Abstracts of the JSP meeting 2013

Oral presentations

1) Effect of cell density on the formation of cannibal giants in *Blepharisma*
Yuna ONO, Mayumi SUGIURA and Terue HARUMOTO

2) Do the nuclear changes occur in interspecific conjugation of *Blepharisma*?
Mao YAMADA, Mayumi KOBAYASHI, Mayumi SUGIURA and Terue HARUMOTO

3) Microtubule dynamics during rapid axopodial contraction in heliozoon *Raphidiophrys contractilis* revealed by immunoelectron microscopy
Risa INOUE and Motonori ANDO

4) Real-time phenomenological analysis of dielectric behavior of *Euglena* cells during their cell-shape changes
Ken HANAHARA and Motonori ANDO

5) γH2AX formation in haploid nuclei of *Tetrahymena thermophila*: A possible involvement of a novel type of DNA double-strand break
Takahiko AKEMATSU, Yasuhiro FUKUDA, Chika TADA, Yutaka NAKAI and Ronald E. PEARLMAN

6) Detection and functional analysis of plant hormones in malaria parasite
Ryuma MATSUBARA, Mikiko KOJIMA, Michiru TAHARA, Syed Bilal Ahmad ANDRABI, Michika FKSHI, Fumiya KAWAHARA, Akinori YAMANO, Hitoshi SAKAKIBARA and Kisaburo NAGAMUNE

7) Analysis of an early stage of the evolution of endosymbiosis between an alga and a ciliate in an experimental microcosm composed of 3 species
Toshiyuki NAKAJIMA

8) The evolution of interspecific relationship between an alga and a bacterium in the long-term culture of experimental microcosm
Masayuki MATSUURA, Yosuke HUJII and Toshiyuki NAKAJIMA

9) Changes in the algal ingestion rate of ciliate cells in an early stage of evolution of algal-ciliate endosymbiosis in the long-term culture of experimental microcosm
Yoichiro OHNISHI, Akiko SANO and Toshiyuki NAKAJIMA

10) Life style and evolution of 'Protozoa' and 'Algae', viewed from freshwater microscopic ecosystem
Masashi M. HAYAKAWA and Toshinobu SUZAKI

11) A study on a rolling structure of plasma membrane isolated from *Amoeba proteus*
Yukinori NISHIGAMI, Atsushi TANIGUCHI, Shigenori NONAKA, Masatoshi ICHIKAWA and Seiji SONOBE

12) The observation of ultrastructure in *Hypophrya* sp.
Go KOBASHIGAWA, Atsushi TANIGUCHI, Tohru YOSHIHISA and Seiji SONOBE

13) Experimental evolution of *Eecherichia coli* toward constructing a novel intracellular endosymbiosis with *Dictyostelium discoideum*
Kayo YAMAMOTO, Kazufumi HOSODA, Makoto SUEYOSHI, Isao KUBO and Tetsuya YOMO

14) Construction of a chloroplast-like novel endosymbiosis between a cyanobacterium and a ciliate
Yuhki AZUMA, Kazufumi HOSODA, Masumi HABUCHI, Kayo YAMAMOTO, Risa TAKAMI and Tetsuya YOMO

15) Theoretical study about evolution of stalk/spore ratio in a social amoeba
Kouki UCHINOMIYA and Yoh IWASA

16) Maltose release mechanism of symbiotic Chlorella isolated from Japanese *Paramecium bursaria*
Aika SHIBATA, Masahiro KASAHARA and Nobutaka IMAMURA

17) Construction of a novel endosymbiosis between *Escherichia coli* and algae-free *Paramecium bursaria*
Risa TAKAMI, Kazuhumi HOSODA, Itsuka KUMANO, Kayo YAMAMOTO and Tetsuya YOMO

18) Effects of the endosymbiosis on phagocytic activities of *Paramecium bursaria*
Takashi MIURA and Sosuke IWAI

19) The stress tolerance of *Paramecium bursaria*
Yuka ADACHI, Ayumi SHIMOMURA, Kozue HAMAO and Hiroshi HOSOYA

20) Infectivity of the symbiotic *Chlorella* spp. of the ciliate *Paramecium bursaria*
Sotaro ARAKI and Yuuki KODAMA

21) Cell signaling pathways and analysis of expressed proteins during the resting cyst formation of ciliated protozoan *Colpoda cucullus*
Yoichiro SOGAME, Katsuhiko KOJIMA, Toshikazu TAKESHITA, Eiji KINOSHITA and Tatsuomi MATSUOKA

