACHI,
Kosuke MATSUBARA, Kozue HAMAO and Hiroshi HOSOYA

(Dept. Biol. Sci., Grad. Sch. Sci., Hiroshima Univ.)

SUMMARY

A medium for the culturing of green paramecia *Paramecium bursaria* has been developed by Weis. It is now used consisting of lettuce infusion inoculated with bacteria. Sometimes, green paramecia are cultured in a mixture of the lettuce infusion and CA medium, which is a widely employed medium used for the culturing of green algae. However, in those culture media, the initial growth rate of paramecia or final concentration of paramecia in the stationary phase are not coincided with each experiments. Here, to elucidate the stable experimental conditions for the culture of green paramecia, we tried to culture green paramecia in several kind of media with or without bacteria. In this meeting, our results obtained will be discussed.

#7

Electrical responses of *Euglena gracilis* in the electromagnetic field at different electrode distances

Lin CHEN and Toshinobu SUZAKI

(Dept. Biol., Grad. Sch. Sci., Kobe Univ.)

SUMMARY

The non-invasive technique of dielectric analysis can be used to monitor changes in electrical characteristics of biological cells or tissues, which is influenced by environmental factors such as toxic substances in the surrounding water. In this study we compared the dielectric behavior of *Euglena gracilis* cells that were confined in parallel-capacitor -like measuring chambers with different electrode distances (8.4 and 7.8 mm). From the observed data set, we obtained characteristic parameters for simulating the observed dielectric behavior of the cell suspension with "dielectric ellipsoidal -shell" models. For the model fitting, the *Euglena* cell was considered as an ellipsoid with a surrounding cell membrane and some cytoplasmic inclusions such as nucleus, chloroplasts and mitochondria, being considered as solid ellipsoids. From the fitting curves, we could obtain a new group of characteristic parameters, which is to be used for real-time biological monitoring of water quality with *Euglena*.

#8

Feeding habit of *Bodo saltans*

Yurie SHIRAKAWA, Osamu NISHIMURA and Ryuichi SUDO

(Grad. Sch. Engn., Tohoku Univ.)

SUMMARY

In this study, we tried to inspect the bacterivory of *B. saltans* isolated from activated sludge. Using with different kinds of fluorescent labeled bacteria, we determined the in situ bacterial abundance those were preyed, and also measured specific growth rate of *B. saltans*. The results of specific growth rate of *B. saltans* were 6.35/day with *Pseudomonas fluorescens*, 4.85 with *Escherichia coli,* 3.08 with *Bacillus subtulis*, 3.28 with *Bacillus cereus,* 3.74 with *Alcalignes faecalis*, 2.93 with *Flavobacterium aquatile*, 2.17 with *Enteroccocus faecalis*, and 1.20/day with *Kocuria varians.* According to the fluorescence labeled bacterial uptake ratio between *P. fluorescens* and *K. varians*, we could not find significant differences within 3 hours. It was concluded that *B. saltans* is able to prey many kinds of bacteria, and compared with other protozoan specific growth rate, it is suggested that *B. saltans* might survive in divers environment. And more, specific growth rate has been changed by bacterial species, some components contained in specific bacteria may increase the growth of *B. saltans*.

#9

Morphological observation of a novel green *Raphidiophrys* sp.

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SUMMARY

Protozoans bearing zoochlorellae (endosymbiotic green algae) have been reported in various protozoan groups. Several centrohelid heliozoan species (e.g. *Acanthocystis turfacea*, *Raphidiophrys viridis*) were reported as "green" hosts for zoochlorellae. We newly found and cultured a strain of unicellular green *Raphidiophrys* from a high moor marsh in Nasu-Shiobara, Japan, last year. While no unicellular strain of green *Raphidiophrys* has been reported so far, this strain is likely to be a new species. Light and electron microscopic observations of this novel strain were carried out. Ultrastructure of the siliceous scales of this strain was examined in details, which resembled those of *R. elegans* (multicellular but not green species) with characteristic parallel striations on the surface of the scale, but different from those of *R. viridis*, a multicellular species of zoochlorellae-bearing *Raphidiophrys*.

#10

Relationships between termite reproductives and symbiotic protists: gut flagellates are not harbored in mature queens and kings

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SUMMARY

The symbiotic relationship between lower termites and cellulolytic flagellate protists in their hindguts is one of the most well-known examples of mutualism. Although the symbiosis in worker termites has attracted much attention, there have been very few studies of protists in other castes. We have performed the first examination of protist population dynamics in queens and kings during termite colony foundation. Protist numbers and reproductive tissue sizes were measured at five time points over 400 days in incipient colonies of *Reticulitermes speratus*, as well as in other castes of mature colonies. The results showed that queens and kings with slightly developed ovaries and testes had large amount of protists from 30 to 100 days compared with alates and workers. On the other hand, they lost protists by day 400, and developed their ovaries and testes. Unlike workers and soldiers, our study showed that symbiotic protists are not harbored in mature queens and kings.

#11

Extension and contraction mechanism of the proboscis in *Lacrymaria* sp.