22) Roles of kinesin14A in micronuclear division in *Tetrahymena*
Yasuharu KUSHIDA, Masak TAKAINE, Kentaro NAKANO, Toshiro SUGAI, Krishna Kumar VASUDEVAN, Mayukh GUHA, Yu-yang JIANG, Jacek GAERTIG and Osamu NUMATA

23) *Tetrahymena* as a production plant of foreign proteins
Kohei MASUDA and Hiroshi ENDOH

24) FAP82 regulates activation of K^+ leak channel in *Paramecium*
Shinobu IZUTANI, Takashi TOMINAGA and Manabu HORI

25) Development of nickel inducible expression system in *Paramecium caudatum*
Yasuhiro TAKENAKA, Nobuyuki HAGA and Ikuo INOUE

26) Molecular composition and the function of plant-vacuole like organelle in extracellular *Toxoplasma gondii*
Michika FKSHI, Kazuhide YAHATA, Takaya SAKURA, Michiru TAHARA, Ryuma MATSUBARA, Osamu KANEKO and Kisa NAGAMUNE

27) Megavirus-infecting *Acanthamoeba* sp. isolated from a keratitis patient
Kenji YAGITA and Yoshitsugu INOUE

28) Species composition change in the intestinal protist communities after community mixing
Osamu KITADE and Yoshihiro KURUSU

29) Development of microscopic system for precise investigation of mobile protists
Ikuko SHIHIRA-ISHIKAWA, Hiroyuki KAWANO, Yasutaka HANADA and Atsushi MIYAWAKI

30) Growth analysis of *Paramecium busaria* and the algal endosymbionts
Sosuke IWAI and Takuro TAMURA

#1

Effect of cell density on the formation of cannibal giants in *Blepharisma*

Yuna ONO[1], Mayumi SUGIURA[2] and Terue HARUMOTO[2]

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SUMMARY

Heterotrich ciliate *Blepharisma* usually feeds on bacteria. When cells are deprived of food, *Blepharisma* often undergoes either "conjugation", "cyst formation" or "cannibalism" as a strategy for surviving starvation. In this study, to characterize giant cells which were generated by cannibalism, we compared the cell size, size of macronucleus and the number of micronuclei of giants, with ordinary (normal sized) cells. Major cell axis, minor cell axis and the size of the macronucleus of giants were significantly larger than the ordinary cells in WK-IV strain of *Blepharisma japonicum*. The volume of the giant was 5–30 times more than the ordinary ones. Most of the giants had more micronuclei than the ordinary ones. We also studied the experimental condition to induce giant formation. The number of giant cells was counted in the different cell densities (800, 1,600, 4,800 and 10,000 cells/ml) for several days. We found that the higher cell density usually induced more giant cells. The observation of giant size and the number of giant cells for successive days suggest that the cannibalism is a transient state for surviving starvation in life cycle of *Blepharisma*.

#2

Do the nuclear changes occur in interspecific conjugation of *Blepharisma*?

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SUMMARY

In the conjugation of *Blepharisma*, mating type I and mating type II cells secrete conjugation-inducing substances, gamone1 and gamone 2, respectively. Cells are stimulated by complementary gamones and induced pair formation. Twenty species so far reported in the genus *Blepharisma* are classified into four groups according to the macronuclear shape. The previous study revealed that gamone 2 is effective to type I cells of any species between the macronuclear group. On the other hand, gamone 1 was not effective to the species which belongs to the different macronuclear group. Recent studies revealed that at least between *B. japonicum* and *B. stoltei*, both belong to the macronuclear group IV, gamone 1 was effective in these species. In this work, we studied whether the pair formation is possible between *B. japonicum* and *B. stoltei*, and whether the nuclear changes characteristic to the conjugation occur in the conjugating pair between these two species. As the results, conjugating pairs formed between these species, and meiosis of micronucleus, subsequent nuclear changes and the formation of new macronuclear anlagen also occurred in the interspecific conjugants almost same as in the intraspecific conjugants.

#3

Microtubule dynamics during rapid axopodial contraction in heliozoon *Raphidiophrys contractilis* revealed by immunoelectron microscopy

Risa INOUE and Motonori ANDO

(Lab. Cell Physiol., Grad. Sch. Educ., Okayama Univ.)

SUMMARY

The centrohelid heliozoon *Raphidiophrys contractilis* (*R. contractilis*) has many radiating axopodia, each containing a bundle of axonemal microtubules as a cytoskeleton. *R. contractilis* shows rapid axopodial contraction induced by mechanical stimulation. The microtubule-containing axopodia were retracted into the cell body within less than video rate. However, the contraction mechanism in *R. contractilis* still remains unresolved. In this study, we investigated the microtubule dynamics during rapid axopodial contraction in *R. contractilis* by conventional electron microscopy and immunoelectron microscopy. Conventional electron microscopy showed that microtubules were well preserved in the cell body in spite of the induction of axopodial contraction. We did not find the distal parts of the axonemal microtubules moved into the axopodial base, and any structures like the contractile tubules which have been reported in actinophryid heliozoon *Actinophrys sol*. Immunoelectron microscopy showed gold particles on the bundles of microtubules radiating from the centrosome in the cell body, and those on the cell surface regions of cytosol, where microtubules were not identified. These results suggest that the axonemal microtubules in *R. contractilis* may be accompanied by microtubule depolymerization during the rapid axopodial contraction, and that a novel disassemble manner of microtubules is involved in *R. contractilis*.

#4

Real-time phenomenological analysis of dielectric behavior of *Euglena* cells during their cell-shape changes

Ken HANAHARA and Motonori ANDO

(Lab. Cell Physiol., Grad. Sch. Educ., Okayama Univ.)

SUMMARY

Euglena cells show high sensitivity towards toxicants and can thus reveal any change in environmental water quality. Dielectric measurements can examine cell-shape dynamics in the flagellate *Euglena gracilis*. The aim of this study was to develop an algorithm for the dielectric analysis of Euglena cell suspensions, and was to apply the algorithm to the description of cell-shape dynamics in real-time. Equivalent parallel capacitances and conductances were measured between 100 Hz and 100 MHz by Agilent 4294A impedance analyzer. Data acquisition and analysis were performed simultaneously by our developed software, and then phenomenological parameters were semi-automatically obtained. Curve fitting was made on the basis of a two-term Cole-Cole equation as modified to include electrode polarization capacitances. The main algorithm of this analysis evaluates the goodness of fitting in terms of the minimized residual between experimental and theoretical curves. The real-time dielectric analysis was performed to monitor cell-shape changes by using Euglena cell suspensions. Among phenomenological parameters, permittivity increments, characteristic frequencies, and the Cole-Cole parameters allowed sensitive detection of cell-shape changes. This study indicates that our developed bioassay system can be applied to monitor for aquatic toxicants in environmental water.

#5

γH2AX formation in haploid nuclei of *Tetrahymena thermophila*: A possible involvement of a novel type of DNA double-strand break

Takahiko AKEMATSU[1], Yasuhiro FUKUDA[2], Chika TADA[2], Yutaka NAKAI[2] and Ronald E. PEARLMAN[1]

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SUMMARY

γH2AX, the phosphorylated form of histone H2AX, is a well-established marker for double-strand DNA breaks (DSB). In *Tetrahymena thermophila*, γH2AX is observed not only in crescent-shaped micronuclei characteristic of meiotic homologous recombination, but also in the developing macronuclear anlagen during genome rearrangement. Here, we demonstrate that *Tetrahymena* conjugation involved alternate γH2AX formation in the haploid micronuclei. This modification was observed in all four nuclei after the completion of meiosis. Subsequently, dephosphorylation occurred in one of the nuclei that localized near the conjugating junction formed between two complementary mating type cells. This nucleus attached to the junction and induced elongation for division, while the other three nuclei with γH2AX migrated to the posterior region of the cytoplasm and eventually disappeared. The somatic knockout mutant (DNA-PKΔ) failed to form γH2AX and was incapable of attachment and elongation of the nucleus. Consequently, these cells did not undergo conjugation and reverted to the vegetative stage. These results suggest that γH2AX formation is critical for conjugation. A novel type of DSB is likely involved in the nuclei in which DNA-PK exerts its function through the formation of γH2AX.

#6

Detection and functional analysis of plant hormones in malaria parasite

Ryuma MATSUBARA[1,2], Mikiko KOJIMA[3], Michiru TAHARA[2], Syed Bilal Ahmad ANDRABI[2,4], Michika FKSHI[1,2], Fumiya KAWAHARA[5], Akinori YAMANO[1,2], Hitoshi SAKAKIBARA[3] and Kisaburo NAGAMUNE[2,6]

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SUMMARY

Plasmodium is an Apicomplexan parasite that causes lethal febrile symptoms called malaria. This parasite belongs to Alveolata superphylum and has extremely different metabolic systems from its host. Recently, we found that *Plasmodium* spp. produced several plant hormones, and in this study, we focused on a dominant one. We succeeded to establish the hormone-deficient mutant genetically. The neural pathogenicity of the mutant to C57BL/6 mice was severer than wild-type parasite. We will discuss the role of this plant hormone from the immunologic and pathologic viewpoints.

#7

Analysis of an early stage of the evolution of endosymbiosis between an alga and a ciliate in an experimental microcosm composed of 3 species

Toshiyuki NAKAJIMA

(Dept. Biol., Ehime Univ.)