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SUMMARY

A ciliate, *Lacrymaria*, has a long proboscis which is highly elastic. It's considered that the extension of the proboscis is due to both microtubule bundles under the pellicle and the long cilia at the oral region of the proboscis. While, the contraction of the proboscis is thought to be due to the myoneme, which contract depending on a calcium ion. However, any direct proofs for these hypothesis have not presented. We examined the relationship between the movement of the proboscis and the cilia by observing the movement of cilia in the presence or absence of the oral region. We also analysed the movement of proboscis by using a high-speed camera in detail and the movement of the proboscis separated from the body of *Lacrymaria*. Moreover, we prepared a detergent-extracted cell models and added a solution containing ATP or a calcium ion. In a solution containing ATP, the movement of cilia occurred but the proboscis didn't extend. In a solution containing a calcium ion, *Lacrymaria* showed the gradual contraction. These results suggested the possibility that the movement of oral cilia decides a direction of the extension of proboscis and the contraction of proboscis is performed by the calcium ion-dependent contractile structure.

#12

A representative model of the gliding motion of a diatom, *Bacillaria paradoxa,* predicted from the action of elastic fibrils

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SUMMARY

Bacillaria paradoxa belongs to pinnate diatom and forms a colony consisting of 2–30 cells. Adjacent cells show active gliding each other, but its mechanism and physiological meanings are not understood. Alexa 488-phalloidin staining revealed that two actin bundles are present along with the raphe, elongated slit in the frustule. Both latrunculin B, an actin polymerization inhibitor, and 2,3-butanedione monoxime, a myosin inhibitor, inhibited gliding motion, suggesting an essential role of actomyosin system. Western blotting using antibodies against tobacco BY-2 actin and against *Amoeba* myosin shows the presence of actin and myosin in *Bacillaria paradoxa*. We found that a single cell separated from a colony shows a motion in back and forth at a point attaching to the substratum, and succeeded in observing a motion of plastic beads attached to the cell surface at the rapha. We believe that this motion reflect the motion of the elastic fibrils extending from raphe. Here, we present a curtain hook model for the gliding motion of *Bacillaria paradoxa*.

#13

Three-dimensional analysis of the plasmalemma and cytoplasm dynamics during locomotion of *Amoeba proteus*

Atsushi TANIGUCHI[1], Yukinori NISHIGAMI[1], Hiroko KOBAYASHI[2], Daisuke TAKAO[2], Teruo SHIMMEN[1], Shigenori NONAKA[2] and Seiji SONOBE[1]

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SUMMARY

During locomotion of *Amoeba proteus*, the plasmalemma is considered essential for amoeboid movement, but its dynamics remains to be explained. To solve this problem, we carried out two-dimensional simultaneous observations of both the plasmalemma and the cytoplasm from a side by a Digital Scanned Light-sheet Microscopy (DSLM). *A. proteus* were stained with 1,1‘-dioctadecyl-3,3,3’,3‘-tetramethylindocarbo-cyanine perchlorate (DiI) for the plasmalemma and with MitoTracker DeepRed for mitochondria. Consequently, both ventral and dorsal plasmalemma showed forward flow, suggesting that the plasmalemma dynamics seemed to be explained by the total folding and unfolding model (TFU model; the plasmalemma has many folds, and the folds are stretched along with locomotion). In addition, the gap between the plasmalemma and ectoplasmic gel was suggested, because mitochondria in the ectoplasmic gel did not move against the substratum. Observation by high-speed imaging DSLM (ezDSLM) also showed that the plasmalemma dynamics of *A. proteus* is basically consistent with TFU model. Interestingly, revolving movement of the plasmalemma at the uroid region was observed.

#14

Localization and properties of *Tetrahymena* formin

Fumihide BUNAI and Osamu NUMATA

(Grad. Sch. Life Environ. Sci., Univ. Tsukuba)

SUMMARY

We suggested that cytokinesis of *Tetrahymena* is occurred by constriction of actin ring just under the division furrow. In animal cells, Adf/cofilin and profilin regulate actin depolymerization and polymerization respectively, and those are essential components of contractile ring. However knockout of *Tetrahymena* Adf/cofilin and profilin genes showed no effects on cytokinesis. So we focused on formins which are involved in the polymerization of actin and are Rho-GTPase effector proteins. Formins regulate the actin and microtubule cytoskeleton and are involved in various cellular functions such as cell polarity, cytokinesis, and cell migration. We found 2 formin-like genes, BNI1 and BNI2, in the *Tetrahymena* gene expression database (http://tged.ihb.ac.cn/Default.aspx). The pull down assay showed that BNI1 directly bound to profilin. Immunofluorescence microscopy with anti-BNI1 and anti-actin antibodies revealed the colocalization of those in the actin ring and BNI1 is involved in the formation of the actin ring. BNI2 was localized in the cell surface structures, contractile vacuole pores and the dividing micronucleus. In the dividing micronucleus, BNI2 colocalized with chromatin, suggesting that BNI2 has roles in chromatin condensation and chromatin segregation.