SUMMARY

The early stage of the evolution of symbiotic relationships was analyzed by conducting a long-term culture of an experimental ecosystem model composed of a green alga (*Micractinium* sp.), a bacterium (*Escherichia coli*), and a ciliate (*Tetrahymena thermophila*). The population dynamics of component species was investigated during three years from its initiation, and interspecific interactions by using isolates from the culture. The results revealed that an early form of algal-ciliate endosymbiotic association and algal-bacterial ectosymbiotic association were evolved, and that these evolutionary changes were closely related in their selection processes in this ecosystem.

#8

The evolution of interspecific relationship between an alga and a bacterium in the long-term culture of experimental microcosm

Masayuki MATSUURA, Yosuke HUJII and Toshiyuki NAKAJIMA

(Nakajima Lab., Ehime Univ.)

SUMMARY

An experimental model ecosystem composed of three species, a green alga (*Micractinium* sp.), a bacterium (*Escherichia coli*), and a ciliate (*Tetrahymena thermophila*) was cultured for about 6 years. During this culture, cell aggregates consisting of *Micractinium* and *E. coli* (called the CE-aggregates) appeared. In order to investigate the relationship between the alga and the bacterium in CE-aggregates, 30 clones of the alga and 30 clones of the bacterium were isolated each from CE-aggregates obtained from the long-term culture of the model ecosystem. The results revealed that phenotypic variation concerning physiological characteristics existed in the *E. coli* population as well as in the *Micractinium* population, and that one type of *E. coli* clones required an amino acid (i.e., isoleucine) for growth, indicating a symbiotic association with *Micractinium* sp. in CE-aggregates.

#9

Changes in the algal ingestion rate of ciliate cells in an early stage of evolution of algal-ciliate endosymbiosis in the long-term culture of experimental microcosm

Yoichiro OHNISHI, Akiko SANO and Toshiyuki NAKAJIMA

(Nakajima Lab., Ehime Univ.)

SUMMARY

An experimental model ecosystem composed of three species, a green alga (*Micractinium* sp.), a bacterium (*Escherichia coli*), and a ciliate (*Tetrahymena thermophila*) was cultured for 5–8 years. Ciliate cells that harbored algal cells emerged during the long-term culture of this system. Our previous studies demonstrated that this algal-harboring ciliate indicated an initial stage of algal-ciliate endosymbiosis. In this study, we investigated the algal ingestion rates of ciliate isolates by using algal and ciliate clones isolated from the model ecosystem in comparison with their ancestral strains. The result revealed that the isolated ciliate strains exhibited higher algal ingestion rates than the rate of the ancestral ciliate strain.

#10

Life style and evolution of 'Protozoa' and 'Algae', viewed from freshwater microscopic ecosystem

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SUMMARY

Protozoa and algae have been classified according to their lifestyles. Both groups are in close relationship with each other, because algae are considered to have appeared through multiple events of secondary endosymbiosis in protozoa. This evolutionary phenomenon is observed in many mixotrophic microorganisms, and probably have played a central role in creating protist diversity. In this talk, we are going to discuss the conceptual confusion in science education concerning “protozoa” and “protists”, and then marshal the evolutionary positions of protozoa and algae in Eukaryota, by using data obtained from freshwater sampling and culturing experiments.

#11

A study on a rolling structure of plasma membrane isolated from *Amoeba proteus*

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SUMMARY

A plasmalemma is complex of cell coat and cell membrane. The Plasmalemma isolated form *Amoeba proteus* forms rolling structure, unlike general structure of isolated plasma membrane. However, the rolling structure, which is made by lipid bilayer, is theoretically unstable. To resolve the confliction, we tried to identify the factor contribute to the stability of the rolling structure. As a result, we isolated a novel protein, which molecular weight was 26 kDa, from the plasmalemma of *Amoeba proteus*. Proteinase K treatment of the isolated plasmalemma abolished the rolling structure and made vesicle structure. In addition, the protein localize in cell coat, suggesting that the protein controls a curvature of the plasmalemma from outside of cell membrane. In this talk, we will discuss about the relation between the rolling structure and behavior of cell membrane in cell locomotion.

#12

The observation of ultrastructure in *Hypophrya* sp.

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SUMMARY

A suctorian, a kind of ciliate, has several thin projections radiating from the cell body, called tentacles. The suctorian catches a prey with in tentacles and sucks endoplasm of the prey. To approach mechanism of predation of the suctorian, we first examined celluar structure of *Hypophrya* sp., forcusing on its microtubule organization in and around tentacles. Immunofluorescence revealed that microtubules extended from a limited area near the nucleus to tentacles, We confirmed microtubule organization by electron microscopy. These results suggested that the microtubules in tentacle of *Hypophrya* emanated from a limited number of microtubule organizing centers. Microtubules in a tentacle were aligned in a highly ordered array forming "MT-tube" and cross-bridges were observed between these MTs. A sleeve-like structure consisting of two MT-tubes was observed near the base of the tentacle, suggesting that tentacle contraction may depend on this structure. At the tip of the tentacle, the cell membrane was folded, and "missile-like bodies," described in Tokophrya, were accumulated. The cell body but not the tentacle was surrounded with a low electron dense layer. Concanavalin A-staining revealed existence of mannoside only around the cell body, so that the low electron dense layer may be a cell body-specific wall-like structure.

#13

Experimental evolution of *Eecherichia coli* toward constructing a novel intracellular endosymbiosis with *Dictyostelium discoideum*

Kayo YAMAMOTO[1], Kazufumi HOSODA[2], Makoto SUEYOSHI[1], Isao KUBO[1] and Tetsuya YOMO[1,3]

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SUMMARY

We want to understand a process toward intracellular endosymbiosis from two organisms by direct observation of the process. Therefore, we are trying to construct a novel intracellular endosymbiosis of a previously non-interacting pair by experimental evolution. In this study, *Dictyostelium discoideum* and *E. coli* are used. Both *D. discoideum* and *E. coli* are prominent model organisms, and they have a predator-prey relationship. If *E. coli*s evolve to live within *D. discoideum* cells by getting the digestion tolerance, a novel intracellular endosymbiosis of *D. discoideum* and *E. coli* would be established. Thus we are trying to make *E. coli* become digestion tolerant by using experimental evolution. In this meeting, we will show you the results of the experimental evolution.

#14

Construction of a chloroplast-like novel endosymbiosis between a cyanobacterium and a ciliate

Yuhki AZUMA[1], Kazufumi HOSODA[2], Masumi HABUCHI[3], Kayo YAMAMOTO[4], Risa TAKAMI[4] and Tetsuya YOMO[3,4]

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SUMMARY

Our goal is to construct a chloroplast-like novel endosymbiosis by making a cyanobacterium live together artificially in a cell of the ciliated protozoan *Tetrahymena* living independently in the nature. Specifically, we plan to make the two organisms coevolve under an experimental environment where *Tetrahymena* depends on the cyanobacterium for survival. Thus, it is first necessary to stably cocultivate the two organisms. Here we investigated the condition for the stable coculture and found some requirements for the stable coculture.

#15

Theoretical study about evolution of stalk/spore ratio in a social amoeba

Kouki UCHINOMIYA and Yoh IWASA

(Kyushu Univ.)

SUMMARY

The social amoeba (or cellular slime mold) lives like a single cell organism while there is enough food. However, when food is depleted from the environment, many social amoebas aggregate and differentiate into either spore cells or stalk cells. These cells make a structure called fruiting body. The stalk cells help dispersal of spore cells, which in turn can produce offspring. Self-generated signaling chemical such as DIF-1 stimulates the differentiation into stalk cell. By considering the mechanism of cell differentiation with a simple mathematical model, we investigate the effect of the capacity to produce a signaling chemical and of the sensitivity to the chemical when making fruiting body. We also discuss the relation between the evolution of stalk/spore ratio and the chemical production.

#16

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

Aika SHIBATA[1], Masahiro KASAHARA[1] and Nobutaka IMAMURA[1,2]

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SUMMARY

The endosymbiotic Chlorella in *Paramecium bursaria* provides a photosynthate, maltose, to maintain the symbiotic relationship and the maltose release is induced by lowering pH. We studied the mechanism of maltose release using Japanese endosymbiont Chlorella F36-ZK. We found its maltose release did not occur without light even in an acidic condition and the effect of light was investigated at first. Blue or red light was effective for the stimulation of the maltose release. These wavelengths were the most important ones for photosynthesis. However, the photosynthesis should not directly promote the maltose release because maltose release was observed under weak light condition and the release was barely affected by light intensity. Furthermore, we studied F36-ZK maltose transport system. Radioactive photosynthate was released even in a buffer with a high maltose concentration, the transport should be an active. The proton gradient was required for maltose transport because uncouplers inhibited the maltose release.