#15

Novel features in the formation and function of the “9 + 2” axoneme revealed by a basal body-deficient mutant of *Chlamydomonas*

Yuki NAKAZAWA, Tetsuro ARIYOSHI, Ritsu KAMIYA and Masafumi HIRONO

(Dept. Biol. Sci., Grad. Sch. Sci., Univ. Tokyo)

SUMMARY

The “9 + 2” structure of ciliary or flagellar axoneme consists of the nine outer doublet microtubules and the central pair microtubules (CP). How this conserved structure assembles and functions is poorly understood. We investigated this question by examining aberrant axonemes with 8, 9, 10, or 11 doublets produced by a basal body-deficient *Chlamydomonas* mutant, *bld12*. Most of the 8-doublet axonemes contained no CP, but those without radial spokes contained CP. Thus the CP formation apparently depends on the central space in the axoneme. In 10- or 11-doublet axonemes, 4 to 6 radial spokes were detached from the CP, resulting in a distorted arrangement of the doublets. The detachments were found predominantly on the C2 side of the two CP microtubules, named C1 and C2. However, when CP projections on C1 were removed, radial spokes frequently attached to a projection on C2. From these observations, we conclude that the radial spokes actually bind to the CP, especially more strongly to projections on C1 than those on C2, while multiple projections on CP interact with the radial spoke. These results thus provide invaluable insights into how the central pair microtubules are formed and how they interact with radial spokes.

#16

Phosphoinositide-specific phospholipase C (PI-PLC) of *Amoeba proteus*

Hitoshi YAGISAWA, Ryoko SHINKI-HORIE, Kayoko MAEDA-AKAHANE, Teruo SHIMMEN and Seiji SONOBE

(Grad. Sch. Life Sci., Univ. Hyogo)

SUMMARY

A free-living amoeba, *Amoeba proteus*, has been used as a model to study locomotion of cells exhibiting the amoeboid movement such as neutrophils and cancer cells. Although mechanisms of regulation of actin-based cell motility by phosphoinositides (PI) in mammalian cells and *Dictyostelium discoideum* have been revealed to some extent, those of free-living protists remain poorly understood. We have examined roles of PI and PI-PLC in the cell movement of *Amoeba proteus*. The movement of the Amoeba was reversibly inhibited by U73122, an "in vivo" inhibitor of PI-PLCs. Conversely, microinjection of Ins(1,4,5)*P*3 into resting cells increased the cell motility and pseudopod extension. We therefore examined whether *Amoeba proteus* have PI-PLC. Lysates from the Amoebae show the PtdIns(4,5)*P*2-hydrolysing activity. Moreover, we cloned a cDNA encoding a mammalian PLC**δ**-like protein from a cDNA library of *Amoeba proteus* by RT-PCR method and designate it as *Applc*. Interestingly, unlike mammalian PI-PLCs, Applc has a C2 domain at the *N*-terminus instead of the pleckstrin homology (PH) domain that generally targets PLCs to the plasma membrane. We have established a bacterial expression system of GST-Applc. Partially-purified GST-Applc required Ca^{2+} for its activity. Nuclear localization of Applc was resolved using an antibody raised against the *N*-terminal Applc.

#17

Determination of Immaturin gene sequence: an intragenic structure responsible for sexual rejuvenescence in *Paramecium*

Nobuyuki HAGA, Toshinori USUI and Tomoaki ABE

(Dept. Biol. Engn., Fac. Sci. Engn., Ishinomaki Senshu Univ.)

SUMMARY

Sexual rejuvenescence is performed in *Paramecium* by two ways. One is conjugation in natural and the other cytoplasmic transfer by microinjection. Sexuality of this organism is defined as the expression of the ability to recognize the cells of complementary mating-types. The life cycle stages are classified in four major phases, such as conjugating processes, immaturity, maturity and senility. We have previously demonstrated that a cytoplasmic polypeptide, Immaturin, induces sexual rejuvenescence in both mature and senescent cells by microinjection. In this study, we have cloned, sequenced and identified the gene which encodes Immaturin. Immaturin gene is composed of two parts those are domains of thioredoxin and GST genes. Transformed-cells of YFP-Immaturin fusion gene indicate that localization of Immaturin is restricted in cytoplasm, but not in cilia. The presence of Immaturin in the macro- or micronucleus is not fully demonstrated, yet. Indirect immunofluoresent signals using anti-Immaturin antibody indicate that Immaturin appears in the cytoplasm of conjugating cells at several hours after the initiation of mating reaction, and the signals are maintained during conjugating processes and immaturity. Our study suggests that *Paramecium* produces Immaturin gene by combining two parts of old genes to perform the molecular regulation of sexuality in whole life cycle phases.

#18

Comprehensive search for the nuclear pore complex proteins of ciliate *Tetrahymena thermophila*

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SUMMARY

In ciliate *Tetrahymena thermophila*, it is known that some of the nuclear pore complex (NPC) components are distinct between macronucleus and micronucleus: among 13 kinds of the identified components, 9 components are common and 4 paralogs of Nup98 are distinct. In order to address how these two distinct NPCs are autonomously assembled in the same cytoplasm, we first examine yet uncovered NPC components of both nuclei of *T. thermophila,* and second understand causative of generating structural difference between macronuclear and micronuclear NPCs. Using mass spectrometry analysis, we identified additional 15 candidates of the NPC components. Among them, we found 2 pairs of plural components localized exclusively to either nucleus by expressing these candidates tagged with GFP. In addition, some scaffold components of the NPC showed biased localization between macronuclear and micronuclear NPCs. These components probably make an important structural contribution in generating fundamentals of distinct NPCs to cause the exclusive localization of nucleus-specific components.