#17

Construction of a novel endosymbiosis between *Escherichia coli* and algae-free *Paramecium bursaria*

Risa TAKAMI[1], Kazuhumi HOSODA[2], Itsuka KUMANO[1], Kayo YAMAMOTO[1] and Tetsuya YOMO[1,3]

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SUMMARY

A lot of changes must have happened to a host and a symbiont in the evolutionary process toward an endosymbiosis from two independent organisms. The purpose of our study is to observe the evolutionary changes that happen to the symbiont. Therefore we try to construct a novel endosymbiosis using *Escherichia coli* and algae-free *Paramecium bursaria*, which do not have symbiotic relationship in the nature. Specifically, we aim to evolve *E. coli* to stay in algae-free *P. bursaria* by using experimental evolution. Here, as an initial state before the experimental evolution, we investigated the engulfment and digestion of *E. coli* by algae-free *P. bursaria*, comparing them with the case of a natural symbiont of *P. bursaria* as a goal of the experimental evolution.

#18

Effects of the endosymbiosis on phagocytic activities of *Paramecium bursaria*

Takashi MIURA[1] and Sosuke IWAI[2]

([1]Grad. Sch. Educ., [2]Fac. Educ., Hirosaki Univ.)

SUMMARY

To elucidate whether endosymbiosis affect phagocytosis of the hosts, we have evaluated phagocytic activities of a ciliate, *Paramecium bursaria*, with or without the algal endosymbionts. To assess the phagocytic activities, *P. bursaria* cells were fed with yeast cells that constitutively express GFP. The fluorescent yeast cells enabled us to readily distinguish between the algal endosymbionts and the ingested prey in *P. bursaria* cells, and also to determine whether the ingested prey was eventually digested or egested. The aposymbiotic *P. bursaria* cells showed significantly enhanced ingestion activity, suggesting that the presence of the endosymbionts affected the phagocytic activities of their host cells. The engulfed yeast cells were mostly digested within 3–4 hours in the food vacuoles and slightly egested. The digestion activity was only slightly affected by the presence of the endosymbionts, suggesting that the digestive activity was not specifically suppressed even under the presence of the endosymbionts. *P. bursaria* cells in stationary phase showed lower ingestion activity than cells in log growth phase, presumably because of the lower expression level of the proteins involved in phagocytosis.

#19

The stress tolerance of *Paramecium bursaria*

Yuka ADACHI[1], Ayumi SHIMOMURA[1], Kozue HAMAO[1] and Hiroshi HOSOYA[1,2]

([1]Dept. Biol. Sci., [2]Marine Biol. Lab., Grad. Sch. Sci., Hiroshima Univ.)

SUMMARY

A single cell of the green paramecia (*Paramecium bursaria*) harbors several hundreds of endo-symbiotic *Chlorella*-like algae in its cytoplasm. The symbiotic association of algae is only established with *P. bursaria* but not with other kinds of paramecium. However, it is not clear how the specific interaction between alga and green paramecia is accomplished. The green paramecium might be resistant against reactive oxygen produced by endosymbiotic algae in the cytoplasm. Then, we elucidated the stress tolerance of *P. bursaria.* Our results obtained revealed that *P. bursaria* with or without algae showed the highest tolerance against H_2O_2 or high temperature in the several kinds of paramecium.

#20

Infectivity of the symbiotic *Chlorella* spp. of the ciliate *Paramecium bursaria*

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([1]Course of Biol. Sci. Biotechnol., Grad. Sch. Life Environ. Sci., [2]Dept. Biol. Sci., Fac. Life Environ. Sci., Shimane Univ.)

SUMMARY

Paramecium bursaria cells harbor hundreds of *Chlorella* spp. in their cytoplasm. Irrespective of the mutual relationships between *P. bursaria* and *Chlorella* spp., both cells retain the ability without their partner. Although their relationship is separable and flexible, it is reported that isolated symbiotic *Chlorella* spp. from European *P. bursaria* cells could not be established endosymbiosis to American alga-free cells. In this study, we examined the infectivity of the symbiotic algae isolated from 6 strains of alga-bearing *P. bursaria* cells to a different strain of alga-removed *P. bursaria* cells which are collected from different areas. As a result, all isolated symbiotic algae examined in this study could localize beneath the host cell cortex and established endosymbiosis with alga-free *P. bursaria* cells. Although these strains could be maintained in the host cells for more than 1 month after mixing, there were some differences in the each algal growth rate in the host paramecium during cultivation under constant light condition. Our study suggests that the host-symbiont compatibility occurs after the establishment of endosymbiosis.