#19

Soil testate amoebae fauna in Japan, re-evaluation

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SUMMARY

Analyses of 100 samples of forest soil each from the following islands: Mt. Tateyama of Honshu, Yaku, Kitadaitou, Minamidaitou and Kume were done. The territory of Japan is an interesting example in protistology. It is made up of large and small islands with a unique island biogeography composed of the subarctic zone, the temperate zone and the subtropics. We identified about 250 taxa of testate amoebae in the samples. More over 100 species of the identified species were new record to Japan. We hereby describe two new species: *Deharvegia japonica* and *Assulina discoides*. Most of fauna belonged to cosmopolitan group of testate amoebae. The following species are not specific to species of the Holarctic, usually from the Indo-Malayan and Neotropical regions: *Centropyxis deflandriana, C. latideflandriana, C. stenodeflandriana, C. sacciformis, Cyclopyxis lithostoma, Distomatopyxis couillardi, Hoogenraadia humicola, Planhoogenraadia daurica, Assulina discoides, Deharvengia japonica, Quadrulella quadrigera, Q. quadrigera v. africana, Wailesella* sp.

#20

Analysis of photosynthetic products in green ciliate *Paramecium bursaria*

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SUMMARY

A green paramecium has several hundreds of endosymbiotic algae. It has been known that those symbiotic algae produce a lot of photosynthetic products, such as glucose and maltose, et al. The algal endosymbionts excrete large amounts of sugars, which might be consumed by the host green paramecia. Interestingly, it has been found that free-living *Chlorella* species, which are believed not to excrete sugars, such as maltose, can experimentally achieve endocytobiosis in the algae-free paramecia. Thus, roles of such photosynthetic products in green paramecia are not well understood. Here, we analyzed the species and concentrations of sugars contained in the green and algae-free paramecia. Further, culture conditions to control the excretion of sugars into the paramecia cytoplasm were elucidated. In this meeting, our results obtained will be discussed.

#21

Specific connections between symchlorosomes and mitochondria in *Paramecium bursaria* as verified by electron tomography and 3D reconstruction from serial sections

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SUMMARY

The spatial relationship between symchlorosomes (symbiotic zoochlorella cells enclosed by peri-algal vacuole membranes, PVMs) and surrounding organelles was carefully examined by transmission electron microscopy after fixing *Paramecium bursaria* cells by a metal-contact quick-freezing procedure in situ, followed by freeze substitution. In contrast to the morphology reported so far by chemical fixation, the PVM was found to be closely apposed to the cell wall of the zoochlorella. Interaction between PVM and other organelles was further investigated by 3-D reconstruction from serial sections and electron tomography by using a high-voltage electron microscope. The PVM was not in direct contact with the host cell cortex, but was associated with trichocysts, mitochondria, and small vesicles in the vicinity of ciliary basal bodies. As trichocysts and small vesicles were directly attached to the inner surface of the cell cortex, all of these organelles constituted a sub-cortical network with symchlorozomes. Mitochondria were always in close contact with the outer surface of the PVM, providing structural scaffold for symchlorosomes to be anchored at the sub-cortical region in the host's cytoplasm. Moreover, the outer membranes of mitochondria were occasionally found to fuse with the PVM, and also with various other membranous organelles including trichocysts and food vacuoles.

#22

New method to eliminate germinal chromosome in *Tetrahymena* and observation of the arrangement of meiotic chromosome using nullisomics and unisomics obtained by the method

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SUMMARY

In a ciliate *Tetrahymena thermophila,* as the macronucleus plays vegetative role, micronuclear chromosomes (2n = 10) can be eliminated, although not all of them. Nullisomic which lacks both copies of homologous chromosomes and unisomic which has only one kind of chromosome were isolated, but the procedure needs long time and a special strain. We developed a new simple and quick method for isolation of nullisomic and unisomic: conjugating pair was fused by hypotonic shock and nuclear change was arrested after meiosis to change diploid to haploid. After division of the fusant, haploid meiosis was induced by conjugation and the nuclear change was arrested again after meiosis to give nullisomic. The procedure was repeated on the nullisomic to give unisomic.

Using nullisomic, chromosomal arrangement in elongated micronucleus (crescent) during meiosis was observed directly. At stage II, both ends of chromosome located to one end of spindle shaped micronucleus. At stage V of triple nullisomic which contains two pairs of homologous chromosomes, two loops were observed at one end of the crescent, suggesting bouquet structure of chromosomes. But two parallel chromatin threads and chromomere-like pattern on them were similar to single pachytene chromosome of other organism.

#23

Prevalence of *Sarcocystis* in slaughtered horses and imported horse meat

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SUMMARY

In recent years, the food borne illness through eating raw horsemeat (Basashi) became a growing problem of food hygiene in Japan. *Sarcocystis fayeri*, a coccidian parasite has been identified in the horsemeat collected from the cases of food borne illness. *S. fayeri* cause a gastrointestinal dysfunction experimentally in animal, suggesting this parasite as a causative agent of the food borne illness. The study of the prevalence of *Sarcocystis* in horsemeat will provide indispensable information for the food poisoning risk assessment in the near future. Thus we determined the amount of *S. fayeri* DNA in 41 meat samples mostly from light horses slaughtered in Japan and 9 samples of dressed horsemeat imported from Canada, using a quantitative PCR assay. The results showed that the amount of *S. fayeri* DNA in domestic horsemeat samples was lower than that of meat samples collected from the cases of food borne illness, while the amount of *S. fayeri* DNA in imported meat samples was almost equivalent to that of the cases of food borne illness. To be noted, the imported meat from Canada is associated with the incidence of the illness in the reported cases.

#24

Transfer mechanism of spasmin–Ca^{2+} binding energy to novel elastic protein spaconnectin

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SUMMARY

In the previous paper (Biophys. J. 1999, 76, 993–1000), we have achieved to measure tension-displacement relationship of isolated giant spasmoneme in *Zoothamnium arbuscula* strain Kawagoe. The tension development of the spasmoneme in the presence of enough Ca^{2+} is due to random coil contraction of the novel protein, spaconnectin. The tension of the spasmoneme in the absence of free Ca^{2+} is due to partial un-folding of α-helix structure of spaconnectin. We found that, at a high tension, the Ca^{2+} binding affinity of spasmin is significantly decreased with increase of tension. The relationship is obeyed to the Le Chatelier-Braun's law. Dimeric spasmins in spasmoneme binds the random coil part and/or α-helix structure in spaconnectin. To confirm this presumption, the various metal ions and lanthanide ions must use to measure tension-displacement relationship of giant spasmoneme.

#P1

Function of glycocalyx in cell adhesion and cell locomotion on *Amoeba proteus*

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SUMMARY

In *Amoeba proteus*, plasma membrane (PM) is coated by glycocalyx, which was mainly composed of polysaccharide. From electron microscopic analysis amoeba glycocalyx was composed of two-layer structure, called amorphous layer (about 0.1 mm thick) and filamentous layer (about 2 mm thick). The amorphous layer was directly attached to PM and filamentous layer was on the amorphous layer. The chemical components of PM-glycocalyx complex were investigated in detail, and most major constituent sugar was mannose. Indicating participation of glycocalyx in endocytosis, however, other physiological significance of glycocalyx has not been clear. Since the glycocalyx is the most external layer, involvement of glycocalyx in cell adhesion and amoeboid movement was predicted. To investigate these possibilities we digested glycocalyx of living *A. proteus* using alpha-mannosidase and measured activity of amoeboid movement and cell adhesion. Treatment with alpha-mannosidase, which deleted of filamentous layer, decreased activity of cell locomotion and cell adhesion. Our results indicated importance of filamentous layer of glycocalyx in cell adhesion and cell locomotion.

#P2

Isolation of actin filaments from a diatom, *Craticura* sp.

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SUMMARY

Pennate diatoms have bilateral symmetry and many genera among them possess an elongated slit in the frustule called a raphe, at which cells adhere to the substratum and glide. Although a model explaining gliding has been presented, the molecular mechanism remained unsolved. It is reported that actin bundles running parallel to the raphe and actomyosin system plays an essential role in gliding. In order to explain the mechanism of bidirectional gliding, determining polarity of actin bundle is essential. Generally, polarity of actin filaments has been determined by myosin S1 fragment to make the arrowhead structure. In application of this method for diatom cells, there are some difficulties; penetration of S1 fragment because of it's large molecular size, many steps required for sample preparation for EM, and sectioning paralleled to actin filament. To avoid these difficulties, we decided to isolate actin filaments from diatoms. Cells of *Craticura* sp., a fresh water diatom, were treated with a buffer containing Triton X-100 and rhodamine-phalloidin and ruptured by voltex with 0.4 mm glass beads. Homogenate was centrifuged with 30% glycerol cushion to remove frustule. Bundles of rhodamine-labeled filaments were collected in the resultant precipitate. In EM, bundles consisting of thin filaments approx. 6 nm in diameter were observed, suggesting that we succeeded in isolating actin filaments from diatoms.

#P3

Ultrastructural changes during rapid axopodial contraction in heliozoon *Raphidiophrys contractilis*

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SUMMARY

The centrohelid heliozoon *Raphidiophrys contractilis* has many radiating axopodia, each containing a bundle of axonemal microtubules as a cytoskeleton. Each axopodium has granular kinetocysts which lie beneath the plasma membrane and have been implicated in the process of food uptake. In this study, we investigated the cell morphology and fine structure of *R. contractilis* during rapid axopodial contraction by light and electron microscopy. Changes in the diameter of axopodia were analyzed using video microscopy. The diameter of axopodia at proximal regions showed a 2-fold increase before and just after induction of rapid axopodial contraction evoked by mechanical stimulation. Electron microscopy revealed that the kinetocysts accumulated into the proximal regions of axopodia after rapid axopodial contraction. To prevent axopodial shortening during glutaraldehyde fixation, we examined the effects of the antitumor-drug paclitaxel (including fluorescence-conjugated drugs) on the microtubular axonemes. The sensitivity of *R. contractilis* to the drug was lower than that of the actinophryid heliozoon *Actinophrys sol* as already reported. These results suggest that a novel mechanism is involved in the rapid axopodial contraction in *R. contractilis*, and that the properties of microtubules and/or microtubule-associated proteins in *R. contractilis* might be different from those in *A. sol*.