#21

Cell signaling pathways and analysis of expressed proteins during the resting cyst formation of ciliated protozoan *Colpoda cucullus*

Yoichiro SOGAME[1], Katsuhiko KOJIMA[2], Toshikazu TAKESHITA[2], Eiji KINOSHITA[3] and Tatsuomi MATSUOKA[1]

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SUMMARY

The molecular events during the resting cyst formation (encystment) of free-living unicellular organisms have not been elucidated, despite a century's research into this biological phenomenon. Recently, our research group has explored the molecular events of encystment including the signal transduction pathways of the ciliate *Colpoda cucullus* by fura-2 (Ca^{2+} indicator) ratiometry, a cAMP enzyme immunoassay, phos-tag/electrochemiluminescence, and two-dimensional electrophoresis prior to a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Cell-to-cell mechanical stimulation promotes an inflow of Ca^{2+}, which activates intracellular signaling pathways for encystment, in which several proteins (histone H4, ribosomal protein P0, ribosomal protein S5, etc.) are cAMP-dependently phosphorylated, and the expression levels of several proteins (actin, elongation factor 1a, heat shock protein, etc.) were enhanced, whereas mitochondria ATP synthase b chain disappeared. Among these proteins, mitochondria ATP synthase b chain was suggested to be involved in the maintenance of the mitochondrial membrane potential, because the knockdown of this protein in *Trypanosoma* reduced mitochondrial membrane potential (Brown S.V. *et al*. 2006; Eukaryot. Cell 5: 45–53), and in encystment-induced *C. cucullus*, the mitochondrial membrane potential disappeared concomitantly with the disappearance of ATP synthase b chain. HSP60 may be involved in the protection of proteins under stressed conditions.

#22

Roles of kinesin14A in micronuclear division in *Tetrahymena*

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SUMMARY

Ciliates possess two functionally differentiated nuclei; the micronucleus (Mic; germline nucleus) and the macronucleus (Mac; somatic nucleus). Mic contains single genome and is transcriptionally silent. Mac is far-polyploid and actively transcribed. Our final aim is to understand how these two nuclei can be maintained through nuclear division during vegetative growth. Here we report the function of microtubule (MT) minus-end motor, kinesin-14s on Mic mitosis and meiosis. One of two homologues of *Tetrahymena* kinesin-14s (*KIN14A*) plays essential role on spindle formation during mitosis and meiosis in dividing Mic. Plus, over-produced *EGFP-KIN14A* accumulates at meiotic spindle poles. These results suggest that *KIN14A* is essential for the formation of spindle pole in *Tetrahymena*. On the other hand, *KIN14A* is not required during Mac amitosis. Some more descriptive research is important to identify the polarity of MTs in dividing Mic and Mac, and other key MT motors governing the arrangement of MT in nucleoplasm. Through these intensive future studies, we believe that one can explain the evolutional process of establishment of 'nuclear dualism' of ciliates.

#23

Tetrahymena as a production plant of foreign proteins

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SUMMARY

Recent genome analyses of ciliates have revealed that relatively more genes are encoded in their genomes than those of other eukaryotes. For instance, *Tetrahymena thermophila* have more than 27,000 protein-coding genes, and *Paramecium tetraurelia* also retain approximately 40,000 genes in their genomes. These observations suggest that ciliates have outstanding abilities such as efficient protein folding and assembly of complicated molecules such as monoclonal antibodies (with the latter, see Cilian AG homepage of German company). In addition, surface display of antigens of viral or parasite on cell membrane of *T. thermophila* was successfully reported. Here we discuss a potential of *Tetrahymena* as a production plant of foreign proteins, based on expression and secretion of some cellulases from symbiotic flagellates of the termites.

#24

FAP82 regulates activation of K^+ leak channel in *Paramecium*

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SUMMARY

Chlamydomonas FAP82, a flagellar protein, is highly conserved from protists to mammals. In *Paramecium*, the ortholog of *Chlamydomonas* FAP82 is highly expressed in cilia. However, the function of FAP82 ortholog in cilia remains unclear. For greater insight into the ciliary function in *Paramecium*, we have studied the function of FAP82 ortholog using RNAi by the feeding method and through analysis of phenotypes. Pt_FAP82 knockdown cells showed no typical escape reaction against mechano-stimulation and cGMP. Moreover, their knockdown enhanced the swimming speed by cAMP. Consequently, our results suggest Pt_FAP82 regulates the activation of cyclic nucleotide dependent K^+-leak channel.

#25

Development of nickel inducible expression system 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 *Paramecium 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+} ions 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 constructed the Ni66 promoter-driven reporter vector harboring secreted luciferase isolated from the marine copepods. Functions of nickel induced genes and future research will be discussed in the oral presentation.