#P4

Screening of microbial anti-trypanosomal compounds using *Bodo saltans*

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SUMMARY

Trypanosoma is a parasitic protozoan that belongs to kinetoplastida: trypanosomatidae. They are parasitic on humans and other animals, and cause various diseases. *Bodo saltans* and trypanosomes are closely related and both of them have an organelle called kinetoplast. *B. saltans* is a free-living flagellate, not parasitic. So, we use *B. saltans* as a model organism to find a new drug for trypanosomiasis. We searched active ingredient from microbial secondary metabolites, and tested its toxicity for HeLa cells. As a result, 468 samples out of 2,231 samples showed activity against *B. saltans*. Further screening using HeLa cells to test toxicity, we selected 3 strains. We will report on the process of search and purification of the active ingredient.

#P5

Gametogenesis in *Noctiluca scintillans* under light-dark cycle

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SUMMARY

Noctiluca scintillans is a heterotrophic dinoflagellate. *N. scintillans* trophonts usually reproduce by binary fission. In such trophont population, a small fraction of cells spontaneously transform into gametogenic cells, which undergo two successive nuclear divisions without cellular divisions. The products of nuclear division are called "progametes" and migrate to cell surface accompanied with a small amount of cytoplasm, and then further divide 6–8 times synchronously, finally reaching 256–1,024 progametes. Thus, numerous mature gametes with two flagella (zoospore) are released from a mother cell ghost. At present, it unknown what triggers differentiation of the gametogenic cells. Here, we report factors/conditions to affect the onset of gametogenesis by successive observations for two weeks after feeding. 1) Culture stage: frequency of the appearance of the gametogenic cells drastically increased in early stationary phase, indicating that starvation is a prerequisite for the differentiation. 2) Light-dark cycle: at the beginning of light period (L0), cells already entered in middle stages (16–64 progamete stages), and then development successively progressed (L4–L12). At the end of light period, cells, which began differentiation anew, were observed, suggesting that differentiation would have begun around at the end of light period (L8–L10). When light-dark cycle was shifted for 4 hour forward or backward, developmental timing followed the cycle within 2 days, suggesting gametogenesis regulation by circadian clock.

#P6

Construction of foreign protein secretion system in *Tetrahymena thermophila*

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SUMMARY

Various heterologous expression and/or secretion systems of foreign protein, e.g. bacteria or fungi, have been used for mass production. However, these systems do not always express correctly and efficiently such proteins due to the difficulties of appropriately folding proteins with complex spatial structures. Now we expect that ciliates have potential to produce foreign proteins, because of high density culture, inexpensive culture media, and remarkably high folding and assembly abilities of foreign proteins. For instance, *Tetrahymena thermophila* is shown to produce a mono-clonal antibody efficiently (see Cilian AG Homepage in Germany). In this study, we constructed a transformation plasmid for convenient expression and secretion systems of foreign proteins in *T. thermophila*, utilizing a pre-propeptide of one of cysteine proteases for extracellular secretion and Neo4 for a selection marker. After introduction of this plasmid into the macronucleus and subsequent selection under Paromomycin, we succeeded in establishing several clones and confirming actual protein expression, secretion into culture medium of two anonymous foreign proteins, and higher enzyme activity of one foreign protein. These results show that *T. thermophila* is one of the most ideal model systems for expression and secretion of foreign proteins in researches and for industrial mass production of useful proteins.

#P7

Measurements of phagocytic activities of *Paramecium bursaria* using GFP-labeled yeast

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SUMMARY

Paramecium bursaria is a ciliate that contains several hundreds of *Chlorella*-like algae in its cytoplasm as endosymbionts. To elucidate whether the presence of these algal endosymbionts affect phagocytosis of the host *P. bursaria*, we have quantitatively estimated the phagocytic activities of *P. bursaria* by using the yeast cells that constitutively express GFP. The GFP-labeled yeast cells were fed to *P. bursaria* cells and the fluorescent yeast cells that remained in the *P. bursaria* cells after various periods were detected by fluorescence microscopy. The use of the GFP-labeled yeast enabled us to readily distinguish between the endosymbiont and the engulfed yeast cells in *P. bursaria* cells, and also to determine whether the engulfed cells were digested or egested. Our observation suggested that once engulfed by *P. bursaria*, yeast cells were mostly digested within 3 hours. Comparison of the phagocytic activities between the aposymbiotic (*Chlorella*-free) and the symbiotic *P. bursaria* cells showed that the aposymbiotic ones engulfed and digested much more yeast cells than the symbiotic ones. These results suggest that the presence of algal endosymbionts may cause reduction in the phagocytic activities of host ciliates.

#P8

Role of the two-headed inner dynein arm in the ciliary movement in *Paramecium tetraurelia*

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SUMMARY

The molecular mechanism of inner dynein arms in the ciliary movement in *Paramecium tetraurelia* is still unclear. Our previous study has shown that the two-headed inner arm dynein heavy chains- and intermediate chains-silenced cells by RNA interference (RNAi) never exhibit backward swimming. To clarify the role of the two-headed inner dynein arm in the ciliary movement in *P. tetraurelia*, we examined the effects of gene silencing of intermediate chain 1 and two heavy chains by RNAi using the feeding method. These three kinds of silenced cells exhibited almost the same characteristic swimming behavior and ciliary response. They swam slowly and meanderingly. The cilia of these silenced cells did not show Ca^{2+}-induced ciliary reversal. To clarify the reason for the defect of ciliary response by the two-headed inner dynein arm related gene silencing, we analyzed the effects of gene silencing on the composition of axonemal proteins. The results indicate that integrity of the two-headed inner dynein arm is essential for creating the effective stroke of cilia.

#P9

Maltose release mechanism of symbiotic *Chlorella* from Japanese *Paramecium bursaria*

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SUMMARY

The endosymbiotic *Chlorella* in *Paramecium bursaria* has been reported to provide a photosynthate, maltose, which is the material to maintain the symbiotic relationship between *Chlorella* and its host. We have studied on the effect of light on maltose release by *Chlorella* F36-ZK from *P. bursaria* F36. It is well known that maltose release of symbiotic *Chlorella* is induced by an acidic condition. F36-ZK also released maltose in pH5 buffer in light condition but did not in dark. However, its maltose release was not inhibited by a photosynthetic inhibitor DCMU and thus it was suggested that photosynthesis did not directly induce maltose release but light stimulated it. From the results using single-wavelength light, it was indicated that blue (450 nm) and red (around 600 nm) light stimulated maltose release in an acidic condition. F36-ZK seemed to release maltose as a sole photosynthate, and it should possess a maltose-specific transport system. Since the amount of released maltose was not affected by the environmental maltose concentration, the maltose should be transported by an active transporter. The maltose release was potently inhibited by the treatment with ionophores, such as CCCP, nigericine and monencine. Therefore, the proton gradient might regulate maltose release on plasma membrane.

#P10

Culturing condition and isolation of micronuclei in *Blepharisma japonicum*

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SUMMARY

Ciliates are presently classified into two large assemblages, Intramacronucleata and Postciliodesmatophora. Most of ciliates represented by *Tetrahymena* and *Paramecium* belong to the former group. The latter group is further divided into two distinct groups, karyorelictids and heterotrichs, which are the most ancestral ciliates that diverged early from the main line of other ciliates. During macronuclear differentiation, ciliates undergo a large-scale genome reorganization, including precise removal of germline-specific sequences (internal eliminated sequences: IES) from the macronucleus. To date, identification of IESs has been restricted to a few representative ciliates, belonging to Intramacronucleata. In contrast, there is no report on any IESs from Postciliodesmatophora. Therefore, they are important groups, when origin of the macronucleus and evolution of IESs are considered. Here we report a new isolation method of micronuclei from the heterotrichous ciliate, *Blepharisma japonicum*, by a combination of a new cell disruption method and the use of nuclepore membrane filter.

Based on sequences of randomly-cloned macronuclear DNA, paired primers were designed on both termini. Using these primer sets, PCR was carried out on total micronuclear DNA to detect products with different length from those on macronuclear DNA. So far, one micronuclear DNA fragment carrying IES has been obtained.

#P11

Do the mating pheromones of genus *Blepharisma* play an important role in speciation?

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SUMMARY

In genus *Blepharisma*, 20 species have been reported, and these species are classified to four groups according to macronuclear shape. We attempt to reveal a role of mating pheromones in speciation. *Blepharisma* has mating pheromones, gamone1 and gamone2 secreted by cells of mating type I and II, respectively. Gamone1 is a glycoprotein, and gamone2 is a derivative of tryptophan. *Blepharisma* forms heterotypic and homotypic pairs by the mating pheromones. In this study, we revealed that gamone1 obtained from macronuclear group II and IV induced mating pairs in strains which belong to the same group. Gamone1 amino acid homology within group II or IV is higher than that between group II and IV. On the other hand, gamone2 induced pairs of any species irrespective of the macronuclear group. Hence, in reproductive isolation, gamone1 plays more important role than gamone2. We speculate that gamone1 promoted macronuclear group differentiation, which is initial stage of speciation in genus *Blepharisma*. Furthermore, type I of macronuclear group II and IV, both stimulated by gamone2, did not form homotypic pairs between group II and IV. Therefore, it is suggested that factors other than gamone1 are also involved in speciation of *Blepharisma*.

#P12

Study on the amino acid residues which are involved in the stop codon recognition in ciliate eRF1s

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SUMMARY

The genetic code of nuclear genes in some ciliates was found to differ from that of other organisms in the assignment of UAA, UAG, and UGA codons, which are normally assigned as stop codons. Eukaryotic release factor 1 (eRF1) is a key protein in stop codon recognition, thus, the protein is believed to play an important role in the stop codon reassignment in ciliates. eRF1 is composed of three domains, and the stop codon recognition site is located in domain1. In this study, we examined the release activity of *Euplotes raikovi* eRF1 by *in vivo* complementation test in yeast and dual-luciferase assay. Our result suggests that *E. raikovi* eRF1 recognizes the stop codons UAA and UAG, but not UGA. We also examined the amino acid I128 in *E. raikovi* (R128 in *Dileptus*) which was previously concluded to play an important role in omnipotent recognition of *Dileptus* eRF1. Our result showed that single amino-acid substitution of I128R slightly increased recognition of UGA codon. The result supports our previous idea that R128 is a key residue important for recognition of the UGA codon in *Dileptus* eRF1.