#26

Molecular composition and the function of plant-vacuole like organelle in extracellular *Toxoplasma gondii*

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SUMMARY

It was previously assumed that *Toxoplasma gondii* did not contain lysosomes, although recent research has revealed that extracellular *T. gondii* form acidic lysosome-like organelle called plant-like vacuoles (PLV) or vacuolar compartments (VAC). To confirm the acidicity of this organelle, we used LysoTracker, which stains acidic organelles like lysosomes. We found a LysoTracker-positive organelle specific for extracellular *T. gondii*. The LysoTracker signal corresponded to the location of a previously described PLV marker whereas the signal did not correspond to the location of a VAC marker. We also found that the PLV marker–positive organelle was divided into two sub-compartment types, i.e., PLV1 and PLV2, by incubation in an extracellular environment. LysoTracker stained only the PLV2 compartment. We also found that PLV stored H^+ and Ca^{2+}. Disrupting PLV-function using primaquine caused decreased tolerance to high NaCl stress; therefore, PLV was inferred to be involved in regulating ionic balance in *T. gondii*. H^+ and Ca^{2+} in PLV2 were released into the cytoplasm after the primaquine treatment, and this treatment also inhibited gliding motility. These results suggest that PLV is a vacuole-like organelle with a formation process similar to that observed for plant vacuoles and a function involving ionic balance regulation in the cytoplasm.

#27

Megavirus-infecting *Acanthamoeba* sp. isolated from a keratitis patient

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SUMMARY

Recently unique and giant nucleocytoplasmic large DNA viruses (NCLDV), such as "mimivirus" have been discovered in *Acanthamoeba* sp., a common free-living amoeba. These giant viruses are beyond the biological concept of "virus" because they are almost the same size with bacteria and have a very large genome (1.0 Mb~). These characteristics of the giant viruses place them at the boundary of living and non-living. The presence of the giant viruses have added to the debate over the origins of life. Beside the impact on biology and evolution, mimivirus has been isolated from a pneumonia patient, suggesting viruses in amoeba are potentially pathogenic to humans. In the study of a molecular epidemiology on *Acanthamoeba keratitis*, we isolated an NCLDV-infecting *Acanthamoeba* sp. from corena of a keratitis patient. An electron microscopy (TEM) for morphological study and preliminary genetic analysis revealed that the virus was a member of megavirus which is bigger in size with larger genome than mimivirus. Megavirus kills the host amoeba and may have a cytopathic effect on human corneal somatic cell culture. Megavirus infecting *Acanthamoeba* is now axenically cultured and maintained by a conventional procedure.

#28

Species composition change in the intestinal protist communities after community mixing

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SUMMARY

Termites in the genus *Reticulitermes* harbor host species-specific symbiotic protist communities in their digestive tracts composed of about 10 excavate species. Using winged reproductives (future kings and queens) of three *Reticulitermes* spp., we made hybrid incipient colonies and investigated the transition patterns of the symbiont species compositions of the colony members. After 800 days of culture, the symbiont compositions of all the hybrid colonies between *R. speratus* and *R. kanmonensis* and those between *R. speratus* and *R. amamianus* became very close to the original symbiont composition of *R. speratus*. Similarly, the symbiont compositions of the hybrid colonies between *R. kanmonensis* and *R. amamianus* approached a novel, specific composition. These results suggest that the symbiotic protist communities are not random assemblages of the element species. The presence of the specific stable compositions may explain the community replacements suggested to have occurred in some termite linages.

#29

Development of microscopic system for precise investigation of mobile protists

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SUMMARY

The flagellated microalgae are difficult to catch the clear images under ordinary microscope because of their extremely rapid movements. We developed a super high speed video-microscope for the visual analysis of their movement, especially of their flagella movement. Based on this microscope, we repeated the improvement of the system with the collaboration of Olympus Corporation and Photoron Limited, searching for better method for visual observation of the outside world of human eyesight. We introduce the various movies of enlarged cell images on screen.

#30

Growth analysis of *Paramecium busaria* and the algal endosymbionts

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SUMMARY

We have investigated growth of *Paramecium bursaria* and its algal endosymbionts. For quantitative analysis of the growth, *P. bursaria* cells were grown in a simple, bacteria-free monoxenic culture system, in which yeast cells were the sole organic nutrients. The population growth analysis using this system showed that the specific growth rate of aposymbiotic (Chlorella-free) cells was comparable to that of wild-type cells hosting the endosymbionts, while the growth yield of the aposymbiotic ones was significantly lower than that of the wild type. Single-cell level growth and survival analysis demonstrated that, under no-food condition, the wild-type cells survived for 30 days, while the aposymbiotic ones for at most 7 days. These results suggest that the benefits of acquiring phototrophy for *P. bursaria* by hosting the algal endosymbionts involve 1) at least in part, obtaining maintenance energy and 2) probably, obtaining carbon sources for cell growth. Furthermore, the simultaneous growth analysis of both the hosts and the endosymbionts within the host cells suggested that the growth of these two species might not be tightly coupled.