#P13

Species identification of wild strains and molecular phylogenetic analysis of *COI* gene in the genus *Blepharisma*

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SUMMARY

Genus *Blepharisma* is classified into 20 species according to morphological characteristics, and these species are divided into four groups by macronuclear shape. However, the traditional definition to distinguish species is often obscure, and only few studies were engaged in molecular phylogenetic analysis so far. Our group previously showed that gamone1, which is a mating pheromone of *Blepharisma*, had an effect on different species in the same group. This result conflicts with the species definition that the strains with reproductive isolation are regarded as different species. In this study, wild strains as well as the laboratory strains were reexamined to identify species in detail, and cell characteristics were expressed as numerical data. We also analyzed *COI* gene and 18S rRNA gene base sequences, and compared the results with those by cell morphology. Homology in the *COI* gene between strains was obviously higher in the same macronuclear group than in different group, and the strains which belong to the same group form a cluster in phylogenetic tree. From these data, it is suggested that grouping by macronuclear shape is appropriate also in molecular level, whereas the traditional species identification by morphological characteristics needs reexamination in further study.

#P14

Nickel-induced genes in *Paramecium caudatum*

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SUMMARY

Inducible gene expression technology is potentially effective for the better expression of proteins that are toxic to a host cell or require a temporal control. In *Tetrahymena*, the Cd^{2+}-inducible metallothionein promoter has been successfully used to control expression of introduced genes. Here we report the isolation of two nickel-induced genes, Ni46 and Ni66, from the subtracted cDNA library of *P. caudatum*. Ni46 and Ni66 mRNAs encode for approximately 16- and 25-kDa proteins, respectively. Both Ni46 and Ni66 mRNAs were remarkably induced by adding nickel sulfate into the culture medium. Cobalt chloride also enhanced slightly the expression of both mRNAs. Inductions of both mRNAs by Ni^{2+} and Co^{2+} were dose-dependent, and their levels were continued to increase up to 3 days after the addition of nickel sulfate. We also cloned ~0.45 kb of 5' flanking region of Ni66 gene, and made the Ni66 promoter-driven reporter construct harboring secreted luciferase coding region. Functions of nickel induced genes and future research will be discussed in the poster session.

#P15

Properties of *Paramecium bursaria* cells in the stationary phase

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SUMMARY

Paramecium bursaria is a ciliate that contains several hundreds of *Chlorella*-like algae in its cytoplasm as endosymbionts. It is well known that *P. bursaria* cells can survive for an extremely long period even after they enter the stationary and/or starvation phase (Karakashian, 1963; Nishihara et al., 1996; Omura et al., 2004). Generally, non-photosynthetic protists withstand starvation by lowering their respiration rate (Fenchel, 1982), and also by forming dormant cysts or spores in some cases. Nevertheless, little is known about the stationary phase of *P. bursaria* bearing algal endosymbionts. Here we describe various activities of *P. busaria* cells that have consumed food yeast cells and thus are in the starvation and stationary phase. The swimming speed and the phagocytic activities of the stationary-phase cells were somehow lower than those of the log-phase cells. Long-term observations of single cells in the stationary phase demonstrated that these cells showed almost no proliferation for at least 1–2 weeks. However, when yeast cells were fed, they resumed growth after a lag of about 24 h. These results suggest that despite of their lower activities, *P. bursaria* cells in the stationary phase still retain fundamental cellular functions, unlike other non-photosynthetic protists.

#P16

Species composition and structure of symbiotic protist community in the termite *Coptotermes formosanus*

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(Coll. Sci., Ibaraki Univ.)

SUMMARY

Termites in the family Rhinotermitidae possess wood-degrading symbiotic protists in their digestive tracts. Three trichonymphid protists have been described from *Coptotermes formosanus*, a notorious pest termite in Western Japan. On the other hand, difference of the symbiont community structures between different termite castes (e.g., workers and soldiers) is not known. In this study we collected ten colonies of this termite from five localities and examined symbiotic protist species composition. For workers and soldiers of two colonies, community structure was investigated by direct counting of the symbionts using a counting chamber. The three trichonymphid species were found from each of five workers in all examined colonies. In Iriomote Is. and Okinawa Is., an undescribed small trichomonad was found. The mean protist number of the soldier was about 2.5% of that of the worker and some individuals lack one of the three trichonyphid species. A principal component analysis exhibited that the community structure of the soldier digestive tract was more variable than that of the worker. Probably the difference in the 'local' protist community size between castes affected its stability.

#P17

New bio-remediation technique for radioactive cesium in contaminated soil using *Paramecium bursaria*

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SUMMARY

We have developed a novel bioremediation system for radioactive cesium using the symbiosis between *Paramecium bursaria* and endosymbiotic zoochlorellae. After adsorbed on kaolin particles as a model soil, cesium was added to the cell suspension of *P. bursaria* with or without containing endosymbiotic zoochlorella cells. Cesium was taken up and accumulated up to 30 times higher than the surrounding medium by paramecia with zoochlorellae, whereas those without endosymbionts showed little accumulation. As *P. bursaria* cells can be easily and efficiently separated from soil particles by galvanotaxis, bioremediation of radioactive cesium-contaminated soil with *P. bursaria* is shown to be a promising method that can be operated on-site at individual farms, without need for costly transportation and time consuming process of decontamination.